Loss Of MHC Class I Related Gene Expression Inhibited The M1 Macrophages Infiltration In Tumor Microenvironment Of Ovarian Serous Cystadenocarcinoma

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Research article

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Abstract

Background: Previous studies have shown that tumor immune microenvironment was an important factor affecting the progression and prognosis of ovarian cancer. The purpose of this study is to explore the prognosis related immune cell types in the immune microenvironment of ovarian cancer by using the ovarian cancer database, and to study the potential factors affecting specific immune cell infiltration.

Method: Based on TCGA ovarian cancer database, CIBERSORT method was used to preliminarily analyze the infiltration ratios of 22 kinds of immune cells around ovarian cancer, and to further evaluate the correlation between infiltration cells and prognosis based on the survival data. In addition, this study will calculate the tumor infiltrating M1 Macrophages abundance (IM1A) to verify the correlation between M1 Macrophages load and the prognosis of ovarian cancer; and to explore the potential pathway of M1 Macrophages infiltration by Pearson correlation analysis.

Results: The results showed that the infiltration proportion of follicular helper T cells and M1 Macrophases was negatively correlated with the poor prognosis of ovarian serous cystadenocarcinoma, while activated Mast cells was opposite. In addition, the overall survival of ovarian cancer patients with high IM1A was significantly longer than that of patients with low IM1A; the enrichment of GSEA KEGG pathway showed that multiple pathways were correlated with M1 Macrophages infiltration (including 67 positive and 1 negative pathways), and the highest correlation was found in antigen processing and presentation pathway. The expression level of some MHC class I related genes (potential target genes of immunotherapy) in antigen processing and presentation pathway was positively correlated with the infiltration ratios of M1 Macrophages in microenvironment, including HLA A, HLA B, HLA C, HLA E, HLA F, B2M, TAP1, TAP2, and TAPBP.

Conclusion: In general, the decreased infiltration of M1 Macrophages indicates poor prognosis of ovarian serous cystadenocarcinoma, and the expression loss of MHC class I pathway gene might be the key factor for the inhibition of infiltration of M1 Macrophages. Altering these key genes expression could improve the infiltration of M1 Macrophages and the overall prognosis of ovarian serous cystadenocarcinoma.

Introduction

According to the latest data, ovarian cancer is one of the most common gynecological malignancies, and the second leading cause of gynecological malignancies related death in the world [1]. In recent years, although the medical personnels specialized in ovarian cancer have made tremendous efforts for the diagnosis and treatment of ovarian cancer, the current status of diagnosis and treatment has not been significantly improved [2–4]. Most patients with ovarian cancer are in the advanced stage at the time of diagnosis. Some research data showed that nearly 80% of epithelial ovarian cancer patients suffered with local progression, abdominal metastasis or other abdominal organ metastasis at the time of diagnosis [5]. At present, the standard therapeutic strategies for these patients is to receive platinum

and/or paclitaxel adjuvant chemotherapy on the basis of tumor reduction surgery [6, 7]. Due to the high chemotherapy resistance rate, other effective treatment strategies for these patients urgently need to be explored, such as targeted therapy, and immunotherapy [8, 9].

In recent years, the roles of tumor immune microenvironment in the occurrence and development of various tumors were gradually revealed. The tumor immune microenvironment mainly includes various types of immune cells, and their related immune molecules (such as cytokines, chemokines, complement fragments, etc.) [10]. Studies have shown that some subtypes of immune cells could play the role of tumor inhibition in tumor progression, such as effector T cells, helper T cells, NK cells, DC cells, M1 Macrophages, etc., while other subtypes could work as tumor promoters, such as Treg cells, M2 Macrophages, bone marrow-derived suppressor cells (MDSC), etc [11, 12]. Except for immune cells, various immune molecules also play corresponding roles in tumor progression, including tumor cell growth, death, metastasis and other biological behaviors [13–15]. In addition, tumor cells could also regulate the function of immune cells through a variety of mechanisms to further promote immune escape. For example, tumor cells could inhibit the growth of effector immune cells by regulating the expression of immune checkpoint molecules [16, 17]; local lactic acid accumulation caused by abnormal metabolism of tumor cells could induce metabolic reediting of immune cells [18].

Immune microenvironment also plays an important role in the progression of ovarian cancer [19, 20]. It has been reported that there were a large number of tumor infiltrating lymphocytes in various pathological types of ovarian cancer (such as high-grade serous ovarian cancer, endometrioid ovarian cancer, mucinous ovarian cancer, etc.) [21, 22]. Among them, CD8+ T cells infiltration was positively associated with the prognosis of ovarian cancer, while the infiltration of CD169 + Macrophages and plasmacytoid dendritic cells was an important factor for increased immune escape, and further lead to poor prognosis of ovarian cancer [23–25]. Although the roles of some subtypes of immune cells in ovarian cancer has been partially revealed, the functions of some other subtypes of immune cells in ovarian cancer still need to be explored. The smooth progress of these works will not only reveal the potential immune escape mechanisms of ovarian cancer, but also provide targets and theoretical basis for related cancer immunotherapy.

Methods And Materials

Data sources

The transcriptome data used in this study were obtained from the RNA-seq data of TCGA ovarian cancer. The downloaded data type was the original count data, and the data version was October 27, 2018. Only 379 cases of ovarian cancer were contained in this database. The matched clinical data were downloaded from the cbioportal website (https://www.cbioportal.org). The gene expression profile data were obtained from the lllumina hiseq2000 RNA sequencer in the University of North Carolina TCGA genome characterization center.

Rna Sequencing Data Preprocessing

Firstly, RNA-seq raw counts of ovarian cancer were downloaded from TCGA database. The R language software (R 3.6.3 GUI 1.70 El Capitan build 7735) was used for subsequent data analysis. And then, according to the gene expression distributed in all samples,low expression genes were eliminated. If the gene expression CPM (counts per million, CPM) in more than 10 samples was less than 0.5, the gene would be deleted. Finally, limma[i] package was used for Standardized treatment (http://bioconductor.org/packages/release/bioc/html/limma.html).

Immune Infiltration Analysis

CIBERSORT software can accurately quantify the relative levels of different cell types in complex gene expression mixture through algorithm simulation. The software was suitable for CHIP data and RNA sequencing data. Pre-processed data were to submitted CIBERSORT, and then, the proportion of immune cell infiltration of ovarian cancer patients were calculated. *P* value less than 0.05 was selected as the screening threshold for predicting reliability.

Analysis Of Immune Infiltration And Tumor Prognosis

Combined with the infiltration ratios of 22 immune cells calculated by CIBERSORT and the clinical survival data, the relationship between the infiltration degree of each immune cell and overall survival was evaluated. According to the proportion of infiltration, the samples were divided into high infiltration (more than median) and low infiltration (less than median). Kaplan Meier (KM) model was used for survival analysis between different groups, and *P* value < 0.05 was used as the significance threshold.

Calculation Of M1 Macrophages Infiltration Score

According to the method listed in the literature(Wang L, et al. EMT- and stroma-related gene expression and resistance to PD-1 blockade in urothelial cancer. Nat Commun, 2018, 9(1):3503), the tumor infiltrating Macrophages M1 cell allowance (IM1A) was calculated. Firstly, 48 gene markers of Macrophages M1 cells were extracted according to the differential gene table of LM22 provided by CIBERSORT. Among them, 4 genes were deleted due to low expression. According to the expression level of the remaining 44 genes, the expression amount of arithmetic mean was calculated as IM1A. According to the method description, the values of IM1A of 379 samples were calculated, and the Pearson correlation between IM1A and all its gene expression was further analyzed. Then, according to Pearson correlation, GSEA KEGG enrichment analysis was performed for positive and negative related genes by R package phenotest. The threshold of pathway enrichment significance was *P* value < 0.05.

Results

The data of training set applied in this study was obtained from TCGA ovarian cancer database, including the RNA-seq raw counts,the basic information, and clinical case information of 379 ovarian cancer patients, as well as the prognosis information of each patient (Table 1). According to CIBERSORT analysis results, the proportion of 22 kinds of immune cells in the tumor microenvironment of ovarian serous cystadenocarcinoma was shown in Supplementary Table 1. Further survival analysis showed that the infiltration of follicular helper T cells, M1 Macrophages, and activated mast cells was significantly correlated with the prognosis of ovarian cancer (Figure 1). Specifically, the prognosis of patients with high infiltration of follicular helper T cells or M1 Macrophages was better than patients with low infiltration of these cells, while patients with high infiltration of activated mast cells were worse than other patients. According to the results of training set, it was suggested that the decrease of M1 Macrophages was a risk factor for poor prognosis of ovarian serous cystadenocarcinoma.

In order to further verify the correlation between M1 Macrophages infiltration and the progression and prognosis of ovarian serous cystadenocarcinoma, as well as its potential influencing factors, we used the expression of M1 Macrophages characteristic genes to calculate the infiltration abundance of this cell (IM1A) in the microenvironment (Supplementary Table 2). Survival analysis based on IM1A revealed that there were significant differences in survival between patients with low and high IM1A score (Figure 2), and the trend was consistent with the results obtained by CIBERSORT method. Analysis of the correlation between IM1A score and ovarian cancer progression showed that there was no significant correlation between IM1A score and pathology parameters of ovarian serous cystadenocarcinoma (Table 2).

Pearson correlation analysis was used to obtain genes related to IM1A score, and further GSEA KEGG enrichment based on these genes showed that 67 pathways were positively correlated with IM1A score, while only 1 pathway was negatively associated with IM1A score (Figure 3). The method of IM1A score calculation was used to evaluate the score of each pathway, and the ovarian serous cystadenocarcinoma patients were divided into two groups according to the median of the pathway score. The survival analysis showed that nine pathways (such as antigen processing and presentation (APP), Alzheimer's disease, toxic phosphorylation, and spliceosome pathway) were significantly correlated with the overall survival of ovarian cancer patients (Figure 3). Further evaluation of the correlation between these nine pathway score and IM1A score showed that there was a significant positive correlation between APP score and IM1A score (Table 3, Figure 4A). According to IM1A score and APP score, ovarian serous cystadenocarcinoma patients were further divided into four groups (APP score high & IM1A score high, APP score high & IM1A score low, APP score low & IM1A score high, APP score low & IM1A score low). Further survival analysis showed that APP score high & IM1A score high group had the best survival, and APP score low & IM1A score low group had the worst survival (Figure 4B). These results not only verified the effect of APP pathway and M1 Macrophages on the prognosis of ovarian cancer, but also partially suggested that APP pathway could affect the infiltration of M1 Macrophages.

Previous studies reported that the inhibited expression of MHC class I related genes in APP pathway was one of the key regulatory factors of tumor immune escape. Therefore, we further evaluated the correlation between IM1A score and MHC class I related gene expression. The analysis results showed that HLA A,

HLA B, HLA C, HLA E, HLA F, B2M, TAP1, TAP2, TAPBP and other gene expression had significant positive correlation with IM1A score, and the correlation coefficient of each gene was shown in Table 4.

Discussion

In this study, we firstly used TCGA ovarian serous cystadenocarcinoma data sets to analyze the infiltration of 22 kinds of immune cells in the immune microenvironment of ovarian cancer by CIBERSORT method. Research data showed that there were many kinds of immune cells infiltrating in ovarian serous cystadenocarcinoma, but the infiltrating proportion of certain immune cell types was significantly different in different samples. Among them, M1 Macrophases has extensive infiltration in the microenvironment, and was closely related to cancer progression and prognosis.

Tumor associated Macrophages evolved from peripheral blood monocytes,and further infiltrated into the tumor tissue [26, 27]. This immune cell was characterized by higher infiltration proportion in the tumor immune microenvironment and diverse functions. Recent studies have shown that the activated Macrophages in tumor immune microenvironment mainly included two subtypes, M1 and M2 Macrophages. The activation of M2 Macrophages depends on the induction of IL-4, IL-10, IL-13, vitamin D3, TGF-β and other molecules; and activated cells promote tumor progression by secreting IL-10, VEGF and other anti-inflammatory cytokines; M1 Macrophages were activated by IFN-γ, GM-CS and other factors, they could exert anti-tumor effect by secreting pro-inflammatory cytokines or mediating tumor antigen-related immune response [28–30]. Previous studies have shown that M1 Macrophages had obvious infiltration in a variety of malignancies, such as gastric cancer, colorectal cancer, lung cancer, etc., and the prognosis of cancer patients with high M1 infiltration or high M1/ M2 infiltration ratio was significantly improved [31–33]. In addition, similar to the results of this study, a study based on more than 2000 patients diagnosed as ovarian cancer found that high-grade serous ovarian cancer existed obvious M1 Macrophages infiltration, and the infiltration level was positively correlated with well prognosis[34]. Besides affecting the prognosis of patients, the infiltration of M1 Macrophages was also significantly associated with tumor progression, the M1 Macrophages proportion in low-grade ovarian cancer was significantly lower than that in high-grade ovarian cancer. In conclusion, the above data suggest that M1 Macrophages was a key factor affecting the progression and prognosis of ovarian cancer. However, the factors responsible for the low infiltration of M1 Macrophages were still unclear, and need to be further explored.

Reviewing previous studies, the mechanisms of Macrophages polarization in tumor immune microenvironment has been partially revealed. For example, it has been reported that the proportion of M2 Macrophages in the microenvironment was increased following the activation of Linc01140/Mir-140-5p/FGF9 axis in bladder cancer [35]; the infiltration of M2 or M1 Macrophages around the tumor was respectively increased or decreased by KRT6A transcriptional regulation in pancreatic cancer [36]; the polarization and function of Macrophages in the microenvironment were also affected by metabolic products such as lactic acid, adenosine, and glutamine [37, 38]. Furthermore, studies on Macrophages polarization regulation in ovarian cancer have also been reported. For example, ovarian cancer cell

inhibited the infiltration of M1 Macrophages in local microenvironment by regulating Wnt5a (atypical Wnt ligand) expression; RNA binding protein SORBS2 bound to the 3 'untranslated regions of WFDC1 or IL-17D to induce Macrophages differentiation into M2 type, and form suppressive tumor immune microenvironment [39]. In order to further explore the interfering factor of M1 Macrophages infiltration in ovarian cancer, we calculated and analyzed the M1 Macrophages infiltration abundance (IM1A) of each ovarian cancer patient, and the analysis results showed that M1 Macrophages infiltration level was negatively correlated with poor prognosis; multiple pathways in ovarian cancer were significantly correlated with IM1A; and the correlation coefficient of Antigen processing and presentation pathway was as high as 0.8, suggested that the polarization of tumor associated Macrophages might be affected by Antigen processing and presentation pathway Presentation pathway regulation.

Antigen processing and presentation is a process in which MHC molecules combine with antigen peptides and then transfer to the cell surface for specific immune cells to recognize, including endogenous antigen presentation mediated by MHC class I molecules and exogenous antigen presentation mediated by MHC class II molecules [40, 41]. Antigen processing and presentation plays an important role in immune cell activation and inflammatory mediators release, phagocyte mediated pathogen killing, tumor cell killing and so on [41]. Previous studies have shown that MHC class I pathway related genes were abnormally expressed in many malignant tumors (including colorectal cancer, melanoma, pancreatic cancer, ovarian cancer, etc.), involving HLA ABC, B2M, antigen presenting machine (APM) and other genes; meanwhile, gene mutations (including HLA haplotype loss, HLA allelic loss, B2M heterozygosity loss, IFN transduction pathway), and hypermethylation (transcriptional factors for the transcription of MHC class I genes) also reduced the expression of HLA, ABC, B2M, and APM genes [42, 43]. The abnormal expression of these genes was involved in the malignant progression of tumor through a variety of pathways, and resulted in poorer prognosis [44, 45]. Among them, the mechanism of tumor immune escape mediated by gene abnormal expression deserves our attention, mainly including the decreased infiltration of T lymphocyte in tumor microenvironment, suppression of immune response, and inactivation of killing [46]. In addition, Francisco Perea and other researchers found that the less infiltration of CD8+ T cells was observed in non-small cell lung cancer with decreased HLA class I expression, as well as the proportion of M1 Macrophages [47]. Combined with our results and previous results, it was suggested that the lower expression of MHC class I genes in ovarian cancer was involved in regulating the polarization of tumor associated Macrophages, which led to the decrease of the proportion of M1 Macrophages in the microenvironment. Reviewing the literature, the mechanism of MHC class I gene regulating TAM polarization was rarely reported. Only Francisco Perea has reported that the regulation of MHC class I genes on TAM polarization was a complex process involving fibroblasts, tumor cells, lymphocytes and other components [47]. We further analyzed the correlation between IM1A score and MHC class I gene expression, and found that IM1A score was significantly correlated with the expression of HLA, HLA B, HLA C, HLA e, HLA F, B2M, TAP1, TAP2, TAPBP and other genes, suggesting that these genes might participate in TAM polarization regulation, but the specific upstream and downstream regulatory mechanisms needed experimental exploration and verification.

In addition, the detection of M1 Macrophages infiltration levels and APP pathway expression levels in ovarian cancer was helpful to assess the prognosis of ovarian cancer patients, and to guide the treatment for some patients. It was reported that the inhibition of MHC class I gene expression could be divided into reversibility and irreversibility. Irreversibility was related to gene mutation, while reversibility was related to transcriptional regulation. Immunotherapy with IFN-γ or other cytokines could promote the MHC class I genes expression and prolong survival of patients with reversible inhibition, but it was not effective for patients with irreversible inhibition. In addition, IFN-γ was also one of the key factors for affecting the polarization of M1 Macrophages. Therefore, ovarian cancer patients with abnormal MHC class I pathway expression caused by transcriptional regulation could attempt IFN-γ based immunotherapy. IFN-γ could reverse MHC class I genes expression and induce TAM to differentiate into M1 Macrophages, so as to maximize the survival benefit of ovarian cancer patients (the data of this study show that IM1A score high & APP score high ovarian cancer patients had the best prognosis, while IM1A score low & APP score low ovarian cancer patients has the worst prognosis); patients with related gene mutations can try adoptive T cell or NK cell therapy, or tumor vaccine therapy. However, the exact therapeutic effect of the above treatment needs to be further verified by animal experiments and clinical trials.

Conclusion

In general, the expression level of APP pathway and the infiltration level of M1 Macrophages were important risk factors for poor prognosis of ovarian serous cystadenocarcinoma. The abnormal expression of MHC class I genes in ovarian serous cystadenocarcinoma was main reason for the decreased infiltration of M1 Macrophages in the microenvironment. Therefore, IFN-γ based immunotherapy might provide a new immunotherapy strategy for partial ovarian serous cystadenocarcinoma patients.

Declarations

We have no conflict of interest. We have full control of all primary data and agree to allow the journal to review the data if requested.

Compliance with Ethical Standards

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Conflict of Interest: Author Lin Xu declares that she has no conflict of interest. Author Hui Zhu declares that she has no conflict of interest. Author Juan Liu declares that she has no conflict of interest. Author Yajuan Fu declares that she has no conflict of interest. Author Lizhou Sun declares that she has no conflict of interest. Author Xuemei Jia declares that she has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Author contributions:

Xuemei Jia Lizhou Sun contributed to the conception of the study;

Lin Xu contributed significantly to analysis and manuscript preparation; Hui Zhu, Juan Liu, Yajuan Fu helped perform the analysis with constructive discussions.

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Tables

Tables 1-4 are available in the Supplemental Files section.

Figures

Figure 1

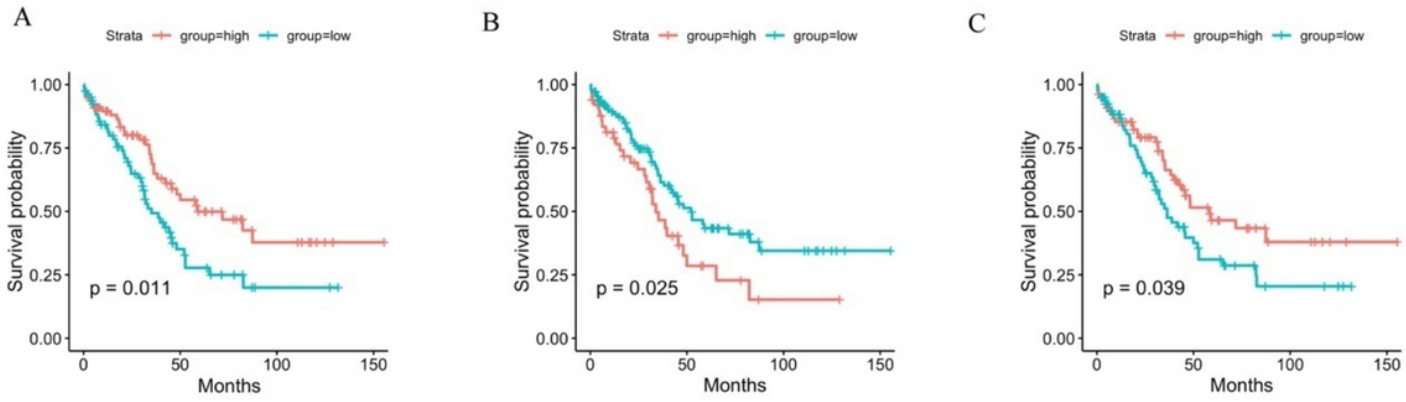

Figure 1

Correlation between multiple infiltrating immune cells and overall survival of patients with ovarian serous cystadenocarcinoma. (A) Correlation between follicular helper T cell infiltration and prognosis of patients with ovarian serous cystadenocarcinoma; (B) Correlation between activated mast cell and prognosis of patients with ovarian serous cystadenocarcinoma; C. Correlation between M1 macrophages and prognosis of patients with ovarian serous cystadenocarcinoma.

Figure 2

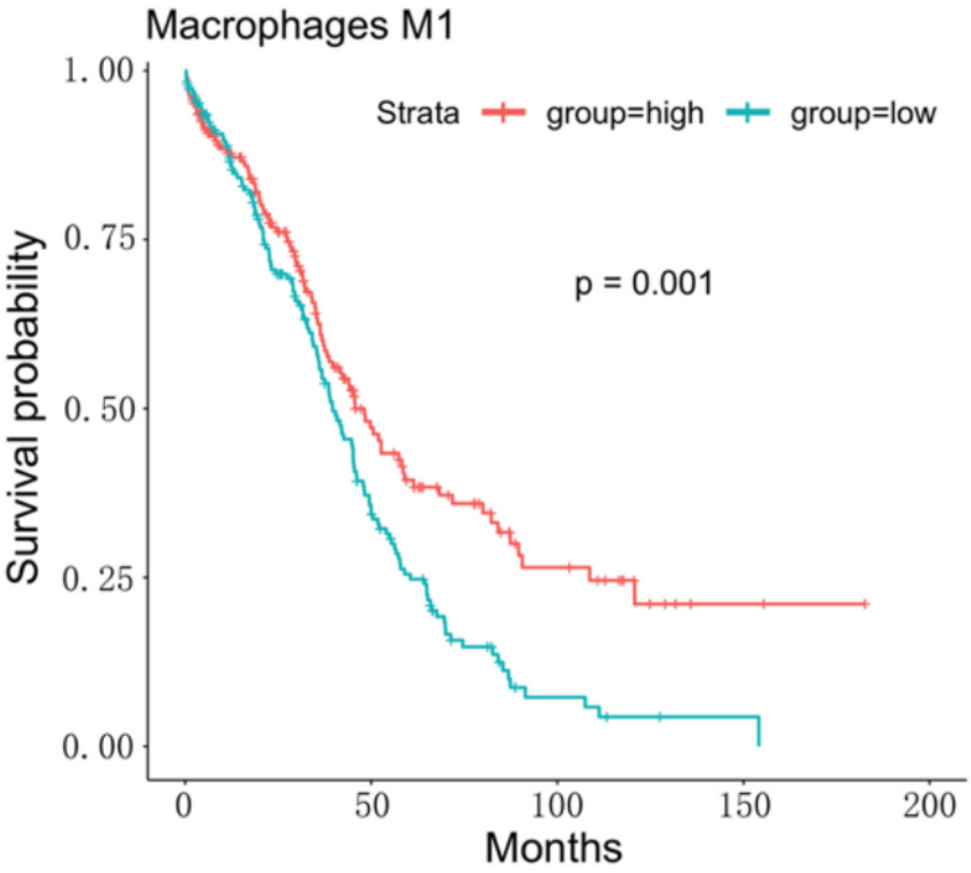

Figure 2

Correlation between M1 Macrophages infiltration score (IM1A) and overall survival of ovarian serous cystadenocarcinoma. The prognosis of patients with high IM1A was significantly better than that of patients with low IM1A.

Figure 3

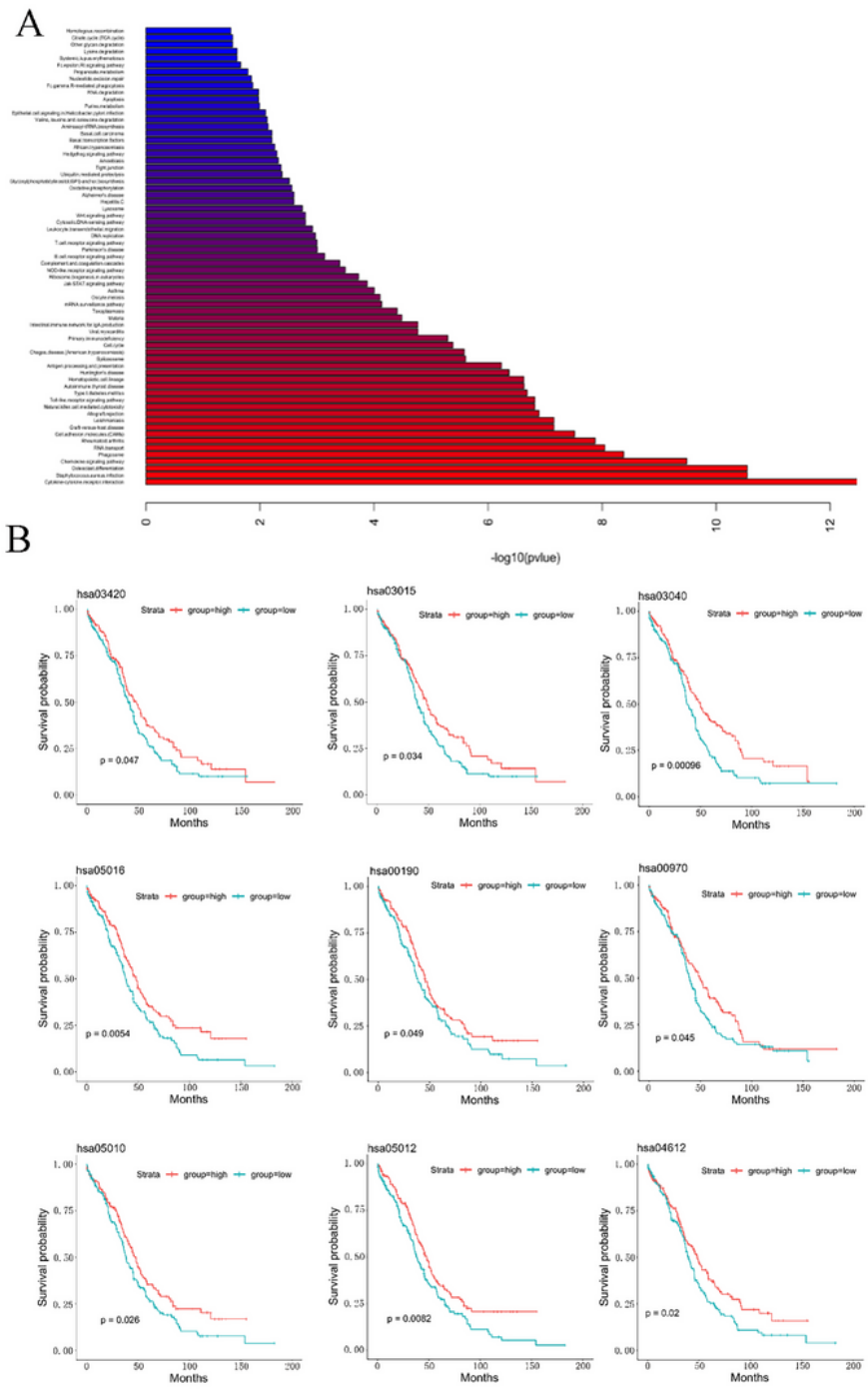

Figure 3

Enrichment results of GSEA KEGG based on IM1A related gene. (A) 67 pathways were positively correlated with im1a scores, such as allograft rejection, graft versus host disease, primary immunodeficiency, Staphylococcus aureus infection, antigen processing and presentation. (B) A total of 9 pathways score were correlated with prognosis. All of them showed that patients with high pathway scores have a good prognosis.

Figure 4

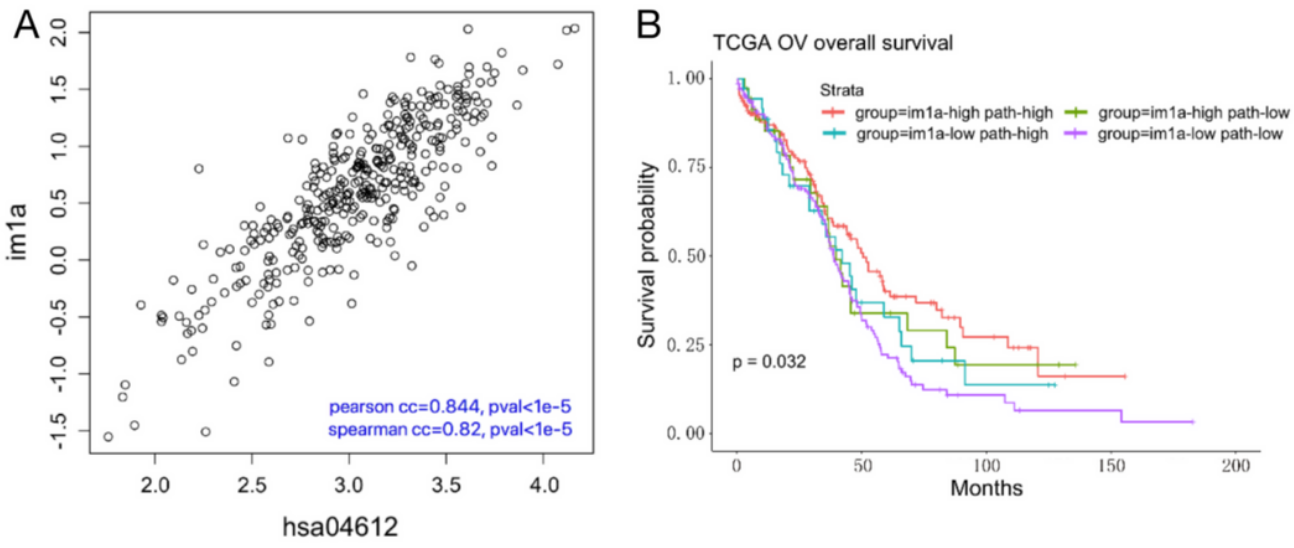

Figure 4

Correlation between hsa04612 (antigen processing and presentation) pathway and M1 Macrophages infiltration. (A) There is an obvious positive correlation between them, with Pearson correlation coefficient of 0.844 and Spearman correlation coefficient of 0.820. (B) The overall survival of high pathway score & high Macrophages infiltration group was the best, and that of low pathway score & low Macrophages infiltration group was the worst.

Supplementary Files

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