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Exosomes released from PD-L1^{+} tumor associated macrophages promote peritoneal metastasis of epithelial ovarian cancer by up-regulating T cell lipid metabolism

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ABSTRACT

Epithelial ovarian cancer (EOC) tends to metastasize to the peritoneum, and the prognosis of patients is poor. In the peritoneum of patients with EOC, TAMs (tumor associated macrophages) regulate the imbalance of T cell ratio and promote the progression and metastasis of EOC. However, the mechanism of peritoneal metastasis in EOC patients remains unclear. Here, we confirmed that the percentages of PD-L1^{+} TAMs in EOC tissues increased significantly, and TAMs-derived PD-L1^{+} exosomes affected the transcription factor PPARα to up-regulate the expression of CPT1A in CD8^{+} T cells, promote fatty acid oxidation, and increase reactive oxygen species to cause cell damage. The apoptosis of CD8^{+} T cells was increased, and the expressions of their exhaustion markers LAG3, TIM-3, and PD-1 were also up-regulated. TAMs affect T cell function through lipid metabolism, leading to peritoneal immune imbalance and promoting peritoneal metastasis of EOC. This study reveals the mechanism by which TAMs in the peritoneal microenvironment regulate T cell lipid metabolism through exosome delivery of PD-L1, and the effect of lipid metabolism on T cell function, reveals the molecular mechanism of tumor immune microenvironment affecting EOC metastasis, and further explores related pathways whether molecular blockade can be used as a means to intervene in disease progression is expected to establish a new strategy for the diagnosis and treatment of EOC.

1. Introduction

Ovarian cancer is the disease with the highest fatality rate among the three major malignant tumors of the female reproductive system, and epithelial ovarian cancer (EOC) is a typical representative [1–4]. Unlike other solid tumors, EOC rarely disseminates via blood vessels but is more prone to peritoneal metastasis. Since the ovary is located deep in the abdominal cavity, the onset features of EOC are hidden [5]. Most patients are at an advanced stage, manifested as peritoneal implantation and metastasis, and the prognosis is often poor [3]. Therefore, exploring the mechanism of EOC peritoneal metastasis is of great significance for the development of EOC targeted therapy and the improvement of long-term survival rate. EOC tends to transfer to the peritoneum, which is already in a state of inflammatory infiltration before EOC cell transfer, and intraperitoneal TAMs and T cells may participate in the construction of the TME before EOC peritoneal transfer [6–8], however, the mechanism of their involvement in EOC peritoneal transfer has not yet been elucidated.

Tumor exosomes play an important role in organ-specific metastasis [9]. Exosomes promote the tumor cell colonization and proliferation by carrying tumor cell-specific proteins and nucleic acids in the TME before metastasis [10]. In addition, exosomes derived from the different cell type in TME mediate cell communications [11], which has a significant impact on tumor progression, for example, macrophage-derived exosomes can induce drug resistance in pancreatic cancer [12], and dendritic cell-derived exosomes caused tumor regression in mouse primary liver cancer [13]. These evidences suggest that exosomes play an important role in the transmission of information between tumors and the TME. There is an interaction between the two main immune cells in the peritoneum, TAMs and T cells, which makes the peritoneum in a

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state of immune imbalance, which is conducive to the peritoneal transfer of EOC. The mechanism of the interaction between them is worthy of further exploration.

Programmed cell death ligand 1 (PD-L1) is an important ligand molecule for immunosuppressive receptors. PD-L1 can be expressed on the surface of various immune cells, epithelial cells, and tumor cells [14]. PD-L1 can bind to programmed cell death protein 1 (PD-1) molecule, inhibiting T cell activation and proliferation, regulating T cell function, consequently affecting $CD8^+$ T cell secretion of cytokines, inhibiting the proliferation and cytotoxicity of $CD8^+$ T cells-mediated the anti-tumor immunity and promote tumor growth [15–17]. At present, the antibodies targeting PD-L1 pathway immunotherapy has been confirmed in a variety of tumors [18,19], but the therapeutic antibody is highly selective for patients, and in-depth research on the mechanism of action of PD-L1 in tumor progression is conducive to targeted and precise therapy.

However, the role of TAMs exosomes in the expression of CPT1A after delivering PD-L1 to $CD8^+$ T cells is unknown. In the present study, the results showed the expression of PD-L1 on TAMs was higher in EOC than in normal OSE patients. In addition, exosomes from PD-$L1^+$ TAMs promote the transcription factor PPARα, up-regulate the lipid metabolism and exhaustion of $CD8^+$ T cells, marked by expression of CPT1A, TIM3, respectively; consequently, resulting tumor metastasis.

2. Results

2.1. PD-$L1^+$ TAMs exosomes influence the effector function of $CD8^+$ T cell

To determine the expression of PD-L1 on the TAMs in the EOC patients, we sorted monocytes ($CD14^+$ cells) from peripheral blood mononuclear cells (PBMCs) of healthy donor by the fluorescence activated cell sorting (FACS), inducing macrophages to TAMs by co-cultured with supernatants derived from SKOV3 cells. The results showed that the expression of PD-L1 on the TAMs in EOC is higher than in benign (Fig. 1A), which indicating that PD-L1 may play a role in EOC.

In the previous study, we have confirmed that there is a large number of immune cell infiltration in the peritoneum of EOC patients, 70% of which are macrophages, called TAMs, and 20% are T cells. Next, we explore whether the exosomes from PD-$L1^+$ TAMs influence the function of $CD8^+$ T cells. Firstly, we isolated exosomes from PD-$L1^+$ TAMs in EOC, co-cultured with $CD8^+$ T cells labeled with CFSE, collected $CD8^+$ T

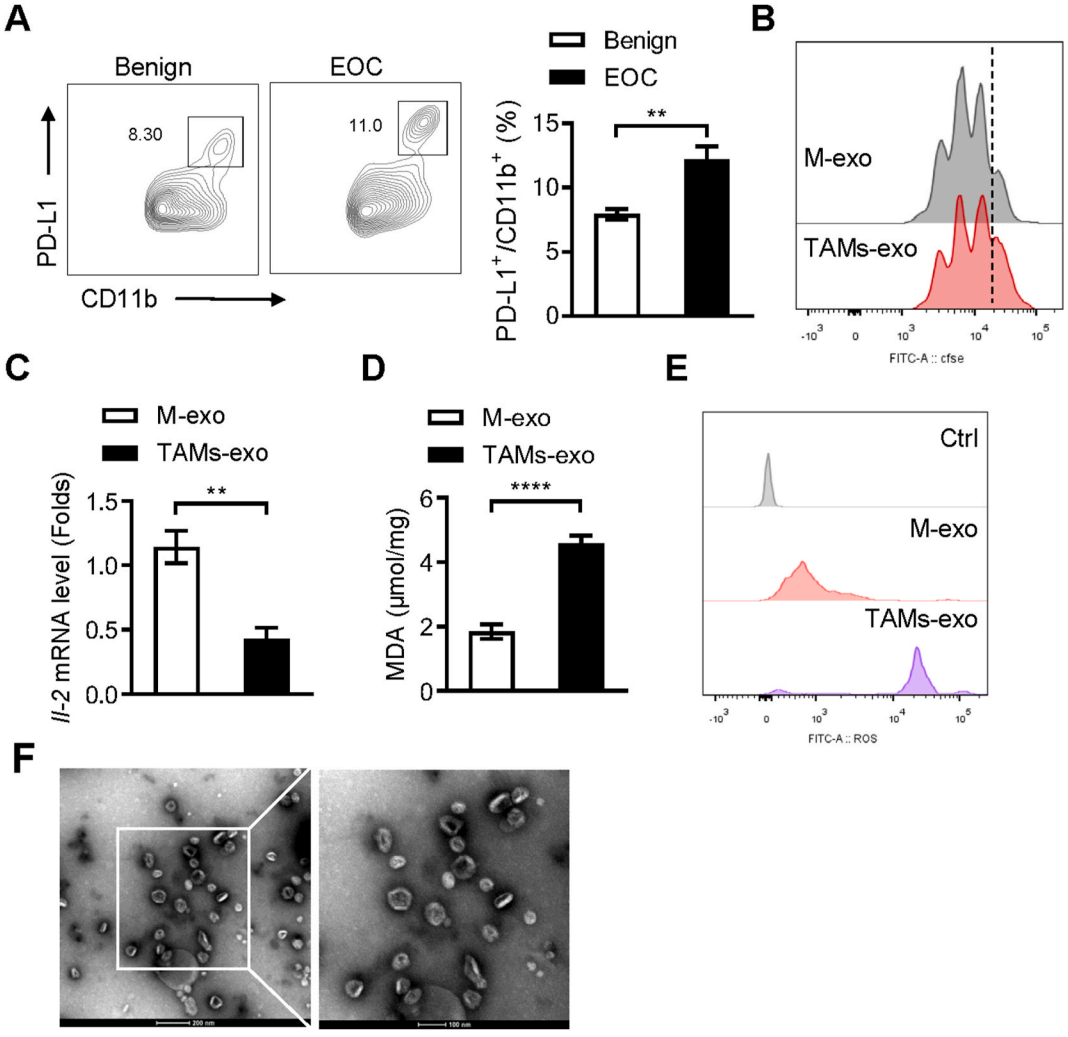

Fig. 1. PD-$L1^+$ TAMs exosomes suppress the effector function of $CD8^+$ T cell. **(A)** FACS analysis of PD-L1 expression of in benign and EOC patients CD11b cells (n = 3). (**B**) CFSE dilution of proliferation of $CD8^+$ T cells treated with M-exo or TAMs-exo for 24 h (n = 3). (**C**) Q-PCR analysis of the mRNA expression of IL-2 in $CD8^+$ T cells treated with M-exo or TAMs-exo for 24 h (n = 3). (**D**) ELISA analysis of the MDA level in $CD8^+$ T cells supernatant treated with M-exo or TAMs-exo for 24 h (n = 3). (**E**) FACS analysis of ROS production in $CD8^+$ T cells treated with M-exo or TAMs-exo for 24 h (n = 3). (**F**) The photograph of exosomes. The picture of scanning electron microscope in exosomes from macrophages. Data are representative of 3 independent experiments, mean ± SD. $^{**}P < 0.01$; $^{****}P < 0.0001$; [unpaired t tests for measurements between the two groups in (**A**), (**C**), and (**D**)].

cells to examine T cell function, marked by the proliferation and *IL*-2 mRNA level. The data showed that PD-L1$^+$ TAMs exosomes inhibited the T cell proliferation and IL-2 production (Fig. 1B and C). Furthermore, we performed ELISA to determine the effect of PD-L1$^+$ TAMs exosomes on the CD8$^+$ T cells lipid metabolism, evidenced by MDA level. PD-L1$^+$ TAMs exosomes from EOC promote the MDA level of CD8$^+$ T cells (Fig. 1D). As reactive oxygen species (ROS) induced lipid peroxidation, which plays an essential role in cell death. Therefore, we performed FACS to explore the influence of ROS level on CD8$^+$ T cells, ROS level was higher in PD-L1$^+$ TAMs exosomes than in macrophages (Fig. 1E and F). These results suggested that PD-L1$^+$ TAMs exosomes promote the lipid metabolism of CD8$^+$ T cells in EOC.

2.2. PD-L1$^+$ TAMs exosomes promote the lipid metabolism through PPARα in CD8$^+$ T cells

We next explored the underlying mechanism of PD-L1$^+$ TAMs exosomes regulating the CD8$^+$ T cells function. CPT1A promote the oxidation of endogenous lipid fatty acids to control T cell metabolism. Firstly, we performed Western blot to determine the effect of exosomes on the CPT1A expression in CD8$^+$ T cells in exosomes-CD8$^+$ T cell co-culture system. The data showed that the mRNA and protein level of CPT1A was higher in PD-L1$^+$ TAMs exosomes than in macrophages-exosomes (Fig. 2A and B). Previously study have demonstrated that PPARα can affect CPT1A to promote fatty acid oxidation in liver cells. We determined the expression and transcription of PPARα in PD-L1$^+$ TAMs or macrophages exosomes on CD8$^+$ T cells. Q-PCR and Western blot showed that PD-L1$^+$ TAMs exosomes promote the mRNA and protein level of PPARα (Fig. 2C and D).

Given that PPARα functions as a transcription, we conducted PPARα-Luciferin plasmid to examine the PPARα transcription. We co-transfected CPT1A and PPARα-Luciferin in HEK293T cells, treated with PD-L1$^+$ TAMs or macrophages exosomes. The luciferin assay demonstrated that PD-L1$^+$ TAMs exosomes promote the transcription of PPARα in the presence of CPT1A (Fig. 2E). To confirm the role of PPARα in the exosomes-induced lipid metabolism of CD8$^+$ T cells, we knock-out the PPARα (KO) in CD8$^+$ T cells. ELISA data showed that deficiency of PPARα abolished the MDA level of CD8$^+$ T cells in the presence of PD-L1$^+$ TAMs exosomes (Fig. 2F). In addition, Western blot showed that knock-out of PPARα inhibited the expression of CPT1A (Fig. 2G), suggesting that PPARα is required in the lipid metabolism of CD8$^+$ T cells induced by PD-L1$^+$ TAMs exosomes.

2.3. PD-L1$^+$ TAMs exosomes promote the CD8$^+$ T cell exhaustion

CD8$^+$ T cells recognize and eliminate pathogen-infected or cancerous cells, however, tumor-infiltrating lymphocytes (Tumor infiltrating lymphocytes, TILs) or T cells in chronic infection sites lead to exhaustion due to their long-term active state [32]. To explore the effect of T cell exhaustion induced by PD-L1$^+$ TAMs exosomes, we examined the expression of PD-1, TIM-3 and LAG3 in exosomes-CD8$^+$ T cell co-culture system by FACS. The data showed that PD-L1$^+$ TAMs exosomes promote the expression of T cell exhaustion, evidenced by upregulation of PD-1, TIM-3 and LAG3 (Fig. 3A–C). In addition, Western blot showed that when co-cultured with PD-L1$^+$ TAMs exosomes, the cleaved caspase-3 was increased (Fig. 3D). These data suggested that PD-L1$^+$ TAMs exosomes promote the dysfunction of CD8$^+$ T cells. Furthermore, we performed FACS to determine the production of IFN-γ in exosomes-CD8$^+$ T cell co-culture system. The data showed PD-L1$^+$ TAMs exosomes inhibit the production of IFN-γ in CD8$^+$ T cells (Fig. 3E). Therefore, these data suggested that PD-L1$^+$ TAMs exosomes may exacerbate the peritoneal metastasis through promoting the exhaustion and dysfunction of CD8$^+$ T cells in EOC.

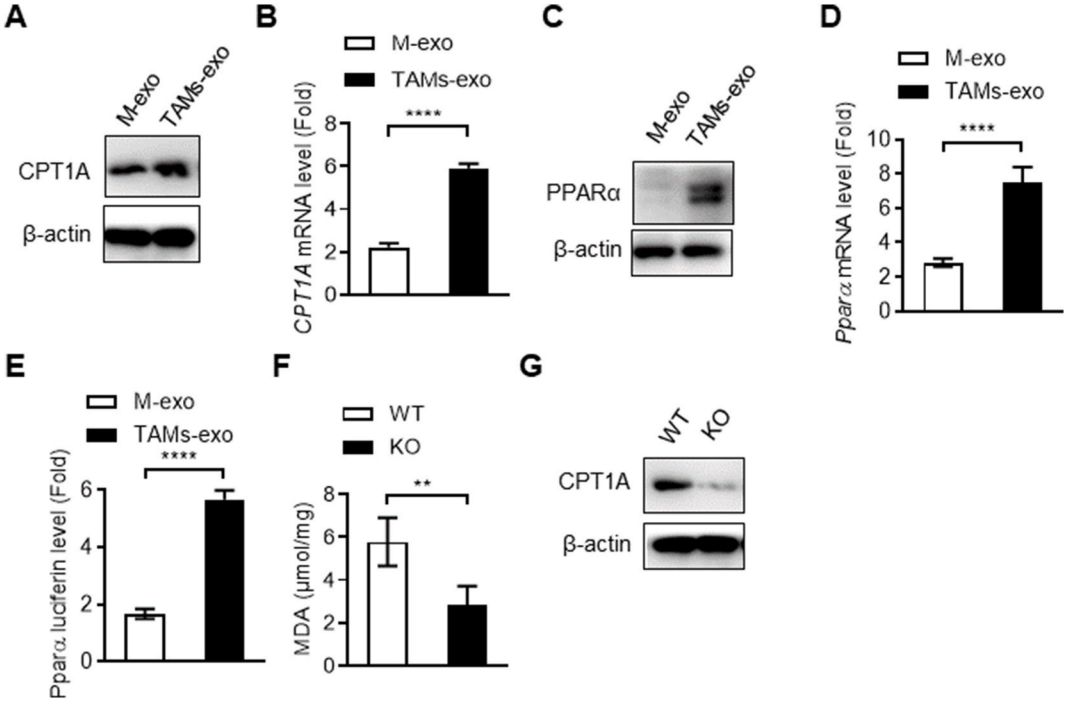

Fig. 2. PD-L1$^+$ TAMs exosomes promote the lipid metabolism through PPARα in CD8$^+$ T cells.(A, B) Western blot analysis of protein level (**A**) or q-PCR analysis of mRNA level (**B**) of CPT1A in CD8$^+$ T cells treated with M-exo or TAMs-exo for 24 h. (**C, D**) Western blot analysis of protein level (**C**) or q-PCR analysis of mRNA level (**D**) of PPARα in CD8$^+$ T cells treated with M-exo or TAMs-exo for 24 h. (**E**) Luciferin activity of PPARα transcription in HEK293T cells co-transfected with CPT1A treated with M-exo or TAMs-exo for 24 h. (**F**) ELISA analysis of the MDA level in control and PPARα-knock-out CD8$^+$ T cells CD8$^+$ T cells supernatant in the presence of PD-L1$^+$ TAMs exosomes (n = 3). (**G**) Western blot analysis of protein level of CPT1A in control and PPARα-knock-out CD8$^+$ T cells CD8$^+$ T cells supernatant in the presence of PD-L1$^+$ TAMs exosomes. Data are representative of 3 independent experiments, mean ± SD. $^{**}P < 0.01$; $^{****}P < 0.0001$; [unpaired t tests for measurements between the two groups in (**B**), (**E**), and (**F**)].

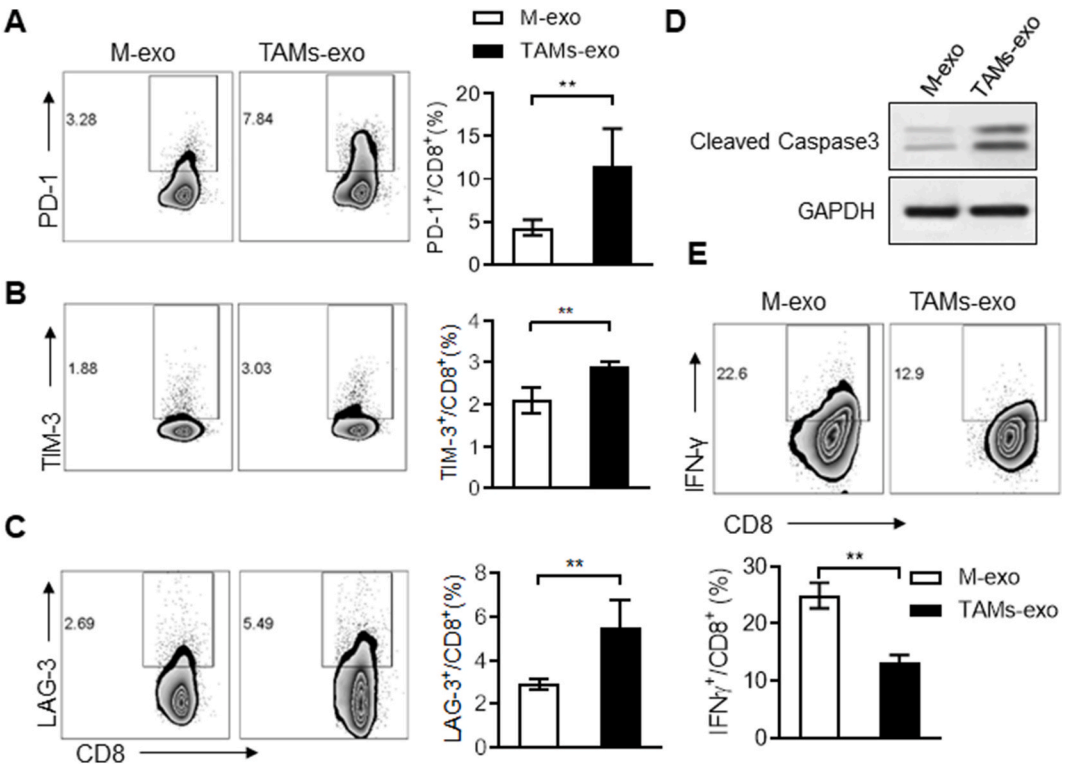

Fig. 3. PD-L1$^+$ TAMs exosomes promote the CD8$^+$ T cell exhaustion. **(A, B, C)** FACS analysis of PD-1 (**A**), TIM-3 (**B**) and LAG-3 (**C**) in CD8$^+$ T cells treated with M-exo or TAMs-exo for 24 h (n = 3). (**D**) Western blot analysis of Cleaved caspase3 in CD8$^+$ T cells treated with M-exo or TAMs-exo for 24 h. (**E**) FACS analysis of IFN-γ in CD8$^+$ T cells treated with M-exo or TAMs-exo for 24 h (n = 3). Data are representative of 3 independent experiments, mean ± SD. $^{**}P < 0.01$; $^{****}P < 0.0001$; [unpaired t tests for measurements between the two groups in (**A-C**) and (**E**)].

2.4. CPT1A was highly expressed on T cells in EOC

To confirm the role of TAMs in EOC, we download single-cell RNA sequencing data from GEO dataset to analyze the immune landscape of TME in relapsed ovarian cancer patient. We found that macrophages and T cells are infiltrated into EOC TME (Fig. 4A). The relationship between macrophages and T cells by CellChat analysis is consistent with our results, which indicating that the cell death pathway (Annexin V pathway) may play a critical role between macrophages and T cells in TME of EOC (Fig. 4B).

Furthermore, integrative unsupervised re-clustering of the macrophage cell population from the EOC samples was performed. From the 770 macrophages, 6 unaligned clusters were identified, including M1 macrophages (C3), M2 macrophages (C0, C2, C4), YBX3$^+$ macrophages (C1) and TAMs (C5) (Fig. 4C). As shown in Fig. 4D, CD163 was characterized as M2 macrophages, as well as CIITA was highly expressed in C0, indicating that C0_M2 macrophages functions as an antigen-presenting cells. In addition, C5_TAMs highly expressed CCL5, which is an inflammatory mediator produced by immune cells, suggesting that TAMs in EOC may play a critical role in pro-inflammatory status (Fig. 4D and E). However, C1 cluster highly expresses YBX3, which does not belong to M1 and M2 macrophages or TAMs, YBX3 is a novel gene that is important for regulating translation and RNA catabolism (Fig. 4D), thus, we proposed that YBX3$^+$ macrophages may be a novel marker that related to EOC.

A total of 950 T cells which characterized by signature gene markers (CD3D, CD4, FOXP3, CD8A, FCER1G, GZMK, IL7R, COTL1, NKG7) were obtained, and grouped into 6 unaligned clusters, including NKT cells (C0), CD4$^+$ T cell (C1, C4, C5), innate-like T cells with high cytotoxic potential (ILTCKs) (C2), CD8$^+$ T cell (C3) (Fig. 4F). As shown in violin plot, IL-7R, COTL1 are highly expressed on CD4$^+$ T cells. There are two subsets of CD8$^+$ T cell, ILTCKs were identified by high expression of FCER1G, which was demonstrated as a marker of ILTCKs. We firstly identified that FCER1G$^+$ T cells in the EOC patients (Fig. 4F).

To explore the T cell exhaustion in EOC patients, we analyzed the exhaustion marker (PD-1, LAG3) and the lipid metabolism in the 950 T cell. PDCD1(PD-1) and LAG3 were highly expressed on NKT and CD8$^+$ T cells (Fig. 4H). Furthermore, ILTCKs and CD8$^+$ T cells expressed high level of CPT1A and PPARα, which are able to promote the T cell exhaustion in EOC (Fig. 4I).

2.5. Discussion

Conceptually, our findings provide an example of applying "exosomes-mediated T cell exhaustion" to promote EOC peritoneal metastasis. We found that the PD-L1$^+$ TAMs exosomes directly enhance the transcription of PPARα to promote CPT1A, thereby regulating T cell exhaustion and aggravating the EOC peritoneal metastasis. PD-L1 was highly expressed on TAMs in EOC peritoneal metastasis patients, exosomes derived from PD-L1$^+$ TAMs influence the CD8$^+$ T cell lipid metabolism and exhaustion, thus exacerbating the peritoneal metastasis in EOC. Single-cell RNA sequencing demonstrated that TAMs and CD8$^+$ T cells are major infiltrated immune cells, thus, it's rational that PD-L1$^+$ TAMs exosomes aggravate the peritoneal metastasis through control CD8$^+$ T cell dysfunction.

Early ovarian cancer has not yet disseminated into the peritoneal cavity, and TAMs are also abundant in peritoneal tissue [20]. This indicates that the peritoneum is already in a state of inflammatory infiltration before EOC cell transfer, and intraperitoneal TAMs and T cells may participate in the construction of the microenvironment before EOC peritoneal transfer, but the mechanism of their involvement in EOC peritoneal transfer has not yet been elucidated. As a carrier of intercellular communication, the role of exosomes in tumor progression has gradually attracted attention [21]. It is further confirmed that there is an interaction between the two main immune cells in the peritoneum—TAMs and T cells, which is conducive to the peritoneal transfer

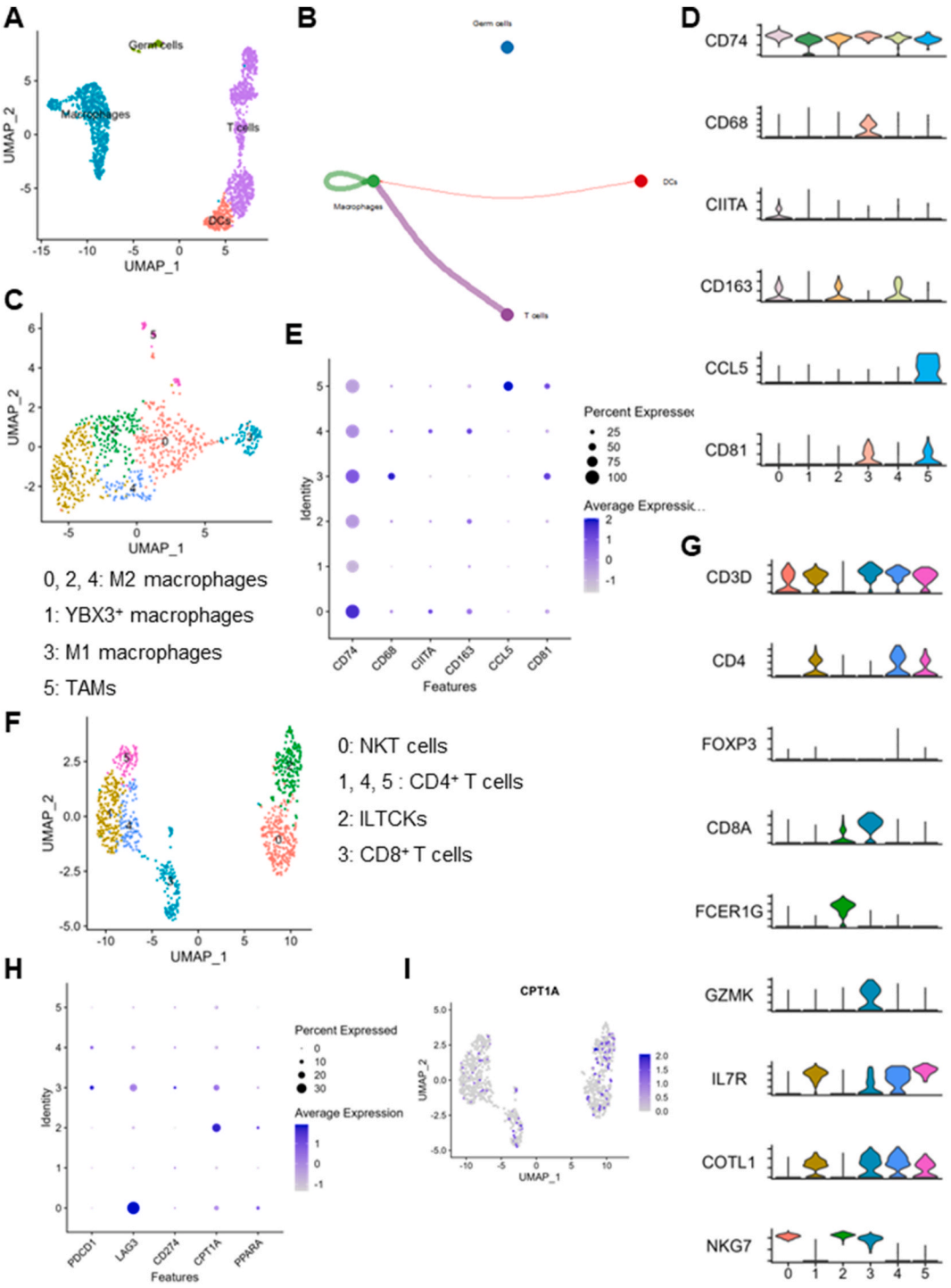

Fig. 4. CPT1A was highly expressed on T cells in EOC. (A) UMAP analysis of immune landscape in relapsed ovarian cancer patient TME. (**B**) CellChat analysis of the cell death pathway (Annexin V pathway) between macrophages and T cells in TME of EOC. (**C**) UMAP analysis of macrophages in relapsed ovarian cancer patient TME. (**D** and **E**) Violin Plot analysis (**D**) and dot plot analysis (**E**) of characterized gene expression in macrophages subsets. (**F**) UMAP analysis of T cells in relapsed ovarian cancer patient TME. (**G** and **H**) Violin Plot analysis (**G**) and dot plot analysis (**H**) of characterized gene expression in macrophages subsets. (I) Feature plot analysis of CPT1A expression in T cells.

of EOC. The mechanism of the interaction between the two is worthy of further exploration.

Immunotherapy focuses on the immune checkpoint pathway, the killing activity of PD-L1^{+}CD8^{+} T cells and the ability to produce cytokines [22,23]. Molecularly targeted tumor therapy drugs targeting the PD-1/PD-L1 signaling pathway have been developed, but for EOC patients, the efficacy of these antibodies is often not significant. Our *in vitro* experiments confirmed that compared with ovarian benign tumor tissues, the percentage of PD-L1^{+} TAMs in EOC tissues were significantly increased, and PD-L1 was also expressed in TAMs exosomes.

Previous studies have confirmed that the PD-L1/PD-1 pathway can control the survival state of T cells by regulating oxidation and increasing the level of reactive oxygen species (ROS) [24], The PD-L1/PD-1 pathway can increase the expression of CPT1A in T cells, thereby promoting the oxidation of endogenous lipid fatty acids to control T cell metabolism [25,26]. CPT1A is the rate-limiting enzyme

that promotes the transport of fatty acids from the cytosol to the mitochondria, and the transfer of long-chain fatty acids from the cytosol to the mitochondria [27]. PPARα is a transcription factor belonging to the PPAR nuclear receptor superfamily, and PPARα can regulate the transport and metabolism of long-chain fatty acids by activating mitochondria [28]. Studies have confirmed that PPARα agonists can affect the metabolism and function of $CD8^+$ T cells by increasing CPT1A [29]; conversely, down-regulating PPARα can affect CPT1A to inhibit fatty acid oxidation, and then play a role in the proliferation and metastasis of liver cancer [30].

We showed after TAMs-derived PD-$L1^+$ exosomes were co-cultured with $CD8^+$ T cells, they affected the transcription factor PPARα, thereby up-regulating the expression of CPT1A in $CD8^+$ T cells. CPT1A promotes fatty acid oxidation, increase reactive oxygen species, lead to cell damage, activate caspase-3 signaling pathway; at the same time, the expression of $CD8^+$ T cell exhaustion markers were increased, and the ability of T cells to secrete inflammatory cytokines decreases. In summary, we proposed that exosomes derived from PD-$L1^+$ TAMs promote EOC peritoneal metastasis by control T cell dysfunction. Furthermore, we found there are two novel cell subsets ($YBX3^+$ macrophages and ILTCKs), it needs to further investigated the role of the novel cell subsets in the EOC peritoneal metastasis.

3. Materials and methods

3.1. Study design

13 patients in Department of Obstetrics and Gynecology of Renji Hospital from January 2022 to December 2022 were selected in the study. There are 6 healthy donors, the average age was (43.00 ± 8.32) years. There are 4 EOC patients, aged from 51 to 68 years, with the average of (58.50 ± 7.68) years; There are 3 benign patients, aged from 53 to 59 years, with the average of (55.67 ± 3.06) years.

3.2. Cell culture

$CD8^+$ T cells isolated from healthy donor PBMC were cultured in complete RPMI 1640 media (10% FBS, 0.05 mM 2-mercaptoethanol, 1 mM NEAA). SKOV3 cells were maintained in McCoy's 5a media containing 10% FBS. TAMs were cultured in complete RPMI 1640 media containing 10% FBS.

3.3. Exosome isolation from PD-$L1^+$ TAMs

Exosomes isolated was described as previously [31]. Briefly, $CD14^+$ T cells were isolated from PBMC of EOC patients, co-cultured with SKOV3 in RPMI-1640, 24 h later, we collected and centrifuged the supernatants two times (1000 g × 10 min and 3000 g × 30 min to deplete the cells or fragments, followed by addition of Total Exosome Isolation Reagent (Life Technologies, 4,478,359) overnight, centrifugation for 10, 000 g × 1 h at 4 °C. Exosomes were re-suspended in PBS and stored at −80 °C. The concentration of exosomes was detected using a BCA Protein Assay.

3.4. FACS analysis of PD-L1 expression and ROS level

TAMs were isolated and enriched from PBMC of Benign and EOC patients, washed cells 2 times with pre-warmed PBS, stained with Aqua (BV510), and anti-CD11b (Percp-cy5.5), *anti*-PD-L1 (APC) for 15 min at 4 °C, then washed cells with pre-warmed PBS for 1 time.

For ROS level detection, cells were stained with surface marker, and then washed with pre-warmed PBS for 2 times, stained with CM-H2DCFDA (Thermo Fisher Scientific, C6827) for 30 min at 37 °C, re-suspended with cold PBS for 2 times. BD LSRFortessa X-20 was used for data acquisition and FlowJo (Tree Star) was used for data analysis.

3.5. CellTrace CFSE analysis of T cell proliferation

$CD8^+$ T cells were treated with exosomes from macrophages (M-exo) or TAMs for 24 h, washed with pre-warmed PBS for 2 times, then re-suspended with 5 μM CFSE (Thermo Fisher, C34554) staining solution for 20 min at 37 °C water bath, incubate with 8 ml pre-warmed complete medium for 5 min, centrifuge 5 min at 300 g and washed with pre-warmed PBS for two times. After 72 h, cells were collected and stained with Aqua (BV510) and anti-CD8 (APC). BD LSRFortessa X-20 was used for data acquisition and FlowJo (Tree Star) was used for data analysis.

3.6. Real-time quantitative PCR (q-PCR) analysis

Cells were collected after treated with M-exo or TAMs-exo, extraction of RNA and operation of q-PCR were performed by TriZOL RNA extraction and YEASEN RT-PCR kits according to the protocols. Sequences of the primers for targeting of indicated gene expression are as follow: Human *IL2RA*: Forward-5′- GTGGGGACTGCTCACGTTC-3′, Reverse-5′- CCCGCTTTTTATTCTGCGGAA-3'; Human *CPT1A*: Forward-5′-TCCAGTTGGCTTATCGTGGTG-3′, Reverse-5′-TCCAGAGTCCGATTGATTTTTGC-3'; Human *PPARα*: Forward-5′- ATGGTGGACACGGAAAGCC-3′, Reverse-5′- CGATGGATTGCGAAATCTCTTGG-3'; Human *GAPDH*: Forward-5′- GGAGCGAGATCCCTCCAAAAT-3′, Reverse-5′- GGCTGTTGTCATACTTCTCATGG-3';. Data was normalized by the level of indicated *Gapdh* expression in each sample.

3.7. Western blot analysis

$CD8^+$ T cells were treated with M-exo or TAMs-exo for 24 h, washed with pre-warmed PBS for 2 times, then re-suspended with RIPA (Containing proteases cocktail and phosphatase Inhibitor Cocktail). The concentration of protein was quantified with the Pierce BCA Protein Assay Kit.

For Western blot, equal amounts of protein were heat in 99.0 °C for 10 min, and then separated on 10% SDS-PAGE, after that, the protein was transferred to PVDF membranes in transfer buffer. Antibodies used for Western blot were as followed: *anti*-CPT1A (Thermo Fisher, PA5-76788), *anti*-PPARα (Santa Cruz Biotechnology, sc-398,394), *anti*-Caspase3 (CST, 9662S), *anti*-β-actin (CST, 4970S), *anti*-GAPDH (ABcam, ab263962). Proteins were detected using ECL Plus (Tanon, 180–5001) through the ChemiDoc Imaging System (AI600).

3.8. Luciferase assay of PPARα transcription activity

HEK293T cells were co-transfected with PPARα-luciferin and CPT1A plasmid, stimulated with M-exo or TAMs-exo for 24 h. Cells were collected after discard the supernatants, the luciferase activity was measured using Dual Luciferase Reporter Gene Assay Kit (Beyotime, RG027). The absorbance was calculated using the SpectraMax i3 (Molecular Devices) and SoftMax Pro 6.3 software.

3.9. Enzyme linked immunosorbent assay (ELISA) analysis for MDA level

$CD8^+$ T cells were plated in 6-well plates with complete growth medium. Cells were treated with M-exo or TAMs-exo for 24 h, MDA level in supernatants was measured by ELISA as instructions. Briefly, collect the supernatant, 3000 rpm centrifuge for 20 min, add 50 μl supernatants or standard for 30 h at 37 °C, wash six times with wash buffer, incubate with 50 μl/well of kinase reagent, wash six times with wash buffer, add 50 μl/well of buffer A and 50 μl/well of buffer B for 15 min at 37 °C, wash six times with wash buffer, stop the reaction with 50 μl/well of stop buffer. The data were collected at 450 nM.

3.10. FACS analysis with T cell effector cytokines

$CD8^+$ T cells were plated in 6-well plates with complete growth medium. Cells were treated with M-exo or TAMs-exo for 24 h. Cells were collected and stained with LIVE/DEAD (BV510)), anti-CD8(FITC), *anti*-IFNγ(APC), *anti*-PD-1(PE-Cy7) and anti-LAG-3(PE) by Foxp3/Transcription Factor Staining Buffer Set (eBioscience, 2,159,394) according to manufacturer's protocol. BD LSRFortessa was used for data acquisition and FlowJo (Tree Star) was used for data analysis.

3.11. scRNA-seq data and analysis

The scRNA-seq data were downloaded from GEO database (10 × Genomics data, GSE213243). The data quality control process was analyzed using the Seurat package (version 4.3.0; https://satijalab.org/seurat/articles/install.html). In brief, after removing low-quality cells (fewer than 200 unique molecular identifiers or mitochondrial gene expression exceeding 60%, we normalized the gene expression using Seurat package. FindVariableFeatures and ScaleData functions were used. Principle component analysis (dims = 20) and FindClusters (resolution = 0.5) were performed to identify cell clusters, and then t-SNE was used to visualize the single cells. We isolated 770 macrophages and 950 T cells, following the Seurat standard process, 6 macrophages and 6 T cell subpopulations were identified. These subpopulations were annotated with data generated from the FindAllMarkers function in the Seurat package, the markers were used from previously reported papers, as mentioned above.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Renji Hospital of Shanghai Jiao Tong University School of Medicine (No. XHEC–NSFC–2019-025).

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Author contributions

Jun Ma: Resources; Formal analysis; Supervision; Investigation; Writing-original draft; Project administration; Writing-review and editing. Qianqian Cen: Investigation. Qingzhu Wang: Software. Li Liu: Conceptualization; Writing-review and editing. Jieru Zhou: Conceptualization; Formal analysis; Investigation; Writing-original draft; Writing-review and editing.

Declaration of competing interest

Authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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