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Spotlight

Tumor derived adiponectin induces immunosuppressive macrophages in upper tract urothelial carcinoma

Cheng-Huang Shen [a,1], PiChe Chen [a,1], Chia-Bin Chang [a], Chih-Chia Chang [b], Meilin Wang [c], Lien-Ping Chou [a], Ya-Yan Lai [d], Ming-Yang Lee [e,*], Shu-Fen Wu [f,*]

[a] Department of Urology, Ditmanson Medical Foundation Chiayi Christian Hospital, Chia-Yi, Taiwan
[b] Department of Radiation Therapy and Oncology, Ditmanson Medical Foundation Chiayi Christian Hospital, Chia-Yi, Taiwan
[c] Department of Microbiology and Immunology, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[d] Ditmanson Medical Foundation Chiayi Christian Hospital, Chia-Yi, Taiwan
[e] Department of Hematology and Oncology, Ditmanson Medical Foundation Chiayi Christian Hospital, Chia-Yi, Taiwan
[f] Department of Biomedical Sciences and Epigenomics and Human Disease Research Center, National Chung Cheng University, Chia-Yi, Taiwan

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ABSTRACT

Upper tract urothelial carcinoma (UTUC) is a rare urologic cancer that is more aggressive and has a poorer survival rate compared to bladder cancer. Previously, we linked the UTUC derived factors with tumor-infiltrating immune cells. Macrophages are considered the most abundant immune cell population within tumors and serve as key regulators of the tumor microenvironment. This study aimed to investigate their role in modulating immunosuppression in UTUC tumors. Our results indicated that UTUC patients had higher proportion of M2-type macrophages in the periphery, and that tumor-infiltrating M2 macrophages were inversely correlated with tumor-infiltrating T lymphocytes within UTUC tumors. The supernatants from UTUC tumor tissue, as well as the predominant factor adiponectin, caused upregulation of arginase-1 and CD206 expression in THP-1 differentiated macrophages. Furthermore, macrophages treated with supernatants from UTUC tumor tissue or adiponectin alone inhibited CD4 T cell and CD8 T cell proliferation, along with reduction of effective cytokines produced by T cells. These results suggest that macrophages modulated by adiponectin are associated with T cell suppression in the UTUC tumor microenvironment, highlighting a potential therapeutic target.

Introduction

Upper tract urothelial carcinoma (UTUC) is a rare urologic cancer but exhibits an aggressive phenotype. Approximately 60 % of cases are muscle-invasive or diagnosed at advanced stage, leading to a poorer survival rate compared to bladder cancer [1,2]. The annual incidence rate of UTUC in Taiwan is approximately 3.14 to 3.41 cases per 100,000 people, which is higher than the global average [3]. Cancer immunotherapy with checkpoint inhibitors has emerged as a treatment option for urothelial carcinoma [4]. Increasing evidence highlights differences in tumor biology and immune microenvironment between UTUC and bladder cancer, suggesting that UTUC may be less responsive to immunotherapy [5]. Understanding the key regulatory factors in UTUC is critical for developing effective and safe therapeutic strategies. The molecular characteristics of UTUC require further investigation, given the limited number of patients studied in existing research on urothelial carcinoma [6].

Tumor microenvironment (TME) is comprised of various cellular components, including tumor cells, fibroblasts, mesenchymal stem cells, adipocytes, endothelial cells and distinct immune cells [7]. Identification of immune signature in the TME of urothelial carcinoma using transcriptional sequence gene expression data has proposed that higher infiltrating immune cells in tumors appears to be associated with a poor prognosis [8]. Macrophages are the most abundant populations among the immune cells in TME, and such the tumor-associated macrophage (TAM) could promote cancer development [9]. In lung cancer and glioma, TAMs exhibit a heterogeneous population, including monocyte-derived and tissue-resident macrophages, which are predominantly located in the tumor core and periphery, respectively [9,10]. Tumor-derived factors affect granulocytic and monocytic cell

* Corresponding authors at: National Chung Cheng University, No. 168, University Road, Min-Hsiung, Chia-Yi, 62102, Taiwan.
E-mail addresses: 05825@cych.org.tw (M.-Y. Lee), biosfw@ccu.edu.tw (S.-F. Wu).
[1] Cheng-Huang Shen and Pi-Che Chen contributed equally to this work and share first authorship.

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Table 1
Characteristics of the study participants.

	UTUC patients (n = 22)	Healthy controls (n = 22)
Age, year (mean±SD)	70.09±9.45	67.09±2.26
Gender, n (%)		
Male	11 (50)	13 (59)
Female	11 (50)	9 (41)
Tumor site, n (%)		
Ureter	15 (68)	
Renal pelvic	10 (45)	
Both	3 (14)	
T stage, n (%)		
≦pT2	13 (59)	
>pT2	9 (41)	

differentiation to achieve the immunosuppressive functions and thus induce protumor (N2 type) neutrophils and (M2-like) macrophages [11]. For example, cytokines (IL-10, CSF-1), proteases (serine proteases and matrix metalloproteinases), and exosomal components can skew monocytes toward an immunosuppressive macrophage phenotype [12]. However, few studies have focused on the modulation of M2-like TAMs in urothelial carcinoma [13]. Evidence from bladder cancer suggests that tumor-derived bone morphogenetic protein and CXCL1 act as direct and indirect mediators of M2-like TAM polarization, thereby promoting tumor progression and metastasis [13]. Recent study reveals that immunoevasive TME of UTUC has higher TAMs and exhausted CD8+ *T* cells, representing inferior prognosis [14]. The M2-like TAMs are known to promote tumor metastasis, recruit regulatory T cells and inhibit T cell activity [15]. In the TME, anti-tumor T cell activity can be modulated by myeloid immune cells [16]. For example, suppressive myeloid cells secrete arginase to deplete the surrounding arginine, which regulates T cell metabolism and anti-tumor activity, contributing to immune evasion of tumor cells [17,18]. Our previous study showed that supernatants from primary UTUC tumor tissues induce immunosuppressive neutrophils, and that the serum level of one highly expressed protein is associated with tumor-infiltrating immune cells [19]. Among the factors present in UTUC tumor tissues [20], several proteins are considered to have potential roles in regulating macrophage functions. Given that macrophages are key regulators for immunosuppression in the TME [21], their role in UTUC tumor should be further studied.

Here, we investigated the association between TAMs and T cell suppression in UTUC. Tumor-infiltrating macrophages exhibited M2-like population and correlated with tumor-infiltrating T cells. Adiponectin protein, a major factor existed in the culture supernatant of UTUC tumor tissue, was found to modulate the change of macrophage phenotype. T lymphocyte proliferation was evaluated after they cocultured with the macrophages treated by adiponectin, aiming to examine the immunosuppressive macrophages in UTUC.

Materials and methods

Study participants

Blood and tumor specimens were obtained from study participants recruited from customary urological practices at the Chia-Yi Christian Hospital. The pathological confirmation of UTUC was performed by endoscopic biopsy and surgical resection of urinary tract cancers. The tumor lesions in the renal pelvis and ureter were confirmed by computed tomography. The characteristics of the study participants, which included 22 UTUC patients and age-matched healthy subjects, are shown in Table 1. This study followed the ethical principles for involving human subjects as outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Chia-Yi Christian Hospital (No. 2020,121 and 2023,003) in Taiwan.

Isolation of peripheral blood mononuclear cell (PBMC), tumor tissue culture supernatants (TTCS) and tumor-infiltrating cells

PBMCs were isolated from heparinized whole blood obtained from the UTUC patients and healthy subjects by centrifugation over a Ficoll–Paque gradient (GE Healthcare). After removal of erythrocytes using ACK lysing buffer, PBMCs were resuspended in RPMI-1640 medium (HIMEDIA) supplemented with 10 mM HEPES, 4 mmol/L-glutamine, 100 units/mL penicillin (HIMEDIA), 100 μg/mL streptomycin (HIMEDIA), 50 μM 2-mercaptoethanol (Sigma-Aldrich), and 10 % heat-inactivated fetal bovine serum (FBS, Gibco).

The collection of tumor tissue culture supernatants (TTCS) was carried out as follows: Surgically isolated tumor tissues were rinsed three times with 1 × PBS to remove blood and mucus. The tumor tissues were minced into small pieces and incubated with RPMI1640 medium containing 10 % heat-inactivated FBS, 100 unit/mL penicillin, and 100 μg/mL streptomycin. After 24 h of incubation, the supernatants were collected following centrifugation. For isolating tumor-infiltrating cells, tumor tissues were digested with collagenase D and DNase I for 50 min at 37 °C. After passing the tissue through 100-μm cell strainers (BD Biosciences), tumor-infiltrating cells were collected and resuspended with culture medium. The resuspended cells were subjected to Ficoll-Paque gradient centrifugation. Cells at the interface were collected and washed with 1x PBS, and the cell count was determined after replacing PBS with culture medium.

In vitro macrophage polarization and the coculture experiment

Human monocytic THP-1 cells were maintained in RPMI 1640 medium containing 10 mM HEPES, 10 % heat-inactivated FBS, 100 unit/mL penicillin, 100 μg/mL streptomycin and 1 mM sodium pyruvate. THP-1 cells were differentiated into macrophages by incubating with 10 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) for 48 h. The cells were then maintained in PMA-free culture medium for an additional 72 h (M0 macrophage). For M2 macrophage polarization, M0 macrophage were further treated with 20 ng/mL of IL-4 and IL-13 (Biolegend) for 48 h. In some experiments, THP-1 differentiated M0 macrophages were treated with culture medium (control, 0 % TTCS), 20 % TTCS, 50 % TTCS, or different concentrations of adiponectin (1 or 5 μg/mL) for 48 h.

CD4 T cells or CD8 T cells from PBMCs of the healthy donors were collected by negative selection using human T lymphocyte enrichment kit (BD Bioscience). The carboxyfluorescein succinimidyl ester (CFSE, ThermoFisher) was used to trace the proliferation of CD4 T cells and CD8 T cells. CFSE-labeled T cells were cocultured with THP-1-differentiated macrophages at a 10:1 ratio in 96-well plates, with 0.05 ng/mL anti-CD3 stimulation. Three days after coculture, the proliferative T cells were analyzed by assessing the CFSE-diluted population using flow cytometry.

Flow cytometry

The cells were resuspended in staining buffer (1 × PBS containing 2 % FBS and 2 mM EDTA) and then stained with fluorescent dye-conjugated antibodies for 30 min at 4 °C in the dark. For intracellular staining, cells were fixed and permeabilized with Cytofix/Cytoperm buffer (BD Biosciences) for 15 min, washed twice with Perm/Wash buffer (BD Biosciences), and then incubated with fluorescent dye-conjugated antibodies. BD FACSAria III flow cytometer was used for evaluating the cell markers, and the data was further analyzed by FlowJo software (version 10). Anti-human CD68-FITC (clone Y1/82A), anti-human HLA-DR-Alexa Flour 488 (LN3), anti-human CD163-PE (GHI/61), anti-human CD14-PE (63D3), anti-human CD45-PECy7 (2D1), anti-human CD33-APC (P67.6), anti-human CD206-APC (15–2), anti-human arginase-1-APC (14D2C43) and anti-human CD11b-APC—Cy7 (M1/70) were purchased from Biolegend. Anti-human CD3-

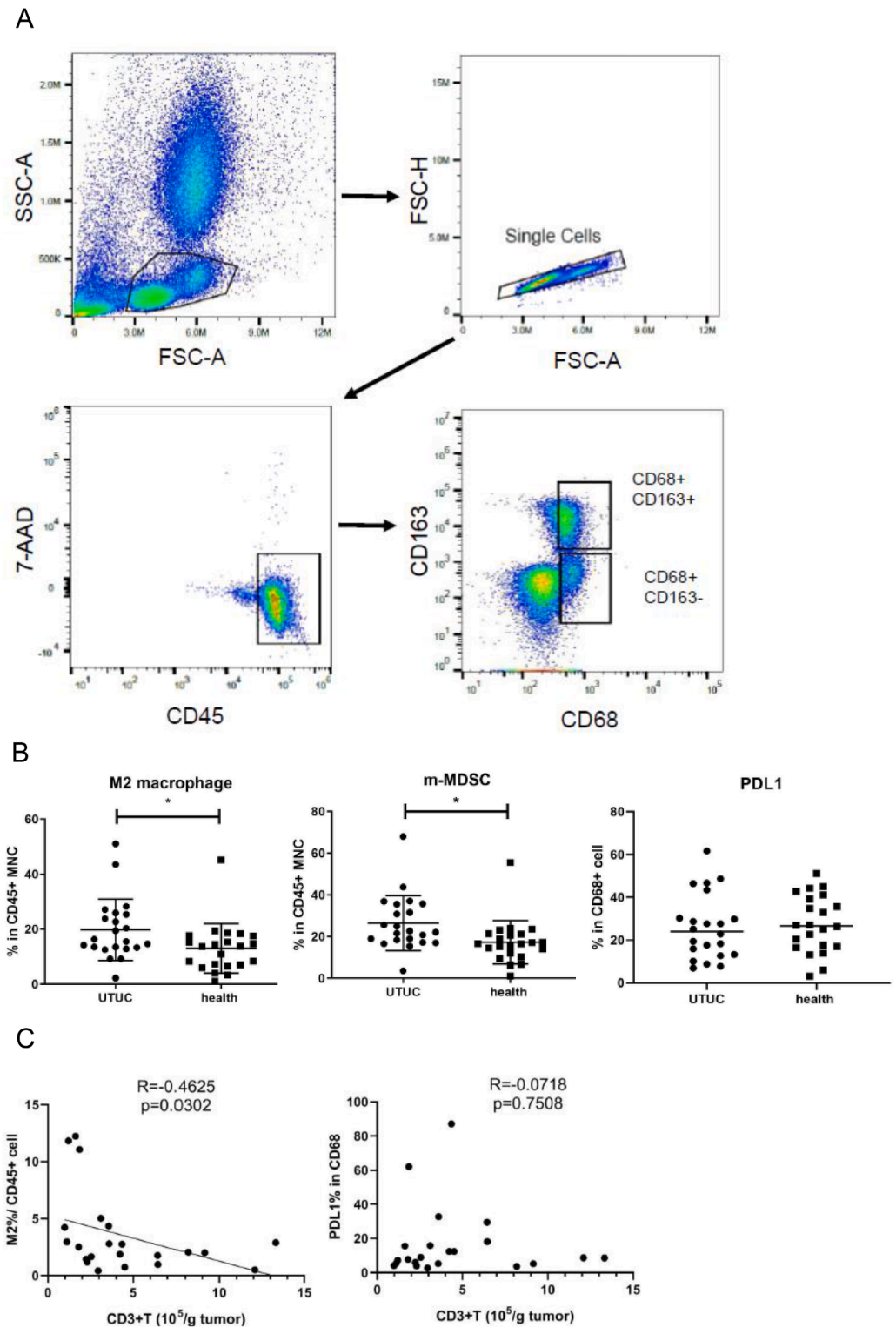

Fig. 1. Tumor associated macrophages and the correlation with T lymphocytes in UTUC. **(A)** Gating strategy for analyzing macrophage populations in the peripheral blood from study participants. After gating mononuclear cell (MNC) population, a single cell population was identified using "FSC-A *vs.* FSC—H" gating. Live leukocytes were then gated using the "$CD45^{+}7\text{-}AAD^{-}$" marker. The analysis displayed the pan-macrophage marker CD68 and its co-expression M2 marker CD163. **(B)** The proportion of M2 macrophage ($CD68^{+}CD163^{+}$ % in $CD45^{+}$ MNC, left panel), monocytic MDSC ($CD11b^{+}HLA\text{-}DR^{-}CD33^{+}CD14^{+}$ % in $CD45^{+}$ MNC, middle panel) or PD-L1 expression on $CD68^{+}$macrophages (right pannel) were represented (UTUC, $n = 22$; healthy subjects, $n = 22$). **(C)** Infiltrating cells isolated from tumor tissues of UTUC patients were counted and subsequently stained with fluorescent-dye conjugated Abs. The infiltrating $CD3^{+}$ *T* lymphocytes and infiltrating $CD68^{+}CD163^{+}$ cells from live leukocytes of single cell population were analyzed. The number of infiltrating $CD45^{+}CD3^{+}$ population per gram of tumor is represented on the x axis, while the percentage of M2 population ($CD68^{+}CD163^{+}$ % in $CD45^{+}$ cells, left panel) or the percentage of PD-L1 in $CD68^{+}$macrophages (right panel) is represented on the y axis. Correlation analysis was performed using the Spearman correlation method. $^{*}p < 0.05$.

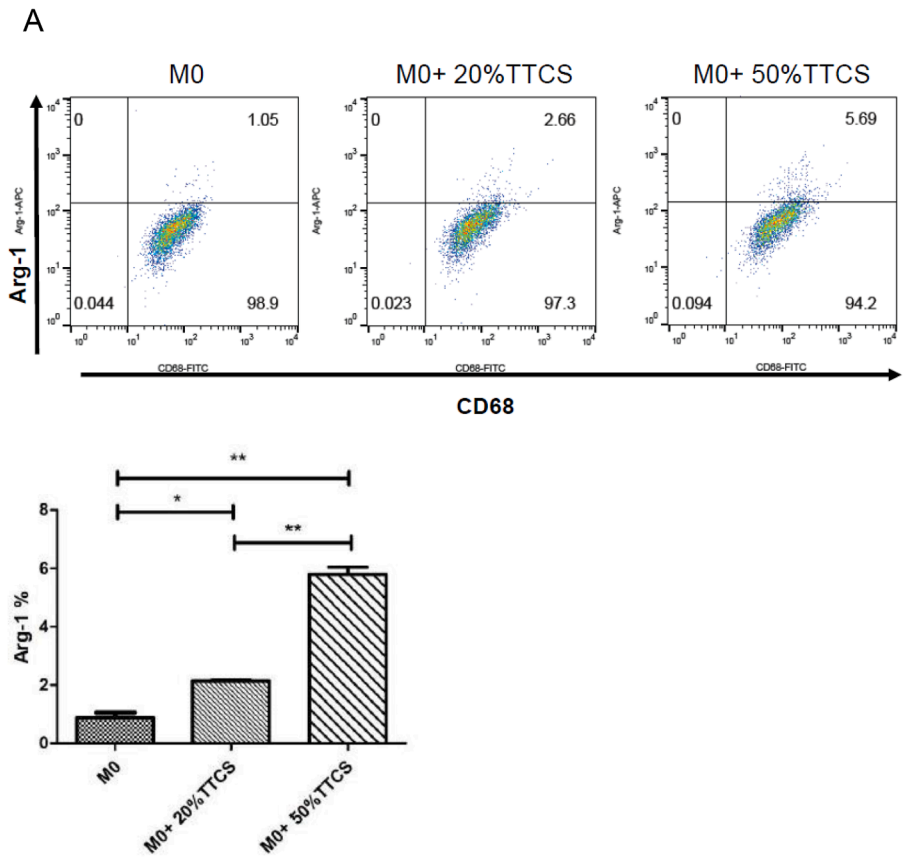

Fig. 2. Supernatant from UTUC tumor tissue increases M2-associated markers in THP-1 polarized macrophages. THP-1 cell line was differentiated to macrophages with 10 ng/mL PMA for 48 h, and subsequently stimulated with or without cytokines for M2- or M0-polarized condition. Tumor tissue culture supernatant (TTCS) was simultaneously added into the culture wells of polarized THP-1 cells, at final 0 %, 20 % or 50 % TTCS treatment. After 48 hour treatment, cells were analyzed after fluorescent-dye conjugated Abs staining using flow cytometry. **(A)** The arginase-1 expression of M0-polarized THP-1 cells in 0 %, 20 % or 50 % TTCS treatment groups are presented in upper panel, and its statistical analysis is presented in lower panel. **(B)** CD68- and CD206-expressing population of 0 % (ctrl medium), 20 % or 50 % TTCS treatment in THP-1 polarized M0 (left) or M2 (right) condition is presented in upper panel, and the statistical analysis is presented in lower panel. One of three independent experiments is represented. *$p < 0.05$ and **$p < 0.01$.

PE (clone UCHT1), anti-human PD-L1-APC (MIH1) and 7-AAD were purchased from BD Biosciences.

Detection of cytokine production

Cytometric Bead Array (BD Biosciences), including Abs for targeting IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF, were used according to the manufacturer's recommendation and analyzed by flow cytometry.

Statistical analysis

GraphPad Prism Software, version 8 (GraphPad Software Inc), was used for statistical analysis. Statistical comparisons were performed using unpaired *t*-test. P-values <0.05 were considered statistically significant. Spearman's rank correlation was used to assess the relationship between two variables. The study participants' characteristics are analyzed as means ± standard deviations.

Results

Cancer associated macrophages are related with T cell infiltration in UTUC tumor

There are three source of tissue infiltrating macrophages: circulating monocyte, tissue resident macrophage and myeloid-derived suppressor cells (MDSCs) [22]. Cancer alters circulating monocyte population and educates TAMs, which correlate with poor clinical outcome [23,24]. It has been examined that $CD163^+$ M2-like macrophage infiltration is positively associated with pathological T stage and poor prognosis in UTUC [25], and that bladder cancer induced PD-L1 (programmed cell death-ligand 1)-expressing TAMs to causes T cell inhibition [26]. In this context, we first evaluated TAM-associated cell populations, such as $CD68^+$ and $CD163^+$ cells, in both peripheral blood (Fig. 1A–B) and tumor tissues from UTUC patients (Fig. 1C). The proportion of $CD163^+$ macrophage or monocytic MDSC (m-MDSC) population among peripheral mononuclear leukocytes was higher in UTUC patients compared to those in healthy subjects, as assessed by flow cytometry (Fig. 1B). The tumor-infiltrating T lymphocytes, assessed by absolute numbers of infiltrating $CD3^+$ *T* cells per gram of UTUC tumor, were negatively correlated with infiltrating $CD163^+$ M2-like macrophages (Fig. 1C). Nevertheless, PD-L1 expression on pan-macrophage ($CD68^+$cells) did not have any significant correlation in our data (Fig. 1B-C). These findings reveal that UTUC exhibits immunosuppressive cell population associating with tumor-infiltrating T cells.

UTUC derived adiponectin induces M2-like macrophage phenotype

Tumor-derived factors could induce protumor M2-like macrophages [11]. Our previous research reveals that UTUC affects neutrophil to inhibit CD3 T cell proliferation [19], and we have further examined that

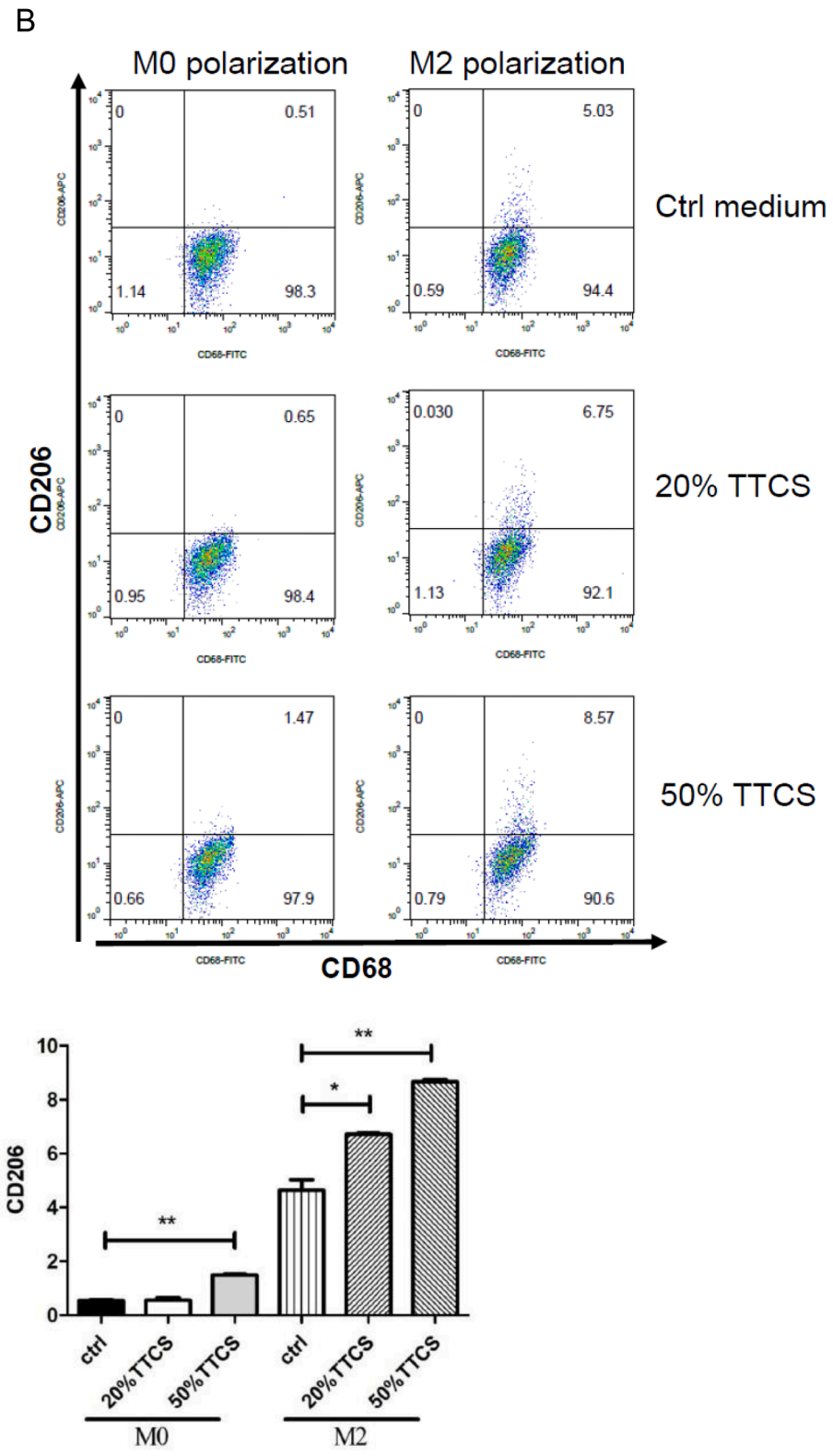

Fig. 2. (*continued*).

arginase-1 expressing N2-like neutrophils induced by tumor tissue culture supernatant (TTCS) of UTUC have the suppressive ability (unpublished data). Arginase-1 and CD206 expression can be induced by type 2 cytokines (IL-4 and IL-13), thus promoting M2 phenotype and causing T cell inhibition in the TME [18]. To investigate whether UTUC derived factors orchestrate immunosuppressive macrophages, TTCS from UTUC patient was collected and used for treatment in this study. We found that treatment of 20 % or 50 % TTCS had higher arginase-1 expression than 0 % TTCS did, in THP-1 differentiated macrophages (Fig. 2A). Furthermore, the M2 marker CD206 expression was also increased after 20 % TTCS or 50 % TTCS treatment, ether in THP-1-polarized M0 and M2 macrophages (Fig. 2B). Above results suggest the possibility of M2

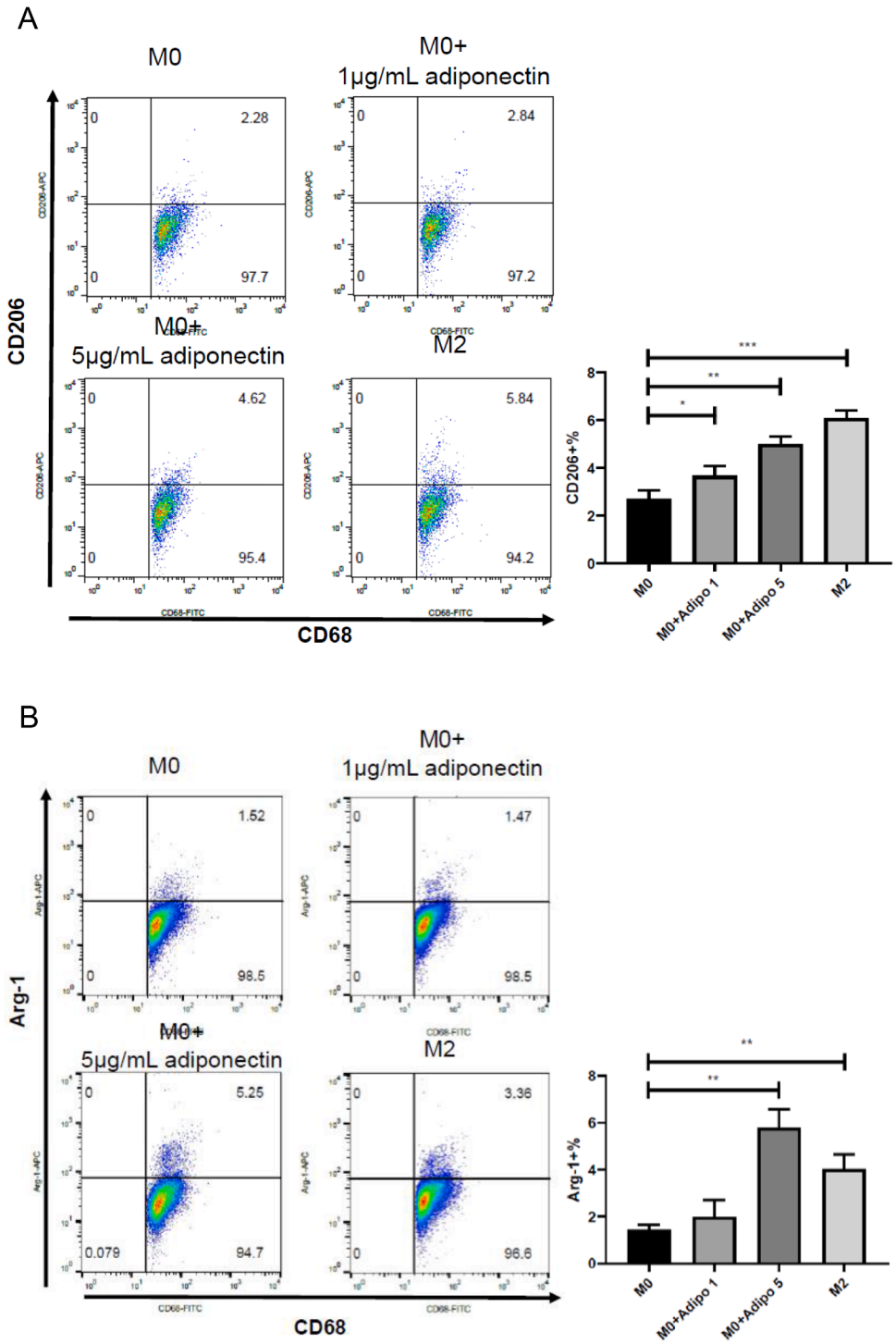

Fig. 3. Adiponectin treatment increases arginase-1 and CD206 protein expression in THP-1 differentiated macrophages. The graphs of arginase-1 expression **(A)** or CD206 expression **(B)** of M0-polarized THP-1 cells in 0, 1, or 5 μg/mL adiponectin treatment groups, as well as M2-polarized THP-1 cells without adiponectin treatment, are presented in the left panel. The statistical analysis of the percentage of arginase-1 or CD206 expression in THP-1 cells is presented in the right panel. One of three independent experiments is represented. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

phenotype macrophages are induced by UTUC TME.

We previous identified 14 differential factors increased in tumor samples of UTUC [19]. Among these factors, two predominant proteins are angiogenin and adiponectin [19], and the latter is considered to have direct impacts on macrophage. It has been examined that adiponectin could reduce the inflammatory cytokines and increase M2 markers, such as IL-10 and arginase-1, in human and mouse macrophages [27,28]. Other research suggest an association between adiponectin and the tumor size or the recruitment of TAM in mouse model of rhabdomyosarcoma, while adiponectin deficiency increases CD8+ *T* cell immunity and promotes the M1-like phenotype of TAMs [29,30]. The associated evidence for urothelial cancer reveal that human bladder tumor tissues have higher adiponectin expression than benign urothelial tissues do [31]. To explore whether M2-like macrophage in TME of UTUC is induced by adiponectin, different dose of adiponectin protein was directly treated with the macrophages. We found that both 1 μg/mL and

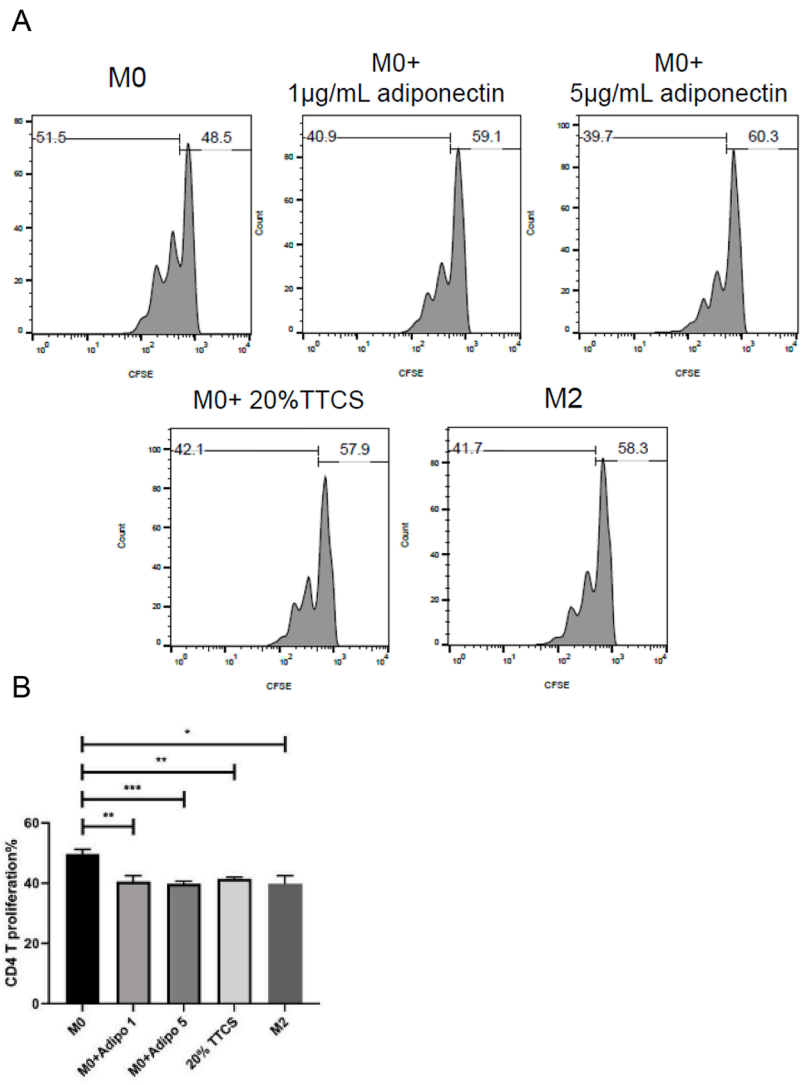

Fig. 4. Adiponectin or TTCS causes suppression of CD4 and CD8 T cell through M2-like macrophages. CD4 T lymphocytes **(A-C)** or $CD8^+$ *T* lymphocytes **(D-F)** from the peripheral blood of the healthy donors were isolated using enrichment kit, respectively. T cells were then labeled with carboxyfluorescein succinimidyl ester (CFSE) cell tracing dye, and sebsequently cocultured with THP-1 polarized macrophages for 72 h. The histograms display the proliferated CD4 T cells **(A)** or CD8 T cells **(D)** identified by diluted CFSE signal ($CFSE^{lo}$ population), and their statistical results for CD4 T cells **(B)** or CD8 T cells **(E)** are presented. **(C, F)** The supernatant from T cell and macrophage coculture well was analyzed using Cytometric Bead Array for detecting cytokine secretion, and the statistical results are presented (Neg, no stimulation with anti-CD3 in the coculture well). Differential cells including M0-polarized THP-1, M0-polarized THP-1 treated with 1 or 5 μg/mL adiponectin, M0-polarized THP-1 treated with 20 % TTCS or M2-polarized THP-1 were used to coculture with CD4 T or CD8 T cells, respectively. One of two independent experiments is represented. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

5 μg/mL of adiponectin enhanced the CD206 expression, likely displaying M2 phenotype in M0 polarized macrophages (Fig. 3A). The arginase-1 protein, which regulates consumption of arginine and leads to T cell suppression, was increased in 5 μg/mL adiponectin treatment but not in 1 μg/mL treatment, in M0 polarized macrophages (Fig. 3B). These results indicate a possibility of T cell activity suppressed by M2-like macrophages modulated by adiponectin protein in UTUC.

T cell suppression by TAMs in UTUC can be modulated by adiponectin

Previous result suggests the inverse correlation between tumor-infiltrating T cells and tumor-infiltrating M2 macrophages in the clinical samples (Fig. 1C). We next investigated whether T cell suppression induced by M2-like macrophages was influenced by UTUC and its key factor adiponectin. Peripheral purified CD4 T or CD8 T cells from healthy donors were cocultured with THP-1 polarized macrophages. Compared to none treatment control, 20 % TTCS- or 1 μg/mL adiponectin- or 5 μg/mL adiponectin-treated M0 macrophages contributed to inhibition of CD4 T and CD8 T cell proliferation, which is similar to the results of M2 polarized macrophages (Fig. 4A-B, CD4 T; Fig. 4D-E, CD8 T). Furthermore, significant reduction of IL-2, IL-6, IFN-γ and TNF produced by CD4 T cells was found in the treatment group of M0-polarized macrophages, including 20 % TTCS, 1 μg/mL adiponectin and 5 μg/mL adiponectin, and even of the M2 polarized macrophages (Fig. 4C). Similar to the results of CD4 T cells, IL-6 and IFN-γ produced by CD8 T cells were also decreased in treatment groups (Fig. 4F). However, other cytokines including IL-2, IL-4, IL-10 and TNF produced by CD8 T cells were not detected in our data. Collectively, these findings suggest that adiponectin exhibits a propensity to modulate macrophage immunosuppression in UTUC through inhibiting T cell activity.

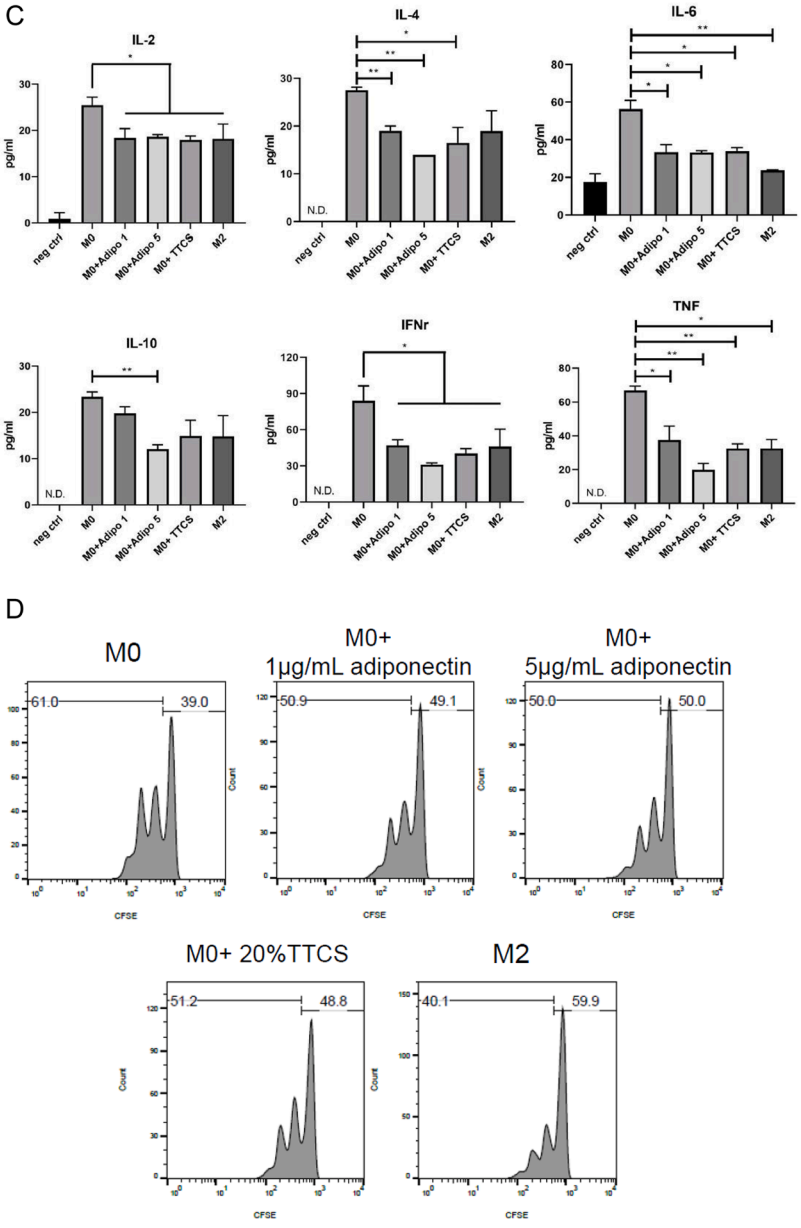

Fig. 4. (*continued*).

Discussion

We examined the relationship between UTUC-derived adiponectin and immunosuppressive macrophages. The M2 markers, such as CD206 and arginase-1, are increased in UTUC tumor-associated macrophages and adiponectin treated macrophages. T lymphocyte proliferation and the production of effective cytokine IL-6 and IFN-γ were inhibited by TTCS- or adiponectin-treated macrophages. These evidences provide an explanation for the phenomenon of inverse correlation between tumor-infiltrating T cells and tumor-infiltrating M2 macropages in our clinical UTUC tumor samples.

Among the factors we previously identified in UTUC tumor tissues [19,20], several proteins have been reported to influence differentiation, chemotaxis, and pro-tumor functions in both neutrophils and macrophages. These factors included the top three highly expressed proteins-angiogenin, adiponectin, and apolipoprotein A1. We previously found that immunosuppressive N2 neutrophil expression could be modulated by apolipoprotein A1, but not by adiponectin [20]. Additionally, in our earlier observations, apolipoprotein A1 did not appear to promote M2-like macrophage expression. In this study, adiponectin is responsible for driving immunosuppressive M2-like macrophage. Collectively, these findings indicate that different UTUC-derived factors impact distinct immune cell populations, even though TTCS treatment can induce both N2 and M2 expression.

Adiponectin is primary known an adipocyte-secreted protein to regulate insulin resistance and energy metabolism [32]. Apart from that, adiponectin also exerts immunomodulatory effects and possess duality in pro- and anti-inflammatory functions to contribute to numerous diseases [33–35]. For anti-inflammatory properties, adiponectin ameliorates the bacterial infection-induced pulmonary diseases via reduction of TNF-α production and NF-κB activity in macrophages, or it seems to prevent acute lung injury during SARS-CoV-2 infection [34]. In the TME, adiponectin is the most abundant adipokine, however, whether adiponectin exerts pro-tumor or anti-tumor effects remains controversial [36]. The epidemiological investigations reveal that circulating adiponectin levels are inversely correlated with the risks of obesity-related cancers [37], suggesting the implication of its anti-inflammatory effects to combat tumorigenesis. On the other hand, several studies indicate that

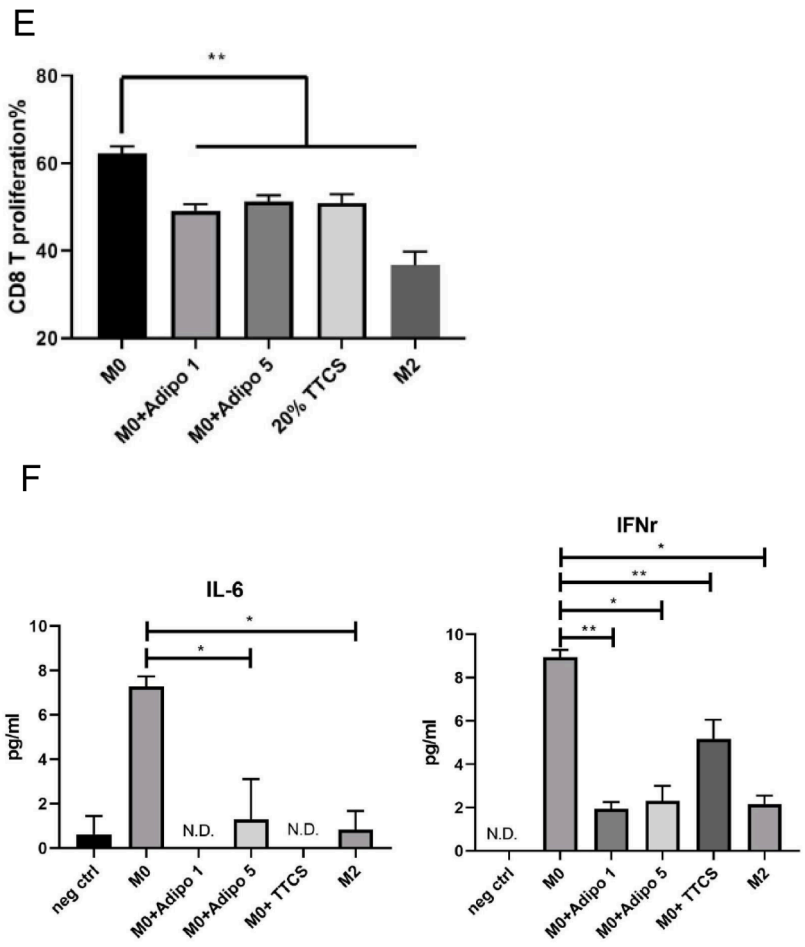

Fig. 4. (*continued*).

adiponectin facilitates tumor progression by promoting angiogenesis and invasion of breast cancer cells [36]. Some evidences indicate that higher serum adiponectin levels are associated with the increase of risk for liver or urothelial bladder carcinoma [38,39]. Higher expressions of adiponectin and its receptors are found in human bladder tumor tissues than in benign urothelial tissues, although *in vitro* treatment of adiponectin could inhibit bladder tumor cell growth [31]. These above findings prompt us to investigate the role of adiponectin in the TME of UTUC.

Unlike the dual effects observed in tumor cells, adiponectin has distinct influences on macrophages. The reduction of adiponectin receptors results in increased production of inflammatory cytokines by monocyte-derived macrophages [40], which may increase the risk of cardiovascular disease. The human lung explant tissues secrete adiponectin to inhibit IL-6, TNF-α and chemokines production by LPS- or poly (I:C)-stimulated lung macrophages [41]. In soft tissue sarcoma, adiponectin is thought to promote M2-like phenotypes in tumor-infiltrating macrophages, which are linked to tumor growth and metastasis [29]. Mechanistically, AdipoR1 and AdipoR2, the two main adiponectin receptors, regulate the functions of monocyte-derived macrophages [41]. Downstream molecules of these receptors, including AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor α (PPARα), have been reported to be involved in adiponectin-mediated M2 macrophage polarization [42,43]. The aforementioned literature clearly outline the molecular signaling pathways underlying adiponectin-mediated M2 macrophage polarization. In this study, we find that adiponectin not only induces M2-like macrophages (Fig. 3) but also orchestrates macrophage immunosuppression (Fig. 4). These results highlight the protumor role of adiponectin in UTUC tumors.

Despite expression of adiponectin in serum and bladder tissue increases the risk for bladder cancer [39], and this literature partially supports our previous finding that highly expressed adiponectin protein was presented in UTUC tumor tissues [19]. Our study still has some limitations. Circulating adiponectin levels are significantly higher in healthy females compared to healthy males, indicating a gender disparity [44]. Due to the limited sample size of UTUC cases within urothelial carcinoma, the number of patients included in studies may be insufficient, even though there is a female predominance of UTUC in Taiwan. Therefore, whether serum adiponectin levels correlate with the proportion of peripheral M2 macrophages or tumor-infiltrating T lymphocytes has not been investigated.

Immune checkpoint inhibitors against PD-1, PD-L1 and CTLA-4 have been used for metastatic urothelial carcinoma immunotherapy over the past decade. Clinical responses to immune checkpoint inhibitor therapy in urothelial carcinoma are associated with higher expression of PD-L1, not only on tumor cells but also on immune cells [45,46]. Recent evidence suggests that TAMs either express PD-L1 themselves or enhance PD-L1 expression on bladder tumor cells, thereby mediating immune suppression [26,47]. However, in this study, we find that PD-L1 is not increased on TAMs (Fig. 1B-C). It appears that T cell suppression by UTUC-educated macrophages is not predominantly mediated through PD-L1 signaling.

In conclusion, we demonstrate that macrophages modulated by adiponectin exhibit M2 markers, which are associated with T cell suppression in the TME of UTUC. These findings provide new insights into immunosuppression in UTUC and could potentially improve disease treatment.

Lists of abbreviations

UTUC, upper tract urothelial carcinoma; TME, tumor microenvironment; TAM, tumor-associated macrophage; TTCS, tumor tissue culture supernatant; PBMC, peripheral blood mononuclear cell; FBS, fetal bovine serum; CFSE, carboxyfluorescein succinimidyl ester; MDSCs, myeloid-derived suppressor cells; PD-L1, programmed cell death-ligand 1.

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Ethics statement

This study involves human participants and was approved by Institutional ethics committee of Chia-Yi Christian Hospital (No. 2020,121 and 2023,003). Participants gave informed consent to participate in the study before taking part.

CRediT authorship contribution statement

Cheng-Huang Shen: Resources, Project administration, Funding acquisition, Data curation. **PiChe Chen:** Validation, Resources, Data curation. **Chia-Bin Chang:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Chih-Chia Chang:** Resources, Data curation. **Meilin Wang:** Methodology. **Lien-Ping Chou:** Resources. **Ya-Yan Lai:** Visualization, Formal analysis, Data curation. **Ming-Yang Lee:** Investigation, Data curation, Conceptualization. **Shu-Fen Wu:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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