Mesenchymal stem cells induce PD-L1 expression through the secretion of CCL5 in breast cancer cells

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Abstract
Various factors in the tumor microenvironment (TME) regulate the expression of PD-L1 in cancer cells. In TME, mesenchymal stem cells (MSCs) play a crucial role in tumor progression, metastasis, and drug resistance. Emerging evidence suggests that MSCs can modulate the immune-suppression capacity of TME through the stimulation of PD-L1 expression in various cancers; nonetheless, their role in the induction of PD-L1 in breast cancer remained elusive. Here, we assessed the potential of MSCs in the stimulation of PD-L1 expression in a low PD-L1 breast cancer cell line and explored its associated cytokine. We assessed the expression of MSCs-related genes and their correlation with PD-L1 expression across 1826 breast cancer patients from the METABRIC cohort. After culturing an ER+/differentiated/low PD-L1 breast cancer cells with MSCs conditioned-medium (MSC-CM) in a microfluidic device, a variety of in-vitro assays was carried out to determine the role of MSC-CM in breast cancer cells' phenotype plasticity, invasion, and its effects on induction of PD-L1 expression. In-silico analysis showed a positive association between MSCs-related genes and PD-L1 expression in various types of breast cancer. Through functional assays, we revealed that MSC-CM not only prompts a phenotype switch but also stimulates PD-L1 expression at the protein level through secretion of various cytokines, especially CCL5. Treatment of MSCs with cytokine inhibitor pirfenidone showed a significant reduction in the secretion of CCL5 and consequently, expression of PD-L1 in breast cancer cells. We concluded that MSCs-derived CCL5 may act as a PD-L1 stimulator in breast cancer.

KEYWORDS
immunosuppression, mesenchymal stem cells, microfluidics, PD-L1, tumor stromal cells

1 | INTRODUCTION

Although in recent years, immunotherapy has become a breakthrough in cancer therapy and clinical trials with antibodies to PD-1/PD-L1 checkpoint inhibitors have shown unprecedented responses in numerous solid tumors; however, few tumor types have shown weak response to this class of immunotherapy, including breast cancer, bladder cancer, pancreatic cancer, and colon cancer (Denkert et al., 2017; Esteva et al., 2019). The tumor microenvironment (TME), especially its cellular components, are now known to interfere with the response to immune checkpoint inhibitors (Binnewies et al., 2018; Quail & Joyce, 2013). Mesenchymal stem cells (MSCs) with multiple differentiation potentials and immune-modulating functions are one of the essential cell components of the TME (Shi et al., 2017). It has been shown that MSCs can colonize in TME and develop tumor-associated MSCs (TAMSCs) and cancer-associated fibroblasts (CAFs; Quante et al., 2011; Ren et al., 2012) and recently emerged as therapeutic targets.
(Hu et al., 2010; Shah, 2012). Besides, in response to paracrine signals from growing tumors in the TME, TAMSCs continuously rewire the TME, facilitating tumor growth, metastasis, and modifying the response to various anticancer treatments, including immunotherapy (Cukierman & Bassi, 2012; Roodhart et al., 2011).

The bidirectional interactions between MSCs and cancer cells can result in the production of various growth factors, chemokines, and cytokines, facilitating cell migration, survival, proliferation, and organization of cancer cells (Shi et al., 2017). Moreover, MSCs have been extensively reported to possess immunosuppressive properties via the modulation of immune cells within both the innate and adaptive systems. Recently, numerous studies highlighted the immunosuppressive potential of MSCs in TME employing induction of PD-1 and PD-L1 expression on T-cells and tumor cells, respectively (Davies et al., 2017; Krueger et al., 2019; O’Malley et al., 2018; Sun et al., 2018; Yang et al., 2019). In the context of breast cancer, besides the activation of PD-1 on T cells, the expression of PD-L1 on tumor cells facilitates immune resistance to immunotherapy.

The role of CAFs in acquired resistance of cancer cells to a wide range of anticancer therapeutics, including immunotherapy, has been demonstrated in previous studies in breast cancers (Costa et al., 2018); however, whether MSCs with immunomodulatory properties able to generate an immunosuppressive TME through induction of PD-L1 expression on breast cancer cells remained to be shown. This study aimed to investigate whether MSCs regulate the PD-L1 expression in breast cancer cells and explore the specific molecular mechanism. Our results evidenced the expression of PD-L1 on PD-L1 low expressing breast cancer cells by MSCs.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Human adipose-derived MSCs (AD-MSCs; Regeneus) were maintained in α-modified minimum essential medium (α-MEM; Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum (FBS; Thermo Fisher Scientific) and 1% pen/strep in a humidified atmosphere of 5% CO2 at 37°C. Human breast adenocarcinoma MCF7 cells were cultured in Dulbecco’s modified Eagle's medium (DMEM; Thermo Fisher Scientific) supplemented with 10% (v/v) FBS and 100 U penicillin/ml and 100 μg streptomycin/ml in the same mentioned condition. The culture medium was changed two to three times per week, and subculture was performed by digestion with 0.25% trypsin/0.02% EDTA when the cells were nearly confluent. AD-MSCs cells from passages 2–5 were used for the experiments.

2.2 | Preparation of conditioned medium

AD-MSCs were cultured at a density of 10,000 cells/cm² in α-MEM medium supplemented with 10% FBS until they reached 90% confluence. Then, the cells were washed three times with phosphate-buffered saline (PBS), and the culture media were replaced with serum-free DMEM and were incubated for an additional 48 h. The concentrated supernatant was collected by centrifuging (Eppendorf) at 1200 RPM for 5 min at room temperature, filtered through 0.45 µm filters, and designated as MSC-conditioned medium (MSC-CM). The MSC-CM was then stored at −70°C until use.

2.3 | Microfluidic device design and cell culture

The microfluidic tissue culture devices used in this study are purchased from AIM Biotech Company. The devices consist of two media channels running parallel to and located on either side of an extended central region containing the extracellular gel matrix like collagen type-I named gel channel. Then, 200 μl collagen gel solution (2.5 mg/ml) at pH 7.4 was prepared on ice with 20 μl 10× PBS was mixed with 4 μl NaOH (0.5 N), 129.2 μl collagen type-I (Corning, Cat# 354236), 10 μl freshly trypsinized and dissociated MCF7 targets at 50 × 10⁶ cells/ml (for 2D studies) or 10 μl cell suspension medium with 50–100 tumor spheroids (for 3D studies), and 22.9 μl cell culture water. The collagen gel solution containing single MCF7 cells or cell clusters designated multicellular aggregates (MCA) was then injected into the dedicated gel region of the device and kept in the cell culture incubator at 37°C and 5% CO2 for 40 min to allow gel polymerization via thermal cross-linking. Devices with gel only (i.e., no embedded cells, control) were prepared similarly by adding collagen gel solution containing 20 μl 10× PBS, 4 μl NaOH (0.5 N), 129.2 μl collagen type-I, and 42.8 μl of deionised water. Immediately after gel polymerization, the two side channels filled with 120 μl of DMEM and MSC-CM.

2.4 | Culturing single and multicellular aggregate in the microfluidic device

To obtain MCAs with the appropriate size for 3D studies, MCF7 cells were trypsinised and resuspended as individual cells at 100,000 cells/ml in DMEM and cultured for three consecutive days onto a 100 mm ultra-low attachment dish (Corning). Aggregates were collected and filtered in two consecutive filtration steps: (a) 40 μm filtration, to exclude all the cell aggregates smaller than 40 μm and (b) 100 μm filtration, to exclude all aggregates larger than 100 μm in diameter, and centrifuged by 250 g for 5 min to separate them from the supernatant. For 2D studies, cells were harvested with Trypsin/EDTA and centrifuged at 200 g for 5 min. Cells were resuspended in growth medium and mixed with collagen type-I solution for a final concentration of 6 × 10⁵ cells/ml total collagen solution. The single cells and aggregates were then mixed with the collagen solution to inject to gel channel of AIM Biotech devices (Aref et al., 2013, 2018).
### 2.5 | Immunofluorescent staining

Cell culture media was removed from the devices, and samples in the microfluidic devices were washed with PBS followed by fixation of samples with 4% paraformaldehyde (PFA; Sigma-Aldrich, Cat# 158127) for 15 min at room temperature. Next, 0.1% Triton-X (Sigma-Aldrich, Cat# X100) was added and incubated for 10 min before blocking by BSA 1% for 2 h. Finally, the samples were stained with E-cadherin (1:200, Biolegend, Cat# 324104), Vimentin (1:200, Biolegend, Cat# 677804), N-Cadherin (1:200, Biolegend, Cat# 350816), and PD-L1 (1:200, Abcam, Cat# ab214958). The mean fluorescent intensity of obtained images was quantified using the ImageJ software. We observed a high expression of NT5E, ENG, and THY1 among patients diagnosed as invasive ductal carcinoma (IDC) type, and in claudin-low subtypes (Figure 2a,b). Moreover, in the claudin-low subtype, tumor samples with the negative status of ER/PR and HER2 showed the highest expression of ENG and NT5E compared to other groups of tumors with at least one positive status in ER/PR or HER2 (Figure 2b), while the expression of THY1 mostly observed in ER+HER2− low proliferative tumor cells (Figure S1).

### 2.6 | Cytokine array

The MSC-CM was analyzed using the Human Cytokine Antibody Array (Abcam, Cat# ab133997) according to the manufacturer’s instructions. Briefly, The MSC-CM was collected by centrifugation and then hybridized to the array membrane overnight at 4°C. A 1× biotin-conjugated anti-cytokines second antibody was used after washing membrane, and cytokines were detected by pipetting HRP-conjugated streptavidin on the membrane. The captured signals were quantified using the ImageJ software.

### 2.7 | Statistical analysis

The results of quantitative experiments were analyzed as mean ± SD. The statistical analysis was performed Using GraphPad Prism 8.0. Statistical significance between experimental groups was evaluated using Student’s *t*-test. *p < .05 was considered as a statistically significant and ****p < .0005 was considered as an extremely significant. Microscopic images are representative images from three independent experiments. The mean fluorescent intensity was analyzed using ImageJ software.

### 3 | RESULTS

#### 3.1 | MSCs in different stages of breast cancer and its correlation with immune suppression

Figure 1 illustrates the workflow of this study, initiating with in-silico data analysis of MSCs-related markers across a large cohort of breast cancer patients, followed by coculturing an epithelial-like/low expressing PD-L1 breast cancer cell line with MSC-CM in a microfluidic device and assessment of released cytokine through the cytokine profiling.

To investigate the association between the presence of the MSCs in TME and stimulation of immune suppression, we analyzed gene expression of three MSCs markers Endoglin (CD105), NT5E (CD73), and THY1 (CD90) among 1826 breast cancer patients registered in METABRIC cohorts (Figure 2). According to the PAM50 subtype classification method provided in this cohort, we clustered patients in five groups namely Basal (n = 209), Claudin-low (n = 218), Luminal-A (n = 700), Luminal-B (n = 475), and HER2 (n = 224; Figure 2a).

We next analyzed the expression of the PD-L1. As depicted in Figure 2c and in line with previous studies (Costa et al., 2018;
Denkert et al., 2017; Emens, 2018), tumors with triple-negative status showed a significantly high level of PD-L1 expression in comparison with other subtypes (Figure 2c). The Pearson correlation coefficient and protein–protein interaction analysis between MSCs-related genes and PD-L1 resulted in the identification of a positive association between THY1, ENG, and NT5E with PD-L1 (CD274; Figures 2d and S2). Together, these data suggest a positive association between MSCs-related genes and induction of an immune suppressive TME through the regulation of PD-L1 expression on cancer cells.

3.2 MSCs induce breast cancer cell proliferation and invasion behavior

To assess the influence of the MSCs on the behavior of breast cancer cells, we cultured a differentiated ER+ and low PD-L1 carcinoma cell line known as MCF7 with MSC-CM to assess potential of MSCs for inducing PD-L1. A significant increase in cell population observed when MCF7 cells cultured in MSC-CM for 72 h in comparison to the control group (Figure 3a,b).

As morphology is closely linked to the cell migratory behavior (Yu et al., 2013), we analyzed the morphology of cancer cells by assessing the circularity and aspect ratio of MCF7 cells which are cultured with MSC-CM. Interestingly, in line with microscopic observations (Figure 3c), we found a significant increase in the aspect ratio, and a reduction in the circularity level of those group of cells cultured with the MSC-CM, indicating morphology changes toward the acquisition of aggressive behavior (Figure 3d).

To investigate the influence of MSCs on the stimulation of invasive behavior in cancer cells, we cultured MCF7 cells in a micro-fluidic device with or without MSC-CM and assessed the expression of epithelial to mesenchymal transition (EMT)-related markers.
FIGURE 3  Effects of MSC-CM on proliferation and invasion behavior of cancer cells. (a,b) Influence of MSC-CM on proliferation rate of MCF7 cells after 72 h (****p < .005). (c,d) Morphology switch of MCF7 cells cultured with MSC-CM. Besides microscopy observation (c), the assessment of circularity, aspect ratio, and cell area showed phenotype changes on MCF7 cells from epithelial-like to mesenchymal-like toward an invasion behavior (d; *p < .05, ****p < .005). MSC-CM, mesenchymal stem cell-conditioned medium.

FIGURE 4  EMT induction effects of MSC-CM on MCF7 cells. (a) The immunofluorescent images of expression of EMT-related marker vimentin (Red) and reduction of E-cadherin (Green) at protein level (Scale bar: 100 µm). (b) Quantification results of mean fluorescent intensity measurement of Vim, N-Cad, and E-Cad (*p < .05). EMT, epithelial–mesenchymal transition; MSC-CM, mesenchymal stem cell-conditioned medium.
(Figure 4a; Aboulkheyr Es et al., 2020). Immunolabeling showed a significant elevation of mesenchymal markers N-cad and Vim, while the expression of epithelial marker E-Cad reduced after 72 h culture of cells with MSC-CM (Figure 4a,b). Similar to previous observations, these data indicate EMT-promoting effects of MSC-CM.

### 3.3 MSCs induce expression of PD-L1 on low PD-L1 breast cancer cells

Various studies highlighted the immunosuppressive role of TME during disease progression in which the infiltration of immune cells and the function of cytotoxic T-cells are limited by tumor cells and tumor stromal cells through the expression of PD-L1. We next asked whether MSCs as immune modulator had the potential to induce immune-suppression effect through the expression of PD-L1 at the early stage of the disease. In this regard, we cultured MCAs derived from MCF7 cells with or without MSC-CM. Figure 5a exhibits the immunofluorescent analysis of PD-L1 and Vim expression in MCAs cultured in MSC-CM for 72 h, demonstrating a significantly elevated level of Vim (Figure 5b). Interestingly, an increase in the expression of PD-L1 was observed in the MSC-CM treated group compared to the control group, although low expression of PD-L1 was observed in this group (Figure 5c). We previously showed that the formation of MCAs could stimulate the expression of numerous stemness-related genes, including CD44 (Azadi et al., 2019). The protein–protein interaction analysis of stemness marker CD44 and EMT-related markers Vim, E-cad, N-cad, and ZEB1 with PD-L1 (CD274), showed positive association between ZEB1, E-Cad, and CD44 with PD-L1, indicating that the expression of ZEB1 and CD44 may induce the level of PD-L1 (Figure 5d). Together, these data suggest that MSC-CM able to stimulate the expression of PD-L1 through the regulation of EMT- and stemness-related genes in a positive manner.

### 3.4 Cytokine expression

Our coculture experiments showed that MSC-CM stimulates the expression of PD-L1 on breast cancer cells. To better characterize this potential inducer, we analyzed the MSC-CM using a cytokine profiling array. Among 42 screened cytokines, high level of several inflammatory cytokines, including CCL2, CCL5, CXCL5, IL-6, IL-8, and GRO-α, were identified from MSC-CM (Figure 6a). The correlation analysis between identified cytokines and PD-L1 showed a positive

![Figure 5](image-url) MSC-CM induces expression of the PD-L1. (a–c) The microscopy images (a) and quantitative analysis of expression of both Vim (b) and PD-L1 (c) in MCF7-MCAs cultured with MSC-CM and stained in a microfluidic device (Scale bar: 100 µm; *p < .05). (d) Protein–protein interaction analysis of EMT- and stemness-related markers with PD-L1, showing a positive association between CD44, ZEB1, and CDH1 with PD-L1. EMT, epithelial–mesenchymal transition; MCA, multicellular aggregate; MSC-CM, mesenchymal stem cell-conditioned medium.
association between IL6, CCL2, CCL5, and CXCL5 with PD-L1 (CD274; Figures 6b and S3).

We next sought to determine how targeting these cytokines may reduce the expression of PD-L1. In this regard, we treated MSCs with pirfenidone (PFD; 100 µM), a well-known cytokine inhibitor for 48 h and generated condition medium from treated cells (MSC-CM + PFD) followed by measuring expression of PD-L1 on MCAs cultured with this conditioned medium. Interestingly, we found that the expression of PD-L1 significantly decreased after replacing MSC-CM with MSC-CM + PFD on MCAs (Figure 6c,d). To better identify suppressed cytokines, we screened released cytokines from MSCs treated with PFD. Remarkably, we observed significant reduction in expression and secretion of CCL5 in the MSC-CM + PFD group in comparison with nontreated samples (MSC-CM; Figure 6ef). Moreover, the production of CCL2, angiogenin (ANG), IL-6, and CXCL5 was also reduced in treated MSCs with PFD (Figure 6g). In line with these observations, the expression profile analysis of identified cytokines between PD-L1-positive and PD-L1-negative samples across 1904 breast cancer samples from the METABRIC cohort revealed the enrichment of these cytokines in PD-L1-positive samples in comparison to the PD-L1-negative samples (Figure 7a–g). Collectively, these data indicate that MSCs as potent immune-modulator able to induce an immune-suppressive TME through the stimulation of PD-L1 expression on cancer cells by releasing various inflammatory-related cytokines, particularly CCL5.

4 | DISCUSSION

It is well established that the expression of PD-L1 plays an essential role in cancer cell-mediated immune response to immunotherapy. The expression of PD-L1 has found in 5%–40% tumor cells (Kulasinghe et al., 2017; Zou et al., 2016), helping tumor cells to escape from immune killer cells (Thorsson et al., 2018).

Herein, we demonstrated immune-suppression potential of MSCs as well-known immune-modulator in TME through the induction of PD-L1 expression on breast cancer cells.

The cellular expression of PD-L1 could be affected by cellular components of TME, particularly tumor stromal cells...
The influence of tumor stromal cells in the regulation of hallmarks of cancer has been highlighted in various studies (De Boeck et al., 2013; Hanahan & Weinberg, 2011; Quail & Joyce, 2013; Zenge et al., 2019). Among tumor stromal cells, MSCs, known as TA-MSCs, have shown direct and indirect effects on each step of tumor progression, metastasis, and drug resistance in various solid tumors (Cukierman & Bassi, 2012; Kalluri, 2016; Mandel et al., 2013; Roodhart et al., 2011; Shi et al., 2017).

In the context of immune-suppression capacity of tumor stromal cells, a large number of studies highlighted the critical role of CAFs in the induction of an immune-suppressive TME through the regulation of the immune checkpoints particularly PD-L1 in both themselves and cancer cells in vitro and in vivo (Aboulkheyr Es et al., 2020; Biswas et al., 2019; Costa et al., 2018; Lakins et al., 2018). Considering the immune-suppression function of MSC within the TME, studies demonstrated that MSC express not only PD-L1 and PD-L2 but also mediate the expression of these proteins on immune compartments of the TME, including T cells (Davies et al., 2017), macrophages (Biswas et al., 2019), dendritic cells (Moravej et al., 2017) and cancer cells (Krueger et al., 2019; O’Malley et al., 2018). However, the role of MSC as an immune modulator in the expression of PD-L1 in breast cancer cells remained to be explored (Shi et al., 2017).
For better understanding of effects of MSCs in the expression of PD-L1 in breast cancer cells, through the analysis of three MSCs-related markers ENG, THY1, and NT5E across 1826 breast cancer samples from the METABRIC cohort, we highlighted a positive association between expression of MSCs-related markers and expression of PD-L1 in different subtypes of breast cancer. A similar gene signature has been used for identification of TA-MSCs across high-grade glioma samples from TCGA (Shahar et al., 2017) and in patient’s tumor specimens (Hossain et al., 2015), in which a negative correlation between expression of MSCs signature and patient survival was reported.

The expression pattern of immune-checkpoint proteins particularly PD-L1 and its association with EMT and stemness feature of breast cancer have been discovered through both in-silico and in-vivo studies, in which tumor stromal cells play a pivotal role for rewiring TME to acquisition of immune-suppressive properties (Malta et al., 2018; Mittendorf et al., 2014). In line with these findings, through correlation and protein–protein interaction analysis between MSCs-related genes and PD-L1, we showed positive association between NT5E, ENG, and THY1 with PD-L1, suggesting that besides CAFs, MSCs may play a critical role in the regulation of PD-L1 in TME of breast cancer (Yang et al., 2019). In support of these data, in a preclinical study on primary and metastatic prostate cancer, Krueger et al. (2019) identified MSCs in prostate cancer TME using an opal multiplex immunofluorescence assay based on NT5E, THY1, and ENG. Interestingly, they found that infiltrating MSCs suppress T-cell proliferation and upregulate the expression of PD-L1 and PD-L2 on their cell surface, indicating that depletion of MSCs from TME not only induce immunologic recognition but also can eliminate prostate cancer cells by cytotoxic T-cells.

The phenotypic transformation, such as EMT, is identified as a critical factor in both stimulation of metastasis behavior and induction of immune suppression within TME. It has been shown that tumor stromal cells can regulate phenotype switch in cancer cells toward the acquisition of invasive phenotype by releasing various cytokines and chemokines in TME (Truong et al., 2019). A large number of studies documented the effects of CAF cells on morphology changes of various types of cancer cells (Fernandez-Nogueira et al., 2019; Miyazaki et al., 2019; Yu et al., 2014; Zhuang et al., 2015).

Similar results observed when we cultured an epithelial-like, noninvasive breast cancer cells with MSC-CM in a 3D microfluidic device (Azadi et al., 2020; Shrestha et al., 2020). Our results also indicated that MSC-CM could stimulate phenotypic transformation, invasive behavior, and immune-suppressive capacity in cancer cells (Truong et al., 2019), through the induction of EMT-related genes, especially Vimentin (Tan et al., 2014). These data indicated that the release of cytokines and chemokines in the conditioned medium of MSCs might mediate the expression of PD-L1 in PD-L1 low expressing breast cancer cells. Our cytokine profiling analysis against 42 different cytokines highlighted the presence of high level of various cancer-promoting and immuno-modulator related cytokines in MSC-CM, including CCL5, CCL2, IL6, IL8, and CXCL5. Similarly, in a syngeneic mouse model of colon adenocarcinoma, O’Malley et al. (2018) highlighted stromal cell-mediated immune suppression and tumor-promoting effects of MSCs through the expression and secretion of numerous cytokines. Moreover, in a study on xenograft and orthotopic mouse models of pancreatic ductal adenocarcinoma (PDAC), it have been shown that Cancer Forkhead Box Protein 3 (C-FOXp3) augments immune evasion of PDAC by recruiting Treg cells into PDAC through the upregulation of CCL5 which in turn, C-FOXp3 directly bound to the promoter region of PD-L1 in pancreatic cancer cells (Wang et al., 2020). They concluded that combined blockade of PD-L1 and CCL-5 axis might provide effective therapy for patients with the high level of C-FOXp3. A similar observation was reported in the colorectal cancer patient, suggesting that CCL5 may provide a potential therapeutic target for the combined PD-1-immunotherapy of CRC (Zhang et al., 2018).

Collectively, these data suggest that targeting these cytokines, particularly CCL5, may impede invasiveness and immune-suppressive characteristics of cancer cells. In this regard, the recent approval of PFD for the treatment of idiopathic pulmonary fibrosis relies on targeting various cytokines. We previously demonstrated that PFD significantly reduced expression of PD-L1 on metastatic cancer cells and able to retard immune-suppressive capacity of CAFs (Aboulkheyr Es et al., 2020). As a proof-of-concept, in this study, we showed that treatment of MSCs with cytokine inhibitor PFD significantly reduce expression and secretion of various cytokines, especially CCL5 and CCL2 and consequently decrease in the level of PD-L1 in breast cancer cells. Although, understanding the underlying mechanism behind the effects of PFD in reversion of tumor immunity and increase sensitivity of tumor cells to immunotherapy remained to be investigated; however, we highlighted that targeting tumor stromal cells using PFD indirectly reduce expression of PD-L1 through the targeting key immune-related cytokines, suggesting an alternative approach for cancer immunotherapy in a combination manner. Collectively, these results support crucial role of tumor stromal cells in mediating and regulation of an immune suppression TME through the secretion of various cytokines.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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