Neoplasma 2021; 68(5): 938–946

doi:10.4149/neo_2021_201226N1404

Tumor-associated macrophages increase COX-2 expression promoting endocrine resistance in breast cancer via the PI3K/Akt/mTOR pathway

Qi QIN[1], Hongfei JI[2], Dongbo LI[1], Han ZHANG[1], Ziwen ZHANG[1], Qingyuan ZHANG[1,2,*]

[1]Department of Medical Oncology, Harbin Medical University Cancer Hospital, Harbin Medical University, Harbin, Heilongjiang, China; [2]Institute of Cancer Prevention and Treatment, Heilongjiang Academy of Medical Science, Harbin Medical University, Harbin, Heilongjiang, China

**Correspondence: zqyHMU1965@163.com*

Received December 26, 2020 / Accepted April 16, 2021

Breast cancer is the most common malignancy in females. The emergence of endocrine resistance is frustrating for estrogen receptor (ER)-positive breast cancer patients even the efficacy of endocrine therapy is acceptable. Our previous study has shown that tumor-associated macrophages (TAMs) are associated with endocrine resistance, yet the mechanism remains unclear. This article is dedicated to discuss the role of TAMs in the endocrine resistance of breast cancer. It was found that tamoxifen-resistant MCF-7 cells induced more macrophages polarized into TAMs. Conversely, TAMs increased the expression of cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2), which promoted tamoxifen resistance through the activation of the PI3K/Akt/mTOR signaling pathway in MCF-7 cells. Furthermore, clinical analysis supported that five-year progression-free survival (PFS) of breast cancer patients with abundant COX-2 expression in TAMs was shorter ($p<0.05$). Therefore, these results show a positive feedback loop between TAMs and breast cancer cells, suggesting that TAMs and COX-2 may be new therapeutic targets for breast cancer patients suffering from endocrine resistance.

Key words: breast cancer, tumor-associated macrophages, COX-2, PGE2, tamoxifen resistance

Breast cancer is the most common malignant tumor among female patients [1], which has become a major health threat to women. Approximately 70% of patients have hormone receptor (HR) positive breast cancer, and endocrine therapy continues to be the cornerstone of treatment for these patients [2]. However, the therapeutic effect of endocrine therapy is limited by primary or acquired drug resistance [3]. Tamoxifen, a synthetic estrogen receptor (ER) modulator, is the international standard treatment of endocrine resistance [4, 5]. Therefore, resistance to tamoxifen in breast cancer patients is a hot topic in clinical research.

The tumor microenvironment (TME) is the internal environment for tumor cells to absorb nutrients and obtain energy. In addition to the tumor cells themselves, the surrounding stromal cells and the cytokines secreted by them together constitute the TME [6], which is considered to be the essential factor in inducing drug resistance [7]. Macrophages, the most abundant inflammatory stromal cells in TME, have a particularly heterogeneous phenotype depending on the different organs and stimuli [8]. M2 macrophages promote tumor growth and metastasis by releasing a variety of tumor-promoting factors, which are generally referred to as tumor-associated macrophages (TAMs) [9, 10]. Some studies have shown that TAMs increase the resistance of breast cancer cells to chemotherapy drugs and improve their survival time [11]. Previously, our research has found that TAMs may play a significant part in endocrine resistance [12], but the underlying mechanism has not been elucidated.

Cyclooxygenase-2 (COX-2) is a key rate-limiting enzyme that catalyzes the conversion of arachidonic acid (AA) into a variety of prostaglandins (PGs) including prostaglandin E_2 (PGE_2) [13]. Moreover, COX-2 is involved in the pathophysiological process of inhibiting tumor cell apoptosis, promoting angiogenesis and metastasis, and improving drug resistance [14]. COX-2 is overexpressed in a variety of malignant tumors [14, 15] and TME, especially TAMs [16]. In addition, studies have shown that the high expression of COX-2 in TAMs is related to the poor prognosis of melanoma, prostate cancer, and breast cancer [15, 17]. But as far as we know, the role of COX-2 in TAMs has not been studied in the field of endocrine resistance in breast cancer up to now.

The mechanism of endocrine resistance in breast cancer involves many molecules and pathways, such as mutations of ER [18], overexpression of EGFR/HER-2/FGFR [19], and activation of PI3K/Akt/mTOR [20] and cell cycle pathways [21]. Among them, the PI3K/Akt/mTOR signaling pathway

plays a major role in resistance to endocrine therapy, which is a crucial bridge linking inflammation and immune response with tumor drug resistance. Due to the fact that the majority of patients will acquire endocrine resistance and disease progression after endocrine therapy [3, 22], it is an important clinical treatment approach to prolong or reconstruct the sensitivity of endocrine therapy.

The aim of this study is to explore the interaction mechanism between TAMs and breast cancer endocrine-resistant cells. We tested the ability of breast cancer cells with different sensitivity to tamoxifen to induce macrophages polarized into TAMs. After that, we verified the underlying mechanism that TAMs promoted tamoxifen resistance in breast cancer cells. Lastly, the correlation between COX-2 expression in TAMs and the five-year progression-free survival (PFS) of breast cancer patients treated with tamoxifen was analyzed by the Kaplan-Meier method. Our results reveal the formation mechanism of the positive feedback loop between TAMs and tamoxifen-resistant cells, further suggesting TAMs and COX-2 may become promising novel therapeutic targets for endocrine-resistant breast cancer patients.

Patients and methods

Cell lines and cell culture. Breast cancer cell line MCF-7 and human monocyte cell line THP-1 were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI-1640 medium (R8758, Sigma, Shanghai, China) with fetal bovine serum (FBS) (FND500, ExCell Bio, China). MCF-7 cells were treated with 1 μM of 4-OH Tam for 3 months to obtain tamoxifen-resistant MCF-7 (MCF-7R) cells. MCF-7R cells maintained their resistance to tamoxifen by growing in RPMI-1640 medium with 5% FBS and 1 μM 4-OH Tam.

The procedure for collecting the conditioned medium. In order to obtain the culture supernatant of the cells, approximately 1×10^6 cells were cultured in a 60 mm diameter cell culture dish (430166, Corning, USA). The new culture medium containing FBS was replaced when the adherent cells grew to 80% confluency the next day, and the cells were continuously cultured for 24 hours. Then the supernatant was collected as the conditioned medium (CM) and used immediately in the later experiments.

The definitions and treatments of cells cultured in CM. Macrophages (MΦ) were defined as THP-1 cells that were treated with 320 nM PMA for 24 h. MS cells were defined as macrophages that were cultured in CM of MCF-7 cells for 24 h. MR cells were defined as macrophages that were cultured in CM of MCF-7R cells for 24 h. Some of the obtained MΦ, MS, and MR cells were used to extract proteins, and the others were used to change a fresh culture medium containing FBS for collecting CM immediately. MCF-7 (MΦ) cells were defined as MCF-7 cells cultured in CM of MΦ for 24 h. MCF-7 (MS) cells were defined as MCF-7 cells cultured in CM of MS cells for 24 h. MCF-7 (MR) cells were defined as MCF-7 cells cultured in CM of MR cells for 24 h. Then the CMs were removed by centrifugation at 1000× g for 5 min, and MCF-7 (MΦ), MCF-7 (MS), and MCF-7 (MR) cells were cultured in a fresh medium containing FBS with 5 μmol/l tamoxifen for 48 h.

Antibodies and reagents. 4-hydroxytamoxifen (4-OH Tam, H113419) was purchased from Aladdin Shanghai, China. PMA (P1585) and PGE_2 (P0409) were purchased from Sigma-Aldrich St. Louis, MO, USA. Celecoxib (T0466), a selective COX-2 inhibitor, which is strong specificity and mild adverse reactions, was purchased from Marget Mol, USA. Antibodies against CD163 (A00812-1), Akt (BM4400), p-Akt (BM4721), mTOR (A00003-2), and p-mTOR (BM4840) were purchased from Boster, Wuhan, China. Anti-Arginase-1 antibody (93668) was purchased from CST, Danvers, MA, USA. Anti-c-Myc antibody (ab32072) and anti-COX-2 antibody (ab179800) were purchased from Abcam, Shanghai, China. Anti-p-JNK antibody (ABP0041) was purchased from Abbkine, CA, USA.

Cell viability assay. Cell proliferation was assessed by Cell Counting Kit 8 (CCK-8, Dojindo, Japan) assays according to the manufacturer's instructions. The cells were incubated for 12, 24, 36, and 48 h with different reagents according to the experimental design. At the end of the respective incubation times, cells were incubated in a complete medium containing 10% CCK-8 reagent for 2 h. Subsequently, absorbance was measured at 450 nm in a microplate reader (Mannedorf, Switzerland). The experiment was repeated three times.

Cell invasion assay. 100 μl cell suspension containing 5×10^5 cells with different drugs and reagents was added into the Transwell upper chamber, while 600 μl of complete medium with FBS was added to the lower chamber, and incubated it in a sterile incubator at 37 °C and 5% CO_2 for 48 h. The cells were dyed with 0.1% crystal violet for 30 min. The cells were observed under the inverted microscope, and 5 fields were randomly selected to count the number of cells and calculated the mean value. The above experiments were repeated three times minimum.

Western blot analysis. The protein concentration was determined by the Lowry protein assay kit (Solaibio, Beijing, China). The proteins were separated by SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% fat-free milk in TBST (50 mM Tris, pH 7.5, 0.15 M NaCl, and 0.05% Tween 20) at room temperature for 1 h, and then incubated with the primary antibodies at 4 °C overnight. The protein bands were detected by ECL chemiluminescence (Promega, Madison, WI, USA), and the gray value was analyzed by ImageJ software (National Cancer Institute, Bethesda, MD, USA).

Patients. From December 2003 to June 2013, 180 patients with ER-positive breast cancer were included in the retrospective study. All the patients received routine endocrine therapy. The fixed paraffin-embedded breast cancer samples were obtained from the Pathology laboratories with a thickness of 4 μm.

Immunohistochemistry. Serial pathological slides of breast cancer samples were incubated overnight at 4°C with anti-CD163 antibody (1:250 dilution) and anti-COX-2 antibody (1:300 dilution), respectively. After which, the slides were washed with PBS 3 times, each time for 3 min. Then the slides were incubated with the secondary antibody (G-21234, 1:500 dilution, Thermo Fisher Scientific, China) for 30 min at room temperature. After color development, the yellow granules represented CD163 or COX-2 positive. CD163 immunoreactivity was scored as the infiltration density of CD163 positive macrophages ranging from 0 (absent) to 3 (dense). Macrophages with a score equal to or greater than 1 were considered as TAMs. COX-2 immunoreactivity was scored by staining intensity (negative, weak, moderate, or strong staining) and the percentage of positive tumor cells per core (≤25%, >25–50%, >50–75%, and >75%). Tissues of COX-2 expression with higher than moderate staining intensity in >25% of the cells examined were regarded as positive. The results were used for statistical analysis.

ELISA. MCF-7 cells were cultured with the CM of macrophages with different degrees of TAM polarization for 48 hours. After that, the secretion levels of IL-1β, TNF-α, TGF-β, and PGE_2 in culture supernatant were detected by ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The sample concentrations were estimated with a microplate reader (450 nm and 630 nm).

Statistical analysis. Numerical results were expressed as mean ± SD. All data and statistic graphs were analyzed using GraphPad Prism software (version 6.0) and SPSS Statistics software (version 19.0). Data from multiple groups were evaluated by ANOVA. χ^2-test was used to analyze the relationship between COX-2 in TAMs and tamoxifen resistance. The survival curves were performed by the Kaplan-Meier method and compared by log-rank test. A p-value <0.05 was considered to be statistically significant different between comparison groups (*p<0.05, **p<0.01).

Ethical statement. The research was approved by the Ethics Committee of Harbin Medical University Cancer Hospital, according to the Declaration of Helsinki. All patients signed informed consent.

Results

CM of tamoxifen-resistance breast cancer cells induces macrophages to polarize into TAMs. Macrophages (MΦ) were cultured in the CM of MCF-7 and MCF-7R cells to obtain MS and MR cells (Figure 1A). In order to analyze the polarization differences in macrophages, western blot

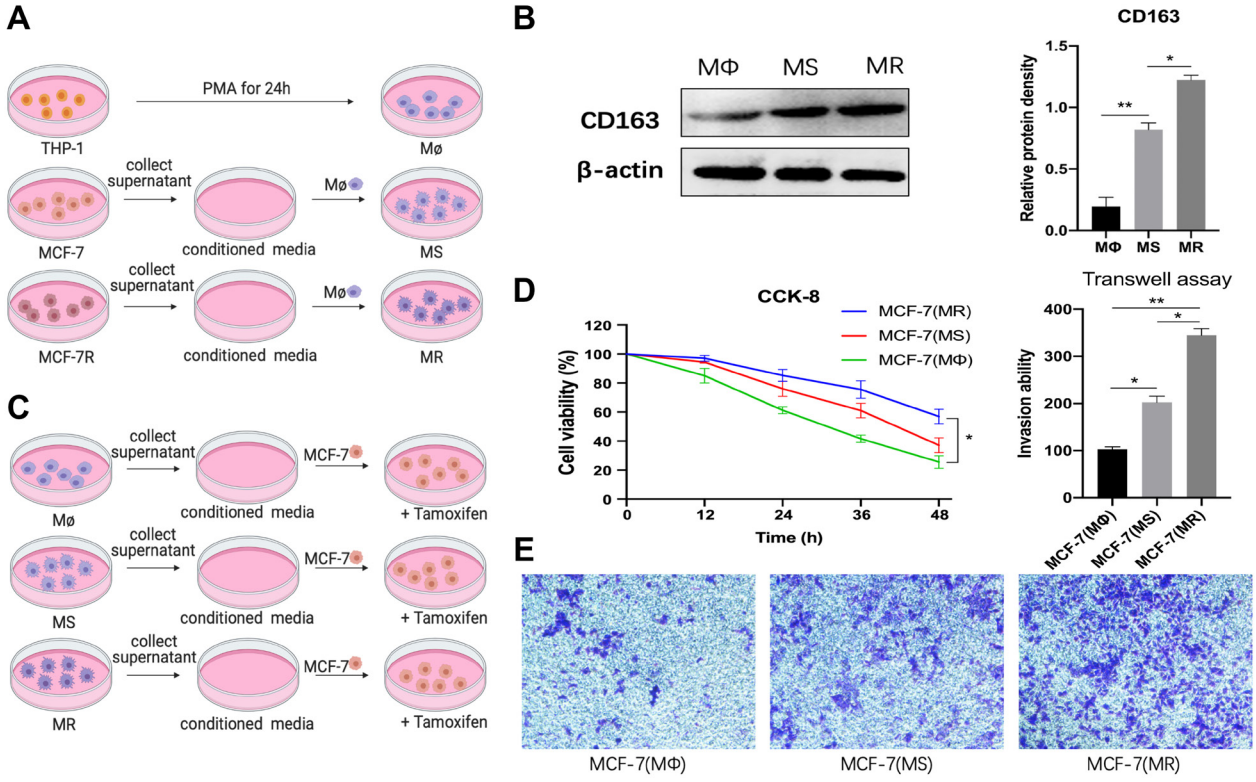

Figure 1. Crosstalk between tumor-associated macrophages (TAMs) and MCF-7 cells. A) Procedure used to obtain macrophages from different conditioned mediums (CMs) ([MΦ] macrophages, [MS] macrophages cultivated in CM from MCF-7 cells, and [MR] macrophages cultivated in CM from MCF-7R cells). B) Western blot analysis of CD163 expression in MΦ, MS, and MR cells. C) Procedure used to testify the effect of CMs from different macrophages (MΦ, MS, and MR) on MCF-7 cells. D) Relative viability of MCF-7 (MΦ), MCF-7 (MS), and MCF-7 (MR) cells treated with 5 μmol/l tamoxifen. E) Transwell assay analysis of MCF-7 (MΦ), MCF-7 (MS), and MCF-7 (MR) cells treated with 5 μmol/l tamoxifen for 48 h.

method was used to detect the expressions of CD163 in MΦ, MS, and MR. CD163 is considered to be a polarization marker for TAMs. The higher expression of CD163 means the more macrophages are induced into TAMs. It was found that CM from breast cancer cell lines increased the expression of CD163 in macrophages. Compared with MS, CD163 expression was higher and TAM polarization was more pronounced in MR (Figure 1B). These results indicate that higher expression of CD163 was induced in macrophages cultivated in the CM from MCF-7R cells.

CM of TAMs induces MCF-7 cells tamoxifen resistance. To verify the function of macrophages with different phenotypes, MCF-7 cells were cultured in the CM of MΦ, MS, and MR (Figure 1C). After that, the cells were treated with 5 μmol/l tamoxifen for 48 h. The CCK-8 analysis was used to evaluate the cell survival rate, and the Transwell assay was used to detect the cell invasion ability (Figures 1D, 1E). MCF-7 cells cultured in CM-TAMs (MS, MR) were observed to have tamoxifen resistance, of which MCF-7 (MR) cells were the most significantly resistant to tamoxifen. Therefore, it indicates that TAMs polarization enhances the ability of MCF-7 to proliferate and invade, and promotes resistance to tamoxifen. Endocrine-resistant cells induce macrophages into TAMs polarization, which in turn promotes endocrine resistance in cancer cells.

TAMs induce endocrine resistance via the PI3K/Akt/mTOR pathway in MCF-7 cells. The above results have shown that CMs of MS and MR are associated with endocrine resistance in MCF-7 cells. As the aberrant activation of the PI3K/Akt/mTOR pathway is a vital process in cancer cell proliferation and drug resistance [23], the effect of TAMs on this pathway was investigated. Western blot results showed that the phosphorylation levels of Akt and mTOR were significantly increased in MCF-7 cells treated with CM of MR, in which macrophages expressed the highest CD163 and polarized into the most TAMs (Figure 2A). It suggests that TAMs promote endocrine resistance in breast cancer by activating the PI3K/Akt/mTOR pathway.

High COX-2 expression in TAMs promotes endocrine resistance by the PI3K/Akt/mTOR pathway. In this study, MCF-7 cells were cultured in a microenvironment of macrophages of different phenotypes, and the microenvironment contained cytokines secreted by them. Therefore, it is likely that a certain cytokine released by TAMs mediates endocrine

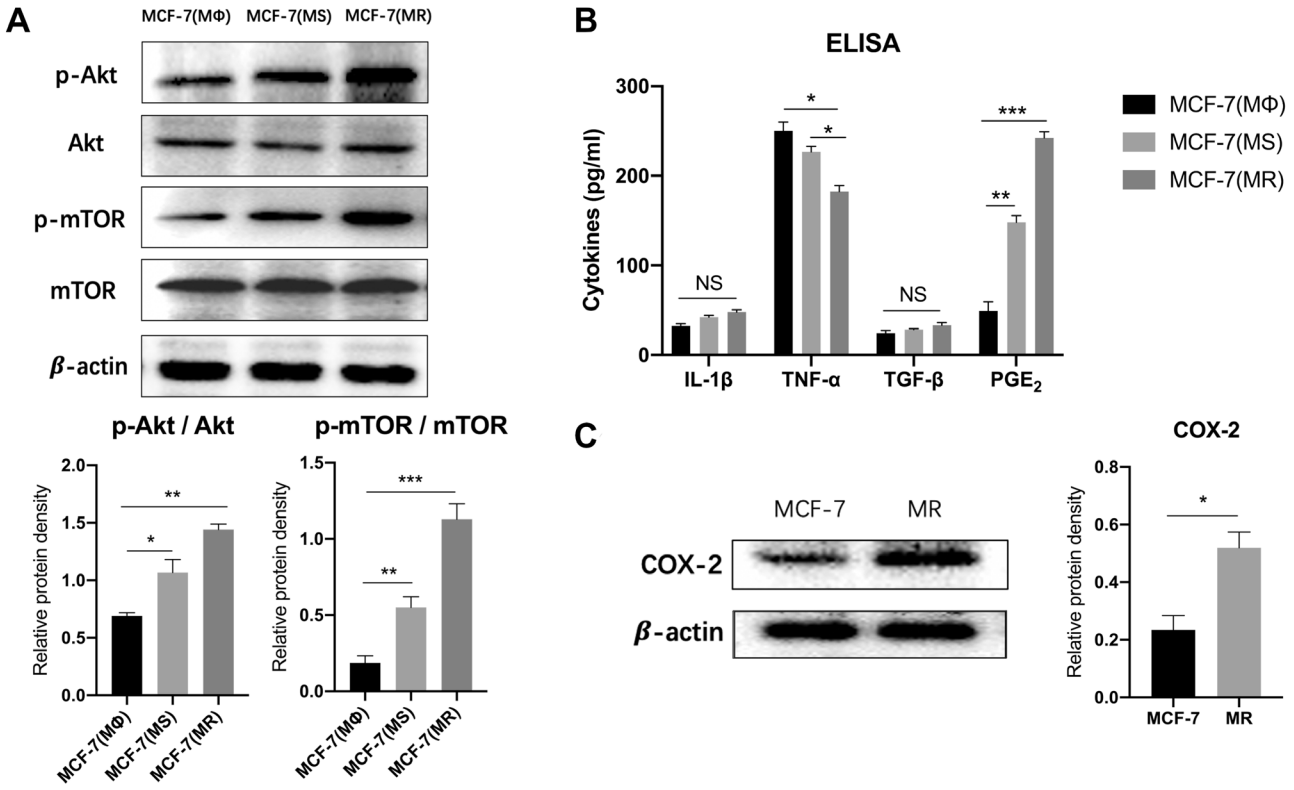

Figure 2. High expression of COX-2 in tumor-associated macrophages (TAMs) induces endocrine resistance by activating the PI3K/Akt/mTOR pathway in MCF-7 cells. A) Western blot analysis of the PI3K/Akt/mTOR pathway. The expressions of p-Akt and p-mTOR were highest in MCF-7 cells cultured in the conditioned mediums (CMs) of MR (TAMs from tamoxifen-resistant microenvironment). B) ELISA shows the secretion levels of four cytokines in CMs of different macrophages (MΦ, MS (TAMs from tamoxifen-sensitive TME), and MR). Only the change trend of PGE_2 was increased significantly. C) The COX-2 expressions in MCF-7 and MR were detected by western blot. It indicates it is TAMs that increase the expression of COX-2/PGE_2 promoting endocrine resistance in MCF-7 cells.

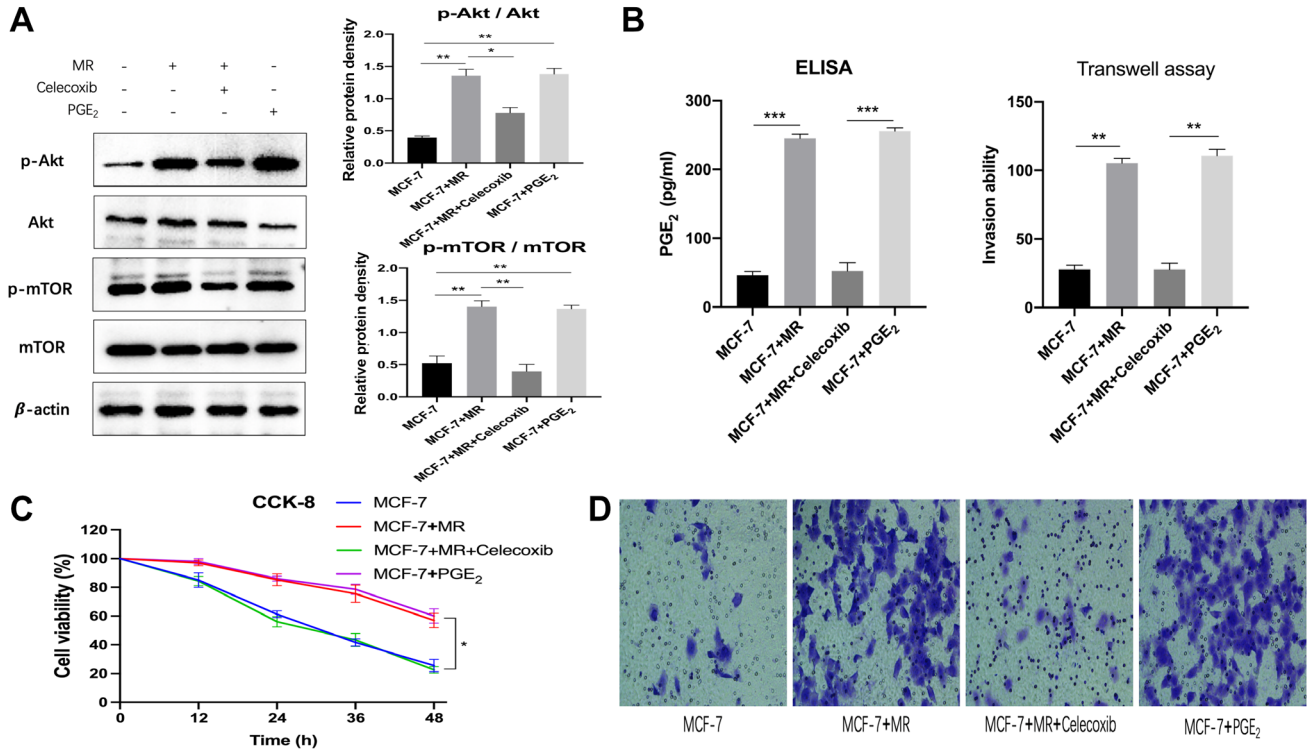

Figure 3. Tumor-associated macrophages (TAMs) and COX-2/PGE_2 promote endocrine resistance, while celecoxib inhibits endocrine resistance in MCF-7 cells treated with 5 μmol/l tamoxifen. A) Western blot analysis of the PI3K/Akt/mTOR pathway. Conditioned mediums (CMs) of MR (TAMs from tamoxifen-resistant TME) and PGE_2 (50 nmol/l) activate the PI3K/Akt/mTOR pathway in MCF-7 cells. The activation of the PI3K/Akt/mTOR pathway by the conditioned mediums (CMs) of MR decreased when MR was treated with 40 μmol/l celecoxib. B) PGE_2 levels in four groups were detected by ELISA. C) Relative viability of MCF-7, MCF-7+MR, MCF-7+MR+celecoxib, and MCF-7+PGE_2 cells treated with 5 μmol/l tamoxifen. D) Transwell assay analysis of MCF-7, MCF-7+MR, MCF-7+MR+celecoxib, and MCF-7+PGE_2 cells treated with 5 μmol/l tamoxifen for 48 h.

resistance, and this cytokine has the ability to activate the PI3K/Akt/mTOR pathway. After reviewing the relevant literature, we selected the key cytokines that may activate this pathway, including IL-1β, TNF-α, TGF-β, and PGE_2 [24]. The secretion levels of these four cytokines in the three CM (MΦ, MS, and MR) were assessed by ELISA. The results showed that with the polarization of macrophages into TAMs, the changes of IL-1β and TGF-β were not obvious, the secretion of TNF-α decreased, while only the secretion of PGE_2 increased significantly (Figure 2B). It indicates that PGE_2 may be an important mediator of endocrine resistance through the PI3K/Akt/mTOR pathway.

The synthesis of PGE_2 requires the role of the key synthase COX-2, which may be expressed in both TAMs and tumor cells. In order to prove that the PGE_2 overexpressed in TME was mainly secreted by TAMs, western blot was used to detect the expression levels of COX-2 in MCF-7 cells and MR (TAMs). The results demonstrated that the expression of COX-2 in MR was higher than that in MCF-7 cells (Figure 2C). It suggests that TAMs overexpress COX-2, inducing the endocrine resistance in breast cancer. The western blot results showed that adding CM from MR to MCF-7 cells enhanced the activation of the PI3K/Akt/mTOR pathway, while celecoxib (40 μmol/l) inhibited this effect. Furthermore, the phosphorylation of Akt and mTOR was also significantly increased when MCF-7 cells were treated with 50 nmol/l PGE_2 (Figure 3A). The levels of PGE_2 in the four groups were analyzed by ELISA, which is consistent with pathway activation (Figure 3B). Besides, MCF-7 cells cultured with MR or PGE_2 acquired a stronger ability of proliferation and invasion and were more resistant to tamoxifen (Figures 3C, 3D). The above results indicate that TAMs overexpress COX-2 increasing the secretion of PGE_2, which promotes endocrine resistance through the activation of the PI3K/Akt/mTOR pathway.

Breast cancer cells induce TAMs polarization through the activation of the JNK/c-Myc/Arginase-1 pathway. The above experiments have proved that the TME induces TAMs polarization, but the relevant mechanism has not been elucidated. Due to the immortal proliferation of tumor cells, the interior of the tumor is often in a state of hypoxia. In this local hypoxic environment, the JNK/c-Myc pathway in macrophages is activated, which is a classic pathway involved in TAMs polarization of macrophages [25]. Therefore, a

western blot was used to detect the activation of this pathway. Moreover, the overexpression of Arginase-1 is the representative marker for macrophages TAMs polarization [8, 26]. The results showed that the expressions of p-JNK, c-Myc, and Arginase-1 were the highest in MR, and the most TAMs were generated (Figure 4A). The reason may be that when breast cancer cells are resistant to tamoxifen, the cell proliferation and invasion ability is still maintained at a high level, and the oxygen content in TME is relatively low. These results indicate that breast cancer endocrine-resistant cells are more capable of mediating the polarization of macrophages into TAMs through the JNK/c-Myc/Arginase-1 pathway. In summary, TAMs promote endocrine resistance in breast cancer, which in turn, endocrine-resistant breast cancer cells mediate macrophages TAMs polarization, forming a positive feedback loop.

COX-2 in TAMs is associated with endocrine resistance in breast cancer patients. In order to investigate the clinical relevance of COX-2 in TAMs and endocrine resistance, pathological paraffin sections of 180 ER-positive breast cancer patients were immunohistochemically stained with anti-CD163 and anti-COX-2 antibody (Figure 5A). In the stroma of 180 patients, 124 (68.9%) CD163-positive patients were included in the final research. Among the 124 patients, 50 (40.3%) were found overexpressing COX-2 in TAMs, while 74 (59.7%) were found under-expressing COX-2 in TAMs. Recurrence and progression were observed in 31 (62%) patients with high COX-2 expression, while 24 (32.4%) patients with low COX-2 expression. Chi-square test statistical analysis was applied to evaluate the relationship between COX-2 expression and endocrine resistance in breast cancer. The results illustrate those patients with high expression of COX-2 in TAMs are more likely to obtain resistance to endocrine therapy of breast cancer, which leads to disease recurrence and progression ($p<0.01$, Figure 5B). To summarize, these results indicate that COX-2 in TAMs plays a key role in promoting endocrine resistance in breast cancer.

High COX-2 expression in TAMs results in a poor five-year PFS for breast cancer patients. The χ^2-test showed that the higher the expression of COX-2 in TAMs, the better chance to develop tamoxifen resistance. As a consequence, it is reasonable to investigate whether the COX-2 in TAMs is relevant to clinical survival. Of the 50 patients with high COX-2 expression in TAMs, 22 (44%) developed progression within five years, with a median follow-up time of 43 months. Of the 74 patients with low COX-2 expression in TAMs, 19 (25.7%) developed progression within five years, with a median follow-up time of 59 months. Kaplan-Meier survival curve was used to analyze the relationship between COX-2 expression and the five-year PFS of these patients. The results indicate that the higher the expression of COX-2 in TAMs, the lower the five-year PFS of the breast cancer patients ($p<0.05$, Figure 5C), and the difference was statistically significant.

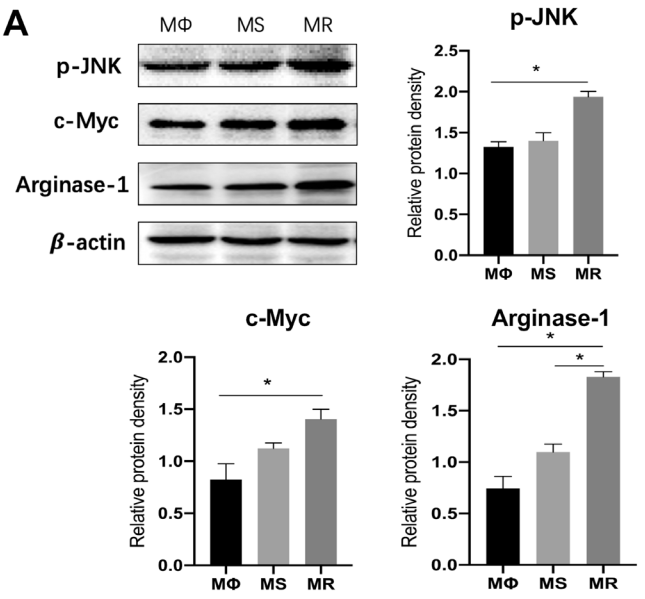

Figure 4. Conditioned mediums (CMs) of tamoxifen-resistant MCF-7 cells (MCF-7R) induce macrophages to polarize into tumor-associated macrophages (TAMs) through the activation of the JNK/c-Myc/Arginase pathway. A) Western blot analysis of the JNK/c-Myc/Arginase pathway. CMs of tamoxifen-sensitive MCF-7 cells (MCF-7S) and MCF-7R activated JNK/c-Myc/Arginase pathway in macrophages. Furthermore, the activation of the JNK/c-Myc/Arginase pathway was more obvious in MR (TAMs from tamoxifen-resistant TME). It suggests that macrophages cultured in CM of MCF-7R are more likely to polarize into TAMs.

Discussion

Endocrine resistance is the main cause of progression or death in patients with ER-positive breast cancer. At present, most researches on the mechanisms of endocrine resistance focus on breast cancer cells, while the study on the role of other cells in TME is less frequent. TAMs are the most abundant infiltrative immune-related stromal cells in TME that promote tumor growth, angiogenesis, invasion, and metastasis, and inhibit apoptosis in a variety of tumors [27]. Our previous study has confirmed that TAMs are associated with tamoxifen resistance in breast cancer patients [12]. This study mainly explored the mechanism of TAMs mediated endocrine resistance in breast cancer cells.

We found that endocrine-resistant breast cancer cells induce macrophages TAMs polarization. When breast cancer cells acquire the ability to resist endocrine therapy, the functions of cell proliferation and anti-apoptosis are not affected, resulting in the local microenvironment of the tumor is often in a state of hypoxia. It is found that macrophages polarize into TAMs through the JNK/c-Myc/Arginase-1 pathway in this specific TME.

It was confirmed that endocrine-resistant breast cancer cells induce the polarization of TAMs, and TAMs in turn further promote endocrine resistance of breast cancer cells. In addition to the overexpression in breast cancer cells, accumu-

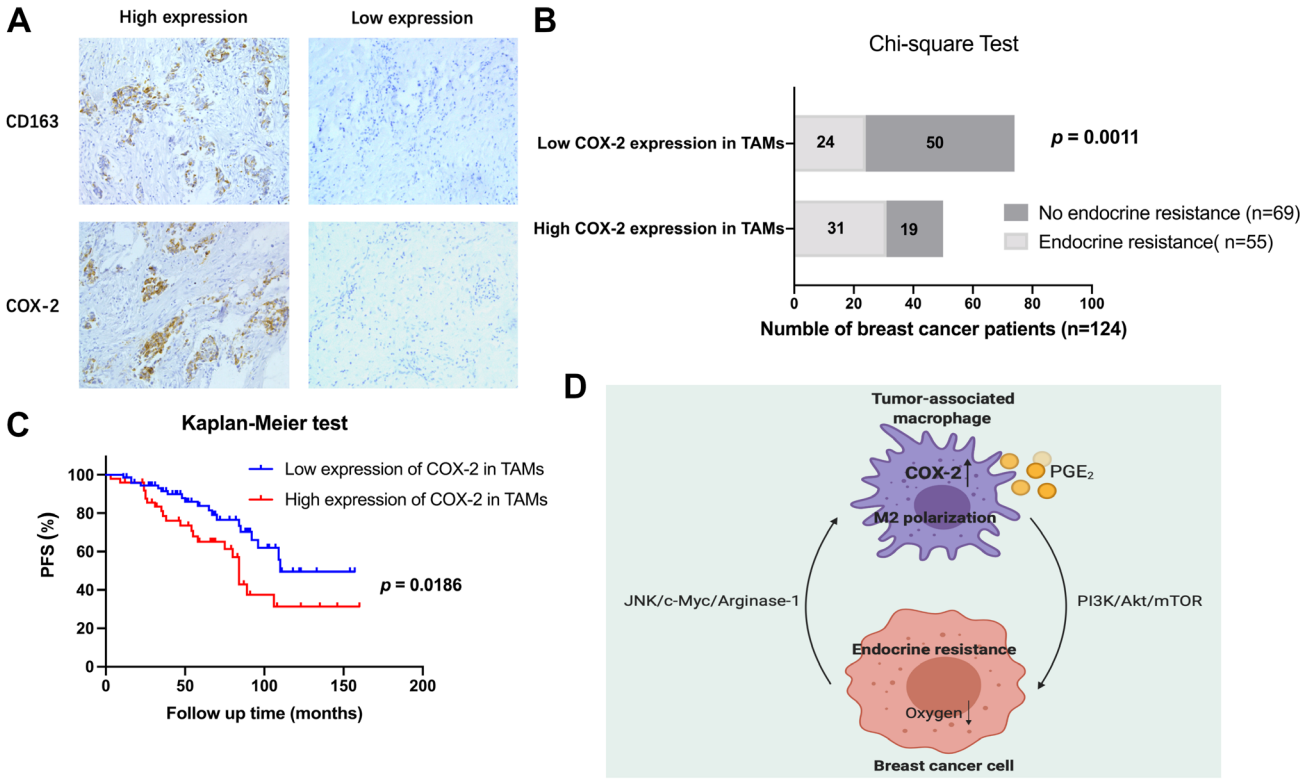

Figure 5. High COX-2 expression in tumor-associated macrophages (TAMs) is related to endocrine resistance and a poor five-year progression-free survival (PFS) in breast cancer patients. A) Representative images immunohistochemical staining for CD163 and COX-2 in serial sections from human breast cancer samples. B) χ^2-test for the correlation between COX-2 expression in TAMs and endocrine resistance in breast cancer patients ($p < 0.0001$). C) Kaplan-Meier five-year PFS curve in estrogen receptor (ER)-positive breast cancer patients with low (n = 74) and high (n = 50) COX-2 expression in TAMs ($p = 0.0186$). D) Proposed model for TAMs increases COX-2 expression activating the PI3K/Akt/mTOR pathway to promote endocrine resistance in breast cancer cells, forming a positive feedback loop between TAMs and breast cancer cells.

lating evidence shows that COX-2 also commonly exists in other components of TME, such as fibroblasts and myeloid cells [28, 29]. Hou et al. [15] observed that macrophages induced COX-2 expression in breast cancer cells, which may result in tumor progression. Moreover, it is also reported that COX-2 inhibition leads to the loss of M2 macrophages' characteristics in TAMs, which may help to prevent metastasis in mouse breast cancer models [30]. Taken together, it indicates that COX-2 in TAMs may participate in the progression and drug resistance of malignant tumors [31]. The activation of the PI3K/Akt/mTOR pathway in tumor cells is the central event of TAMs-mediated cancer progression, because the chemokines or cytokines secreted by TAMs may be the effective PI3K/Akt/mTOR activators [23, 32]. In this study, we found that COX-2 in TAMs increases the release of PGE_2 and promotes endocrine resistance in breast cancer cells by activating the PI3K/Akt/mTOR pathway, which is the classical signaling pathway that mediates the endocrine resistance [20, 21]. We also investigated that breast cancer patients with COX-2 overexpression in TAMs are more likely to develop endocrine resistance. More importantly, the high expression of COX-2 in TAMs is related to the poor five-year PFS, which is consistent with a previous study [11].

The crosstalk between TAMs and cancer cells is complex, involving exosomes, cytokines, and metabolites [33]. Considering that TAMs are a kind of stromal cells in TME, other stromal cells and various cytokines may also have potential impacts on the endocrine resistance of breast cancer cells. In future research, it is necessary to further investigate these issues.

In conclusion, our results indicate that endocrine-resistant breast cancer cells prefer to mediate TAMs polarization of macrophages through the JNK/c-Myc/Arginase-1 pathway. In turn, TAMs highly express COX-2, which further promotes the endocrine resistance of breast cancer cells by activating the PI3K/Akt/mTOR pathway, thereby forming a positive feedback loop between TAMs and breast cancer cells (Fig. 5D). These findings suggest that COX-2 and TAMs may be potential therapeutic targets for endocrine-resistant breast cancer patients. Our results further improve the possibility of reversing the endocrine resistance of breast cancer and improve the clinical treatment effect of these patients.

Acknowledgments: This study was financially supported by the National Natural Science Foundation of China (Grants No. 81730074 and 81672599).

References

[1] SIEGEL RL, MILLER KD, JEMAL A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7–34. https://doi.org/10.3322/caac.21551

[2] DAVIES E, HISCOX S. New therapeutic approaches in breast cancer. Maturitas 2011; 68: 121–128. https://doi.org/10.1016/j.maturitas.2010.10.012

[3] ZHOU J, TENG R, WANG Q, XU C, GUO J et al. Endocrine resistance in breast cancer: Current status and a perspective on the roles of miRNAs. Oncol Lett 2013; 6: 295–305. https://doi.org/10.3892/ol.2013.1405

[4] LUQMANI YA, ALAM-ELDIN N. Overcoming Resistance to Endocrine Therapy in Breast Cancer: New Approaches to a Nagging Problem. Med Princ Pract 2016; 25: 28–40. https://doi.org/10.1159/000444451

[5] CHENG R, LIU YJ, CUI JW, YANG M, LIU XL et al. Aspirin regulation of c-myc and cyclinD1 proteins to overcome tamoxifen resistance in estrogen receptor-positive breast cancer cells. Oncotarget 2017; 8: 30252–30264. https://doi.org/10.18632/oncotarget.16325

[6] HANAHAN D, COUSSENS LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 2012; 21: 309–322. https://doi.org/10.1016/j.ccr.2012.02.022

[7] OSTMAN A. The tumor microenvironment controls drug sensitivity. Nat Med 2012; 18: 1332–1334. https://doi.org/10.1038/nm.2938

[8] MURRAY PJ, ALLEN JE, BISWAS SK, FISHER EA, GILROY DW et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 2014; 41: 14–20. https://doi.org/10.1016/j.immuni.2014.06.008

[9] QIAN BZ, POLLARD JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010; 141: 39–51. https://doi.org/10.1016/j.cell.2010.03.014

[10] NOY R, POLLARD JW. Tumor-associated macrophages: from mechanisms to therapy. Immunity 2014; 41: 49–61. https://doi.org/10.1016/j.immuni.2014.06.010

[11] TARIQ M, ZHANG J, LIANG G, DING L, HE Q et al. Macrophage Polarization: Anti-Cancer Strategies to Target Tumor-Associated Macrophage in Breast Cancer. J Cell Biochem 2017; 118: 2484–2501. https://doi.org/10.1002/jcb.25895

[12] XUAN QJ, WANG JX, NANDING A, WANG ZP, LIU H et al. Tumor-associated macrophages are correlated with tamoxifen resistance in the postmenopausal breast cancer patients. Pathol Oncol Res 2014; 20: 619–624. https://doi.org/10.1007/s12253-013-9740-z

[13] VO BT, MORTON D JR, KOMARAGIRI S, MILLENA AC, LEATH C et al. TGF-β effects on prostate cancer cell migration and invasion are mediated by PGE2 through activation of PI3K/AKT/mTOR pathway. Endocrinology 2013; 154: 1768–1779. https://doi.org/10.1210/en.2012-2074

[14] LIU B, QU L, YAN S. Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. Cancer Cell Int 2015; 15: 106. https://doi.org/10.1186/s12935-015-0260-7

[15] HOU Z, FALCONE DJ, SUBBARAMAIAH K, DANNENBERG AJ. Macrophages induce COX-2 expression in breast cancer cells: role of IL-1β autoamplification. Carcinogenesis 2011; 32: 695–702. https://doi.org/10.1093/carcin/bgr027

[16] MISRA S, SHARMA K. COX-2 signaling and cancer: new players in old arena. Curr Drug Targets 2014; 15: 347–359. https://doi.org/10.2174/1389450115666140127102915

[17] TSAI CS, CHEN FH, WANG CC, HUANG HL, JUNG SM et al. Macrophages from irradiated tumors express higher levels of iNOS, arginase-I and COX-2, and promote tumor growth. Int J Radiat Oncol Biol Phys 2007; 68: 499–507. https://doi.org/10.1016/j.ijrobp.2007.01.041

[18] HERYNK MH, FUQUA SA. Estrogen receptor mutations in human disease. Endocr Rev 2004; 25: 869–898. https://doi.org/10.1210/er.2003-0010

[19] DOWSETT M. Overexpression of HER-2 as a resistance mechanism to hormonal therapy for breast cancer. Endocr Relat Cancer 2001; 8: 191–195. https://doi.org/10.1677/erc.0.0080191

[20] CIRUELOS GIL EM. Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. Cancer Treat Rev 2014; 40: 862–871. https://doi.org/10.1016/j.ctrv.2014.03.004

[21] MILLS JN, RUTKOVSKY AC, GIORDANO A. Mechanisms of resistance in estrogen receptor positive breast cancer: overcoming resistance to tamoxifen/aromatase inhibitors. Curr Opin Pharmacol 2018; 41: 59–65. https://doi.org/10.1016/j.coph.2018.04.009

[22] GONZALEZ-VILLASANA V, GUTIÉRREZ-PUENTE Y, TARI AM. Cyclooxygenase-2 utilizes Jun N-terminal kinases to induce invasion, but not tamoxifen resistance, in MCF-7 breast cancer cells. Oncol Rep 2013; 30: 1506–1510. https://doi.org/10.3892/or.2013.2549

[23] SU S, LIU Q, CHEN J, CHEN J, CHEN F et al. A positive feedback loop between mesenchymal-like cancer cells and macrophages is essential to breast cancer metastasis. Cancer Cell 2014; 25: 605–620. https://doi.org/10.1016/j.ccr.2014.03.021

[24] GEORGE RJ, STURMOSKI MA, ANANT S, HOUCHEN CW. EP4 mediates PGE2 dependent cell survival through the PI3 kinase/AKT pathway. Prostaglandins Other Lipid Mediat 2007; 83: 112–120. https://doi.org/10.1016/j.prostaglandins.2006.10.005

[25] ZHANG Y, HUANG T, JIANG L, GAO J, YU D et al. MCP-induced protein 1 attenuates sepsis-induced acute lung injury by modulating macrophage polarization via the JNK/c-Myc pathway. Int Immunopharmacol 2019; 75: 105741. https://doi.org/10.1016/j.intimp.2019.105741

[26] BENNER B, SCARBERRY L, SUAREZ-KELLY LP, DUGGAN MC, CAMPBELL AR et al. Generation of monocyte-derived tumor-associated macrophages using tumor-conditioned media provides a novel method to study tumor-associated macrophages in vitro. J Immunother Cancer 2019; 7: 140. https://doi.org/10.1186/s40425-019-0622-0

[27] TANG X. Tumor-associated macrophages as potential diagnostic and prognostic biomarkers in breast cancer. Cancer Lett 2013; 332: 3–10. https://doi.org/10.1016/j.canlet.2013.01.024

[28] EREZ N, GLANZ S, RAZ Y, AVIVI C, BARSHACK I. Cancer associated fibroblasts express pro-inflammatory factors in human breast and ovarian tumors. Biochem Biophys Res Commun 2013; 437: 397–402. https://doi.org/10.1016/j.bbrc.2013.06.089

[29] CHEN EP, MARKOSYAN N, CONNOLLY E, LAWSON JA, LI X et al. Myeloid Cell COX-2 deletion reduces mammary tumor growth through enhanced cytotoxic T-lymphocyte function. Carcinogenesis 2014; 35: 1788–1797. https://doi.org/10.1093/carcin/bgu053

[30] NA YR, YOON YN, SON DI, SEOK SH. Cyclooxygenase-2 inhibition blocks M2 macrophage differentiation and suppresses metastasis in murine breast cancer model. PLoS One 2013; 8: e63451. https://doi.org/10.1371/journal.pone.0063451

[31] YANG C, HE L, HE P, LIU Y, WANG W et al. Increased drug resistance in breast cancer by tumor-associated macrophages through IL-10/STAT3/bcl-2 signaling pathway. Med Oncol 2015; 32: 352. https://doi.org/10.1007/s12032-014-0352-6

[32] ZHANG B, YIN C, LI H, SHI L, LIU N et al. Nir1 promotes invasion of breast cancer cells by binding to chemokine (C-C motif) ligand 18 through the PI3K/Akt/GSK3β/Snail signalling pathway. Eur J Cancer 2013; 49: 3900–3913. https://doi.org/10.1016/j.ejca.2013.07.146

[33] GOSSELIN D, LINK VM, ROMANOSKI CE, FONSECA GJ, EICHENFIELD DZ et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell 2014; 159: 1327–1340. https://doi.org/10.1016/j.cell.2014.11.023