FPR2 Participates in Epithelial Ovarian Cancer (EOC) Progression Through RhoA Mediated M2 Macrophage Polarization: An Invitro Experimental Study

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Research

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Abstract

Background: In our previous study, we found Formyl peptide receptor 2 (FPR2) promoted the invasion and metastasis of EOC and it could be a prognostic marker for EOC. In this study, we aimed to study the possible mechanism of FPR2 in promoting EOC progression.

Methods: The FPR2 ectopic expression and knockdown EOC cell lines as well as their control cell lines were established and the expression change of RhoA in each cell lines was evaluated by RT-qPCR and Western-blot. Wound healing and Transwell assays were performed to detect the migrational ability of EOCs that affected by FPR2 and RhoA. The supernatant of each EOC cell lines were used to co-culture with the macrophages, and tested the M1 and M2 macrophges biomarkers by flow cytometry. THP-1 cell line was also indcued to differentiated to M1 and M2 macrophages, FPR2 and RhoA expression in each macrophage cell lines were detected by RT-qPCR and Western-blot.

Results: RhoA expression was significantly increased in EOCs along with the overexpression of FPR2, which showed a positive correlation by Pearson correlation analysis. FPR2 ectopic expression would contribute to the migrational ability of EOCs, and RhoA inhibitor (C3 transferase) would impare EOCs migration. Furthermore, FPR2 stimulated the secretion of Th2 cytokines by EOCs, which induced macrophages differentiate to M2 phenotype, while RhoA inhibitor stimulate the secretion of Th1 cytokines and induce macrophages differentiate to M1 phenotype. Moreover, compared with M1 macrophages and THP-1 cells, FPR2 and RhoA expression were significantly up-regulated in M2 macrophages.

Conclusion: FPR2 stimulated M2 macrophage polarization and promote invasion and metastasis of ovarian cancer cells through RhoA.

Background

Ovarian cancer is one of the most lethal gynecology malignancies in the world. 75% of patients were first diagnosed with ovarian cancer in their advanced stage because of lacking clinical symptoms. What's worse, ovarian cancer was characterized by rapidly progression, poorly prognosis and highly rates of recurrence and metastasis. At present, in addition to the classical treatments including operation and postoperative chemotherapy, neoadjuvant chemotherapy, immunotherapy and molecular targeted therapy were recommended as well. Nevertheless, the five-year survival rate for ovarian cancer had not been improved in the past 20 years, which was fluctuated between 30% and 35% [1, 2].

Formyl peptide receptor 2 (FPR2) has been identified as a member of G protein-coupled chemoattractant receptor (GPCR) family. It is a seven-transmembrane receptor with 351 amino acids and its gene is located on human chromosome 19q13.3-q13.4 [3]. FPR2 is a multi-functional receptor that is associated with diverse pathophysiologic processes, such as inflammation, cancer, amyloidosis, neurodegenerative diseases, wound healing, diabetes and AIDS [4]. In previous study, FPR2 was illuminated overexpressed in ovarian cancer tissues and was located on the cell membrane. Also, knocking down FPR2 was available

to inhibit the invasion and migration of ovarian cancer cells, which was indicating potentially that FPR2 plays a key role in cancer cell metastasis. Moreover, FPR2 was closely related to the clinical prognosis of ovarian cancer patients [5]. It is reported that Small GTPases of Rho family were involved in FPR signaling [6, 7]. The most noticeable member of Rho family is RhoA, which acts as a “molecular switch” to activate the downstream signaling pathways. Here, whether RhoA is involved in the role of FPR2 in ovarian cancer invasion and migration, was investigated in this study.

In recent years, tumor microenvironment which is composed of tumor cells, fibroblasts, immune cells, endothelial cells, extracellular matrix, cytokines, etc, was considered vital in malignant development. It is reported that macrophages are major tumor-infiltrating immune cells that are associated with tumor progression, and it accounts for almost 50% of total immune cells in tumor microenvironment [8]. Blood-derived monocytes in tumor tissues would differentiate into macrophages and then further developed into M1 or M2 phenotype depending on different conditions. When stimulated by Th1 cytokines, such as IFN-γ, LPS, TNF-α, ROS, IL-1, IL-12, infiltrating macrophages would differentiate into M1 phenotype which showed an cytotoxic effect, promoted immune activation and inhibited malignant progression. When stimulated by Th2 cytokines, including M-CSF, GM-CSF, IL-4, IL-10, TGF-β, macrophages would differentiate into the M2 phenotype that equipped with the funcitons of immune suppression, facilitating tumor progression, cell proliferation and angiogenesis [9]. It is suggested that M2 macrophages could play a role in ovarian cancer progression and the larger M1/M2 ratio is, the greater the overall survival rate and progression-free survival rate of patients could be [10, 11]. However, whether FPR2 has an influence on M2 macrophages differentiation is still controversial [12, 13].

In this study, RhoA expression was positively correlated with FPR2 in EOCs, and FPR2 ectopic expression promoted the migrational ability of EOCs, wherease RhoA inhibitor (C3 transferase) diminished the migration. What’s more, FPR2 stimulated the secretion of Th2 cytokines by EOCs, which induced macrophages differentiate to M2 phenotype, while RhoA inhibitor stimulate the secretion of Th1 cytokines and induce macrophages differentiate to M1 phenotype. Thus we suggested that FPR2 stimulated M2 macrophage polarization and promote invasion and metastasis of ovarian cancer cells through RhoA.

Methods

Cell cultures.

The human ovarian cancer cell lines SKOV3, OVCAR3, A2780 and Caov3 were obtained from the America Type Culture Collection (ATCC; Manassas, VA, USA), and HO-8910 cell lines, Hosepic cell lines and THP-1 cell lines were obtained from the China Center for Type Culture collection (CCTCC). The cell lines were cultured in either Dulbecco's modified Eagle's medium (DMEM, Hyclone, Cat.No.SH30022.01B) or RPMI-1640 medium (Hyclone, Cat.No.SH30809.01B) supplemented with 10% fetal bovine serum (FBS, Hyclone,Cat.No.SH30256.01B) and antibiotics (penicillin 100 U/mL, streptomycin 0.1 mg/mL and amphotericin B 0.25 lg/mL) and maintained in a 37°C incubator containing 5% CO2. THP-1 cells was

treated with 10 ng/ml phorbol 12-myristate 13-aceteat (PMA; SigmaAldrich, Germany) for 72 h to induce differentiation of M0 macrophage. M0 macrophages were treated with 100 ng/ml LPS (#L2880, sigma) plus 10 ng/ml IFN-γ (#BEK-2026, 4A Biotech, Beijin) for 48 h to induce M1 phenotype differentiation or with 10 ng/ml IL-4 (#214 − 14, Peprotech, Germany) for 48 h to induce M2 phenotype differentiation. Moreover, M0 macrophages were also treated with the supernatant of each ovarian cancer cell line for 48 h. Cells were collected for flow cytometry analysis, RT-qPCR or Western blot assays.

Real-time quantitative PCR(RT-qPCR).

Total RNA was extracted from cells using TRIzol reagents (Pufei Biotechnology, Shanghai, China). Reverse transcription was performed using M-MLV reverse transcriptase (Promega, Madison, USA).

The primer sequences were designed as follows:

FPR2:forward5'-TTTGGCTGGTTCCTGTGTAAG-3',reverse5'-GGTCCGACGATCACCTTCAT-3';RhoA:forward5'-TGGATGGAAAGCAGGTAGAGT-3',reverse5'-CTATCAGGGCTGTCGATGGA-3';18sforward5'-CCTGGATACCGCAGCTAGGA-3',reverse5'-GCGGCGCAATACGAATGCCCC-3'

Quantitative PCR was performed using SYBR-Green RealTime PCR Master Mix (Toyobo,Osaka,Japan) according to the manufacturer's protocol. Data were analyzed by Sequence Detection Software for the threshold cycle(Ct), and the comparative Ct (ΔΔCt) was used to calculate the difference between samples by relative qualification.

Western blot.

Total cell lysates were harvested in NP-40 lysis buffer (150 mM NaCl, 1% Nonidet P-40, 50 mM Tris, pH 8.0, protease inhibitor cocktail) and protein concentrations were determined by BCA protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Equal amounts of proteins from each lysate were submitted to SDS-PAGE for protein separation and then transferred to PVDF membranes. Membranes were blocked with buffer containing 5% skim milk and 0.1% Tween-20 in PBS for 1 h at room temperature with gentle shaking. Primary antibodies (FPR2,RhoA and GAPDH) were incubated overnight at 4˚C with gentle shaking, followed by secondary antibody incubation at room temperature for 1 h with gentle shaking. The following antibodies were used: FPR2 (#sc-66898; Santa Cruz Biotechnology, Santa Cruz, CA, USA); NF-κB (#ab54835; Abcam, Cambridge, MA, USA); GAPDH (#KC-5G5; AKsomics,ShangHai); Goat Anti-Rabbit IgG (H + L), ads-HRP (#4050-05; southern biotech, Birmingham, AL, USA).

Vector construction and plasmid transfection.

PCR product and pcDNA3.1 vector (invitrogen) were both treated by double digestion of XhoI and HindIII. Target fragments were separated and purified, and the recombinant plasmid was constructed. T4-DNA ligase was used to combine the vector and the target gene. Caov-3 cell lines and OVCAR-3 cell lines were transefected with pcDNA3.1-shFPR2 vector and pcDNA3.1-FPR2 + vector respectively; as well as both of the corresponding control cell lines were constructed. The shRNA sequences for FPR2 knock down were

as follows: shRNA-, ccggGGCCAAGACTTCCGAGAGAGActcgagTCTCTCTCGGAAGTCTTGGCCtttttg; The FPR2 over expressed RNA sequence were: TCACCTCCTGCAGAGACTGAGTTACAGGCAATGTGA.

ELISA analysis.

Concentration of cytokines including TGF-β, IL-4, and IL-10, was determined using ELISA kits from Solarbio (Cat. No. #SEKH0316, #SEKH0011, and #SEKH0018, respectively). Cell culture supernatants were collected and centrifuged at 4 °C at 1000 *× g* for 10 min before analyzed according to the manufacturer's protocol.

Flow cytometry.

For fow cytometric analysis, cells were stimulated as described above. 1 × 106/cells per sample were collected and stained with antibody in 100 μl PBS + 1% BSA for 30 min at 4 °C in the dark, followed by wash with PBS. Fuorescence was detected on a BD FACSCan (Becton, Dickinson and Company, MD, USA). All antibodies, except Dectin-PE (R&D Systems, Germany), were obtained from BD Bioscience (New Jersey,America).

Wound-healing assay.

Cells (3 × 104 /well) were seeded on 96-well plates and grown to 90% confluence, after which a scratch was made in the monolayer using a 10-μl pipette tip. Then, the cells were incubated at 37℃ in 5% CO2 for another 4 h according to the result of the pre-experiment, and the images were obtained at the time points of 0, 4 and 8 h. Each experiment was performed three times.

Transwell assay.

The assay was performed using a pre-coated cell invasion kit (pore size, 8.0 μm; Corning Inc., Corning, NY, USA) and Matrigel (BD Biosciences, Bedford, MA, USA) was inserted in the upper chambers. Approximately 1 × 105 cells in 100 μl serum-free medium were placed into the upper chambers, the cells were cultured in 5% CO2 at 37℃ for 16 h (according to the pre-experiment). The lower chambers contained 30% FBS, thus, the cells migrated to the lower chambers. The cells remaining in the upper chambers were removed with a cotton swab and the cells that migrated through the membrane to the lower surface were stained with Giemsa's staining for 3–5 min at room temperature. The number of cells that migrated through the lower membrane of the inserts was counted under a light microscope. Each experiment was performed three times.

Statistical analysis.

Statistical analysis was performed using IBM SPSS Statistics 23.0 (IBM SPSS, Armonk, NY, USA). Statistics of continuous data were performed using AVOVA or Kruskal-Wallis test; correlation analyses were performed using Pearson correlation analysis. At least three independent experiments for each

group were conducted, and differences between groups were assessed by variance analysis and Student's t-test. P-value < 0.05 was considered to indicate a statistically significant result.

Results

FPR2 and RhoA were high expressed and correlated in EOC cell lines.

First, we used RT-qPCR to examine the expression of FPR2 and RhoA in five EOC cell lines (Caov-3, SKOV3, A2780, HO-8910, OVCAR-3) and one normal ovarian epithelial cell line (Hosepic). We found that FPR2 and RhoA were both significantly high expressed in EOCs, compared with Hosepic cells. Caov-3 cell line showed the highest expression, compared with other EOC cell lines. Subsequently, we analyzed the correlation between FPR2 and RhoA by Pearson correlation analysis, the results showed that FPR2 expression was positively correlated with RhoA in mRNA expression level. (Fig. 1-A,B,C)

RhoA expression was upregulated in OVCAR3 $^{-FPR2+}$ cells and downregulated in Caov3$^{-shFPR2}$ cells

To futher study the role of FPR2 in RhoA expression, we established FPR2 overexpressed OVCAR3 cell line (OVCAR3^{-FPR2+}) and FPR2 knockdown Caov3 cell line (Caov3$^{-shFPR2}$), as well as their control cell lines (OVCAR3^{-NC}, Caov3^{-NC}). We treated each EOCs with MMK-1(Potent and selective FPR2 agonist) for 24 h. Then RT-qPCR and Western blot were performed to detected FPR2 and RhoA expression. RT-qPCR results showed that FPR2mRNA was remarkably increased in the OVCAR3^{-FPR2+} cell lines and decreased in the Caov3$^{-shFPR2}$ cell lines compared with their respective control group. After stimulated by MMK-1, FPR2mRNA was also increased in OVCAR3^{-FPR2+} cell lines compared with MMK1-treated OVCAR3^{-NC}; however no statistical significance was shown between MMK1-OVCAR3^{-FPR2+} cells and OVCAR3^{-FPR2+} cells (Fig. 2-A). RhoA expression was upregulated in OVCAR3^{-FPR2+} cell lines and downregulated in Caov3$^{-shFPR2}$ cell lines compared with their respective control group, and significantly increased after stimulated by MMK-1 in OVCAR3^{-FPR2+} cell lines compared with MMK1-stimulated OVCAR3^{-NC} cells (Fig. 2-B). Western-blot results also showed that FPR2 protien was remarkably increased in the OVCAR3^{-FPR2+} cell lines and decreased in the Caov3$^{-shFPR2}$ cell lines compared with their respective control group, either after stimulated by MMK-1 or without treatment (Fig. 2-C). RhoA expression was also prominently decreased in Caov3$^{-shFPR2}$ cell lines with MMK-1 stimulated or not. In OVCAR3^{-FPR2+} cells, RhoA was highly expressed compared with its control group after stimulated by MMK-1 (Fig. 2-D).

FPR2 and RhoA inhibitor (C3 transferase) had an adverse role in the motility of ovarian cancer cells.

Wound healing and Transwell assays were performed to clarify the migrational ability of EOCs. Wound healing assay showed the average migration rate had no significant difference in each group of OVCAR3 cells lines (Fig. 3A). Transwell assay revealed that the number of EOCs that penetrated the Matrigel was significantly increased in OVCAR3^{-FPR2+} group when compared to the OVCAR3^{-NC} group; however, It showed no significant difference among the OVCAR3 cells lines that treated with C3 transferase or not (Fig. 3B). Wound healing assay revealed that the migration rate was significantly decreased after treated

with C3 transferase both in Caov3^{-NC} cell lines and Caov3$^{-shFPR2}$ cell lines (Fig. 3C). The number of transmembrane cells were decreased in Caov3$^{-shFPR2}$ cell lines than in the control group, but showed no significant difference. After treated with C3 transferase, the number of transmembrane cells was evidently decreased in C3 + Caov3^{-NC} cell lines than that in the Caov3^{-NC} cell lines, while C3 transferase had non-effect in FPR2 knockdown Caov3 cell lines (Fig. 3D).

The role of FPR2 stimulated Th2 cytokines secretion in EOC cells

The expression of TGF-β1, IL-4, IL-10, IL-12 and TNFα in EOC cell supernatant collected from each group was examined by ELISA kits. The results showed that IL-4 and IL-10 expression was higher in the supernatant of OVCAR3^{-FPR2+} cell lines than in the control group, and IL-10 secretion of OVCAR3 cell lines was distinctly reduced after treated with C3 transferase. TNFα expression was significantly lower in supernatant of OVCAR3^{-FPR2+} cell lines than its control group, and C3 transferase would increase TNFα secretion of OVCAR3^{-FPR2+} cell lines. While IL-4, IL-10 and TGF-β1 expression was significantly lower in Caov3$^{-shFPR2}$ cell supernatant than its control group, and C3 transferase would diminish the secretion of IL-4, IL-10 and TGF-β1 in Caov3 cell lines. TNFα expression was evidently increased in supernatant of Caov3$^{-shFPR2}$ cell lines than its control group, and C3 transferase would decrease TNFα secretion of Caov3 cell lines. (Fig. 4-A,B).

The Effects Of Fpr2 On Macrophage Polarization

As reported, polarization of M2 macrophages are induced by Th2 cytokines. We treated macrophages with cell supernatant of each cell lines that we mentioned above. Subsequently, we detected M1 (iNOS) and M2 (CD206) markers on macrophages by Flow cytometry. The results showed that iNOS expression was significantly upregulated in Caov3$^{-shFPR2}$ group and was downregulated in OVCAR3^{-FPR2+} group; conversely, CD206 expression was significantly upregulated in OVCAR3^{-FPR2+} group and was downregulated in Caov3$^{-shFPR2}$ group. (Fig. 5-A,B)

FPR2 and RhoA expression on M1 and M2 macrophages.

We also examined the expressions of FPR2 and RhoA on macrophages of different phenotypes. THP-1 cells was induced to M0 macrophages by PMA, then M0 macrophages were induced to M1 or M2 macrophages by LPS + IFN-γ or IL-4, respectively. Flow cytometry was performed to detect the biomarker of different phenotype of macrophages, and RT-qPCR and western-blot were used to detect the expressions of FPR2 and RhoA in THP-1 cell line, M1 and M2 macrophages, respectively. The results showed that FPR2mRNA expression increased significantly in M1 and M2 macrophages compared with THP-1 cells; and M2 macrophages showed higher expression than M1 macrophages (Fig. 6-B). RhoAmRNA was significantly upregulated in M2 macrophages compared with THP-1 cells and M1 macrophages, and no siginicant difference was shown between THP-1 cells and MI macrophages (Fig. 6-B). Western-blot results showed that FPR2 protein was significantly upregulated in M1 and M2

macrophages compared to the THP-1 cell line; RhoA protein expression was significantly decreased in M1 macrophages compared with THP-1 cells, and increased in M2 macrophages than in THP-1 cell line and in M1 macrophages. (Fig. 6-C)

Discussion

In this study, we first demostrated the interactions between FPR2 and RhoA in epithelial ovarian cancer cells. We found that RhoA expression in EOCs was increased with the overexpression of FPR2, whereas with the knock down of FPR2 on EOCs, RhoA expression was decreased correspondingly. FPR2 is one of the members of Formyl peptide receptors family, which belongs to G-protein coupled receptors family (GPCRs)[14]. As we know, GPCRs play the key role in regulating the sensitivity to chemokines and the signaling of other GPCRs involved in migration, migration requires the coordinated activation of hundreds of proteins in distinct compartments of the cell[15]. Rho GTPases family is part of the Ras superfamily, and Rho GTPases are highly conserved and found in nearly all leukaryotes, which act as molecular switches and cycling between an active GTP bound form and an inactive GDP bound form. GPCRs are activated through binding to intracytoplasmic Rho proteins and stimulate the downstream signaling transduction. They contribute to several cellular processes and pathological processes including, cell morphogenesis, cell polarity or migration, cancer progression, inflammation and wound repair[16, 17].

It is suggested that FPR2 mediated the activation of Rho proteins and affected its downstream signaling transduction. According to the study by Faour WH et, al, FPR2 agonist fMLP activated ERK1/2 and Akt pathways through specific activation of FPR2/ROS/RhoA-GTPase pathway and stimulated H_2O_2 release by monocytes[6]. In bone marrow PMNs, RhoA/ROCK pathway was activated in the respiratory burst via mFPR1 and mFPR2, RhoA was consodered to be one of the regulatory and signal transduction components in the respiratory burst through FPRs and both mFPR1 and mFPR2 binding with a ligand trigger the activation of RhoA and regulated NADPH-oxidase activity[7]. In this study, we preliminarily clarified the positive correlation of FPR2 and RhoA in ovarian cancer cells, and RhoA inhibitor could reverse the migration aibility of EOCs, which was promoted by ectopic expression of FPR2. Meanwhile, futher studies revealed that secretion of Th2 cytokines was increased by ovarian cancer cells with high FPR2 expression, which induces the M2-like macrophages polarization, and C3 transferase would partly inhibite the polarozation of M2 macrophages. .

Macrophages play an indispensable role in defensing against microbial infections and tumor cells. Tumor associated macrophages (TAMs) constitute a significant part of the tumor-infiltrating immune cells, predominantly resemble with M2-like polarized macrophages and produce a high amount of anti-inflammatory factors that contribute to the development of tumor[18]. It is reported that interferon regulatory factor 5 (IRF5) and IRF4 act as two key transcriptional regulators in regulating the polarization of macrophages to the M1 and M2 phenotypes, respectively. IRF5 expression drives M1 macrophage polarization by directly inducing the expression of pro-inflammatory cytokines and repressing the transcription of anti-inflammatory cytokines such as IL-10. In contrast, IRF4 has been shown to be a crucial mediator of M2 macrophage polarization[19]. Moreover, The status of macrophage polarization

can be also polarized or reversed by cellular signaling pathways. JNK signaling pathway activation would induce the macrophages polarized to M2 phenotype, while activation of Notch signaling pathway would promote M1 macrophages polarization[20].

IL-10 is an anti-inflammatory cytokine that plays a critical role in the control of immune responses both in inflammation and cancer. It is a cytokine whose levels have been shown to be elevated in the tumor microenvironment and blood of tumor bearing mice as well as ovarian cancer patients. IL-10 also acted as a critical regulator of PD-1/PD-L1 axis for immunosuppression in ovarian tumor microenvironment[21]. Studies showed that IL-10 regulated metabolic processes of glycolysis and oxidative phosphorylation in macrophages and inhibited the switch to the metabolic program induced by inflammatory stimuli in macrophages[22]. In lung cancer, IL-10 was considered to suppresse the inflammatory macrophage-Th17 cell axis that is critical to tumorigenesis, and may be used to prevent against lung cancer in high-risk patients[23]. IL4/IL4R signaling act as pro-metastatic phenotypes in epithelial cancer cells including enhanced migration, invasion, survival, and proliferation as well. Studies revealed that IL-4 antibody neutralization enhances anti-tumor immunity and delays tumor progression. IL-4 blockade also alters inflammation in the tumor microenvironment, reducing the generation of both immunosuppressive M2 macrophages and myeloid-derived suppressor cells, and enhancing tumor-specific cytotoxic T lymphocytes[24]. In our study, we found that FPR2 played an auxo-action on secretion of IL-10 and IL-4 in ovarian cancer cells, and induction of M2 macrophages polarization. Currently, the effect of FPR2 on macrophage polarization is unclear yet. Studies showed that FPR2 plays a critical role in anti-tumor host immunity by limiting macrophage recruitment into the tumors and sustaining macrophages in an M1 phenotype[13]. In Mice, FRP2 deletion reduces tissue and systemic inflammation by inhibiting macrophage infiltration and M1 polarization[25]. FPR2 was also considered as a mediator that leading to macrophage skewing in a model of skeletal muscle injury and repair, which accelerating muscle regeneration[26]. The results in this study demonstrated that FPR2 participated in M2 macrophages differentiation. It has been generally accepted that M1 macrophages are responsible for stimulation of immune system and inflammation while M2 macrophages play a role in cancer and tissue repair. Our results showed that FPR2 was expressed differentially between M1 and M2 macrophages, which may indicate that FPR2 might play a role in both M1 and M2 macrophage phenotype and function, while RhoA might be inclined to performe an M2 macrophage phenotype, which still needed a further study.

Conclusion

In this study, RhoA expression was significantly increased in EOCs along with the overexpression of FPR2, which showed a positive correlation by Pearson correlation analysis. Moreover, FPR2 promoted the migrational ability of EOCs, and RhoA inhibitor played an adverse effect in EOC migration. In addition, FPR2 stimulated the secretion of Th2 cytokines by EOCs, which induced macrophages differentiate to M2 phenotype, while RhoA inhibitor stimulate the secretion of Th1 cytokines and induce macrophages differentiate to M1 phenotype. Therefore, we suggested that FPR2 stimulated M2 macrophage polarization and promote invasion and metastasis of ovarian cancer cells through RhoA. Futher study of

the mechanisms on FPR2-regulated macrophages polarization and its role in ovarian cancer progression was need.

Declarations

Declarations

Acknowledgements

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Authors' contributions

Shi Kun designed the study and approved the final version of the manuscript; Xie Xiaohui executed cell cultures, analyzed datas and drafted the manuscript; He Juan executed the Wound healing assays, Transwell assays and Flow cytometry; Liu Yaqiong and Chen Weiwei executed the qPCR,Western Blot and ELISA experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Matulonis UA, Sood AK, Fallowfield L, Howitt BE, Sehouli J, Karlan BY. Ovarian cancer. Nat Rev Dis Primers. 2016;2:16061.
2. Bast RJ, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer. 2009;9:415-428.

3. Richard D Ye, François Boulay, Ji Ming Wang, Claes Dahlgren, Philip M Murphy. International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. Pharmacol Rev. 2009;61:119-161.
4. Li Y, Ye D. Molecular biology for formyl peptide receptors in human diseases. J Mol Med (Berl). 2013;91:781-789.
5. Xie X, Yang M, Ding Y, Yu L, Chen J. Formyl peptide receptor 2 expression predicts poor prognosis and promotes invasion and metastasis in epithelial ovarian cancer. Oncol Rep. 2017;38:3297-3308.
6. Faour WH, Fayyad-Kazan H, ElZN. fMLP-dependent activation of Akt and ERK1/2 through ROS/Rho A pathways is mediated through restricted activation of the FPRL1 (FPR2) receptor. Inflamm Res. 2018;67:711-722.
7. Filina JV, Gabdoulkhakova AG, Safronova VG. RhoA/ROCK downregulates FPR2-mediated NADPH oxidase activation in mouse bone marrow granulocytes. Cell Signal. 2014;26:2138-2146.
8. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19:1423-1437.
9. Mills CD, Ley K. M1 and M2 macrophages: the chicken and the egg of immunity. J Innate Immun. 2014;6:716-726.
10. Mingzhu Yin, Xia Li, Shu Tan, Huanjiao Jenny Zhou, Wang Min. Tumor-associated macrophages drive spheroid formation during early transcoelomic metastasis of ovarian cancer. J Clin Invest. 2016;126:4157-4173.
11. Xia Yuan, Jing Zhang, Dan Li, Ye Mao, Fei Mo, Wei Du, et al. Prognostic significance of tumor-associated macrophages in ovarian cancer: A meta-analysis. Gynecol Oncol. 2017;147:181-187.
12. Y Li, L Cai, H Wang, P Wu, W Gu, Y Chen, et al. Pleiotropic regulation of macrophage polarization and tumorigenesis by formyl peptide receptor-2. Oncogene. 2011;30:3887-3899.
13. Ying Liu, Keqiang Chen, Chunyan Wang, Wanghua Gong, Teizo Yoshimura, Mingyong Liu, et al. Cell surface receptor FPR2 promotes antitumor host defense by limiting M2 polarization of macrophages. Cancer Res. 2013;73:550-560.
14. Hilger D, Masureel M, Kobilka BK. Structure and dynamics of GPCR signaling complexes. Nat Struct Mol Biol. 2018;25:4-12.
15. Cleghorn WM, Bulus N, Kook S, Gurevich VV, Zent R, Gurevich EV. Non-visual arrestins regulate the focal adhesion formation via small GTPases RhoA and Rac1 independently of GPCRs. CELL SIGNAL 2018, 42: 259-269.
16. Haga RB, Ridley AJ. Rho GTPases: Regulation and roles in cancer cell biology. Small GTPases. 2016;7:207-221.
17. Hodge RG, Ridley AJ. Regulating Rho GTPases and their regulators. Nat Rev Mol Cell Biol. 2016;17:496-510.
18. Antonio Sica, Paola Larghi, Alessandra Mancino, Luca Rubino, Chiara Porta, Maria Grazia Totaro, et al. Macrophage polarization in tumour progression. Semin Cancer Biol. 2008;18:349-355.

19. Thomas Krausgruber, Katrina Blazek, Tim Smallie, Saba Alzabin, Helen Lockstone, Natasha Sahgal, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. Nat Immunol. 2011;12:231-238.
20. Cheng H, Wang Z, Fu L, Xu T. Macrophage Polarization in the Development and Progression of Ovarian Cancers: An Overview. FRONT ONCOL 2019, 9: 421
21. Lamichhane P, Karyampudi L, Shreeder B, Krempski J, Bahr D, Daum J, et al. IL10 Release upon PD-1 Blockade Sustains Immunosuppression in Ovarian Cancer. CANCER RES 2017, 77(23): 6667-6678.
22. Ip W, Hoshi N, Shouval DS, Snapper S, Medzhitov R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science. 2017;356:513-519.
23. Li Q, Anderson CD, Egilmez NK. Inhaled IL-10 Suppresses Lung Tumorigenesis via Abrogation of Inflammatory Macrophage-Th17 Cell Axis. J Immunol. 2018;201:2842-2850.
24. Ito SE, Shirota H, Kasahara Y, Saijo K, Ishioka C. IL-4 blockade alters the tumor microenvironment and augments the response to cancer immunotherapy in a mouse model. Cancer Immunol Immunother 2017, 66(11): 1485-1496.
25. Xiaofang Chen, Shu Zhuo, Tengfei Zhu, Pengle Yao, Mengmei Yang, Hong Mei, et al. Fpr2 Deficiency Alleviates Diet-Induced Insulin Resistance Through Reducing Body Weight Gain and Inhibiting Inflammation Mediated by Macrophage Chemotaxis and M1 Polarization. Diabetes. 2019;68:1130-1142.
26. Machado MG, Tavares LP, Souza G, Queiroz-Junior CM, Ascencao FR, Lopes ME, et al. The Annexin A1/FPR2 pathway controls the inflammatory response and bacterial dissemination in experimental pneumococcal pneumonia. FASEB J 2020, 34(2): 2749-2764.

Figures

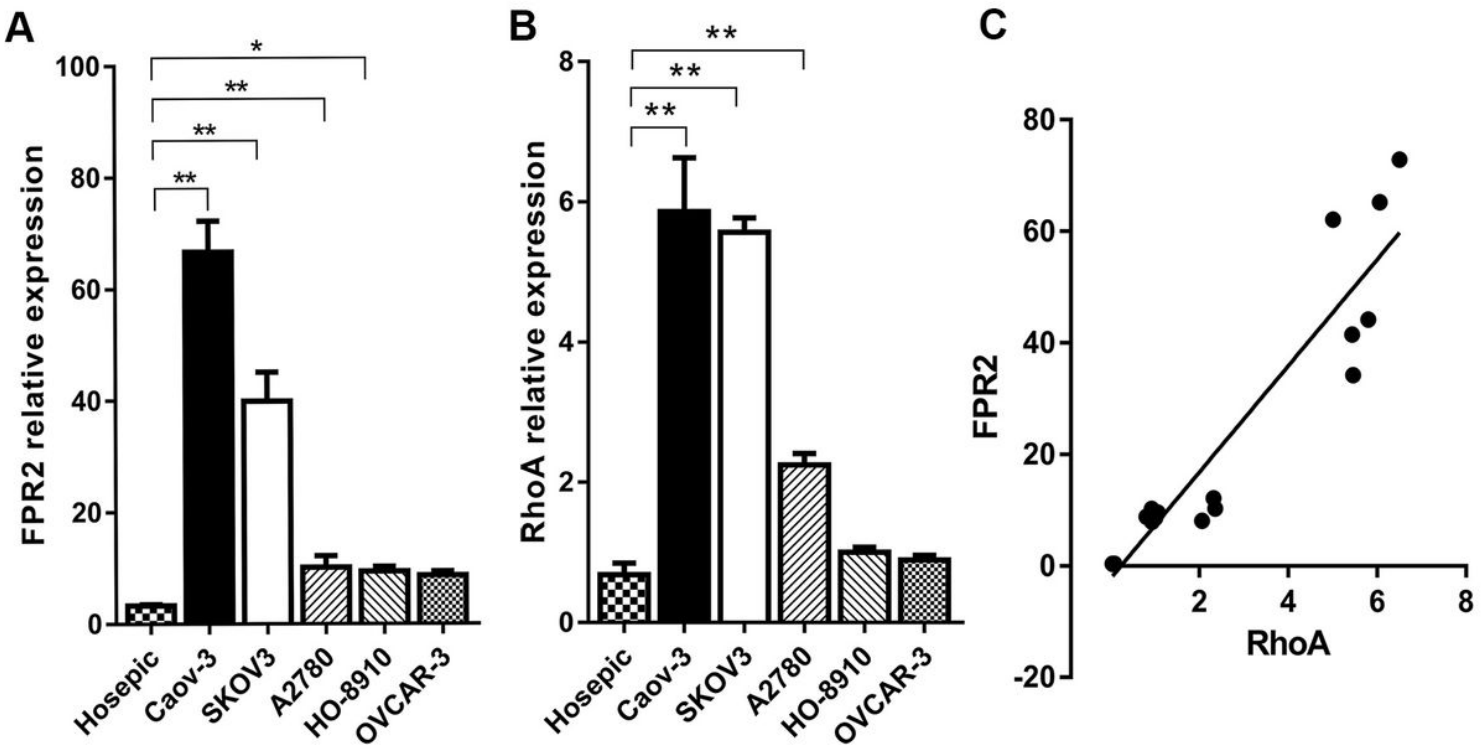

Figure 1

(A) FPR2mRNA was upregulated in EOCs than in normal ovary cell line(*P□0.05), (**P□0.01). (B) RhoAmRNA was upregulated in EOCs than in normal ovary cell line(**P□0.01). (C) Positive correlation between FPR2 and RhoA, Pearson correlation coefficient was 0.936.

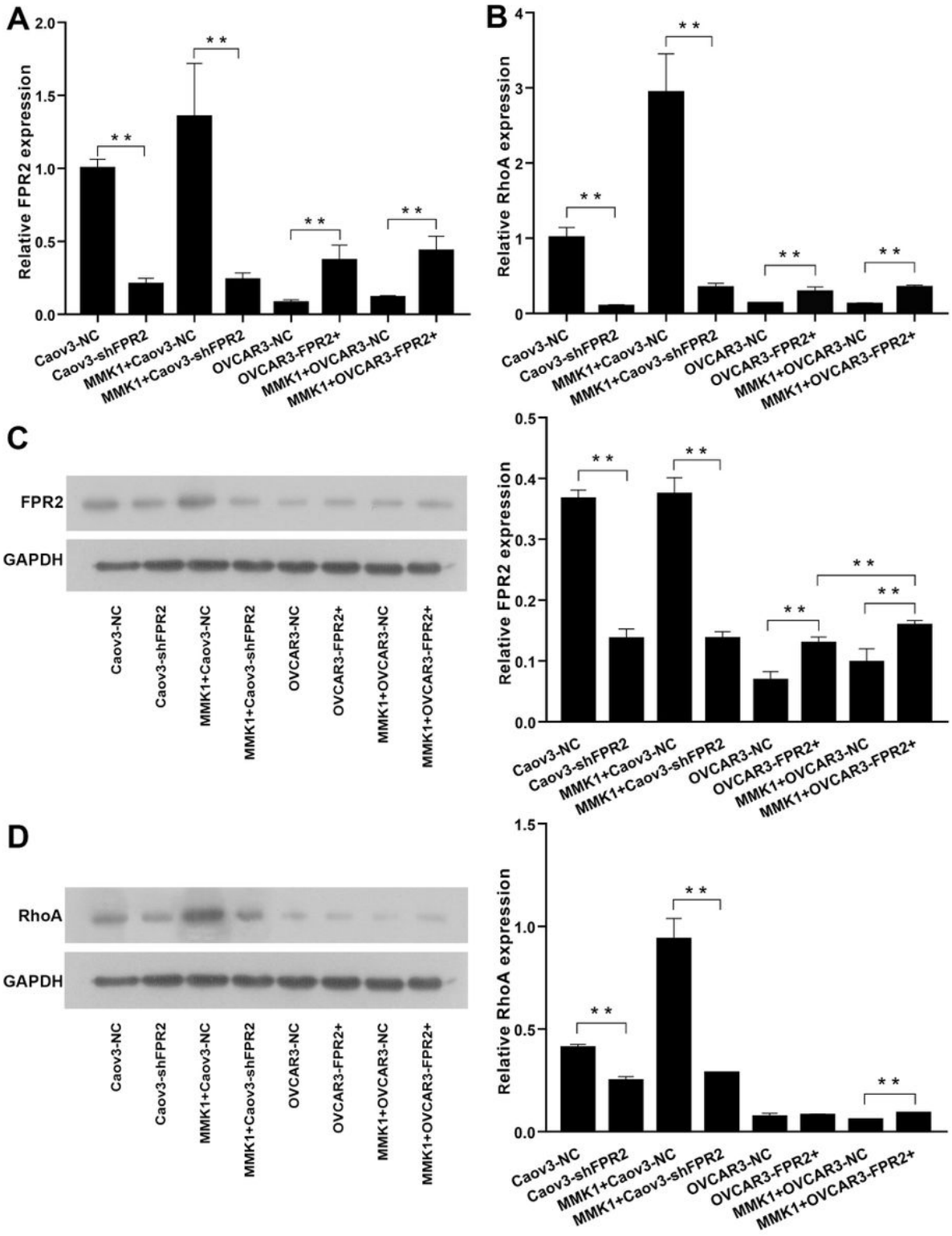

Figure 2

(A,B) RT-qPCR showed that FPR2mRNA and RhoAmRNA were significantly increased in OVCAR3-FPR2+ cell lines and decreased in Caov3-shFPR2 cell lines whether MMK-1 was treated or not (**P□0.01). (C) Western-blot showed that FPR2 protein was significantly increased in OVCAR3-FPR2+ cell lines and decreased in Caov3-shFPR2 cell lines, and was remarkably increased after stimulated by MMK-1 in OVCAR3-FPR2+ cell lines (**P□0.01). (D) Western-blot showed that RhoA protein was significantly

decreased in Caov3-shFPR2 cell lines, while showed no statistical significance in OVCAR3-FPR2+ group and the control group; whereas RhoA was significantly increased in OVCAR3-FPR2+ cell lines when stimulated by MMK-1 (**P□0.01).

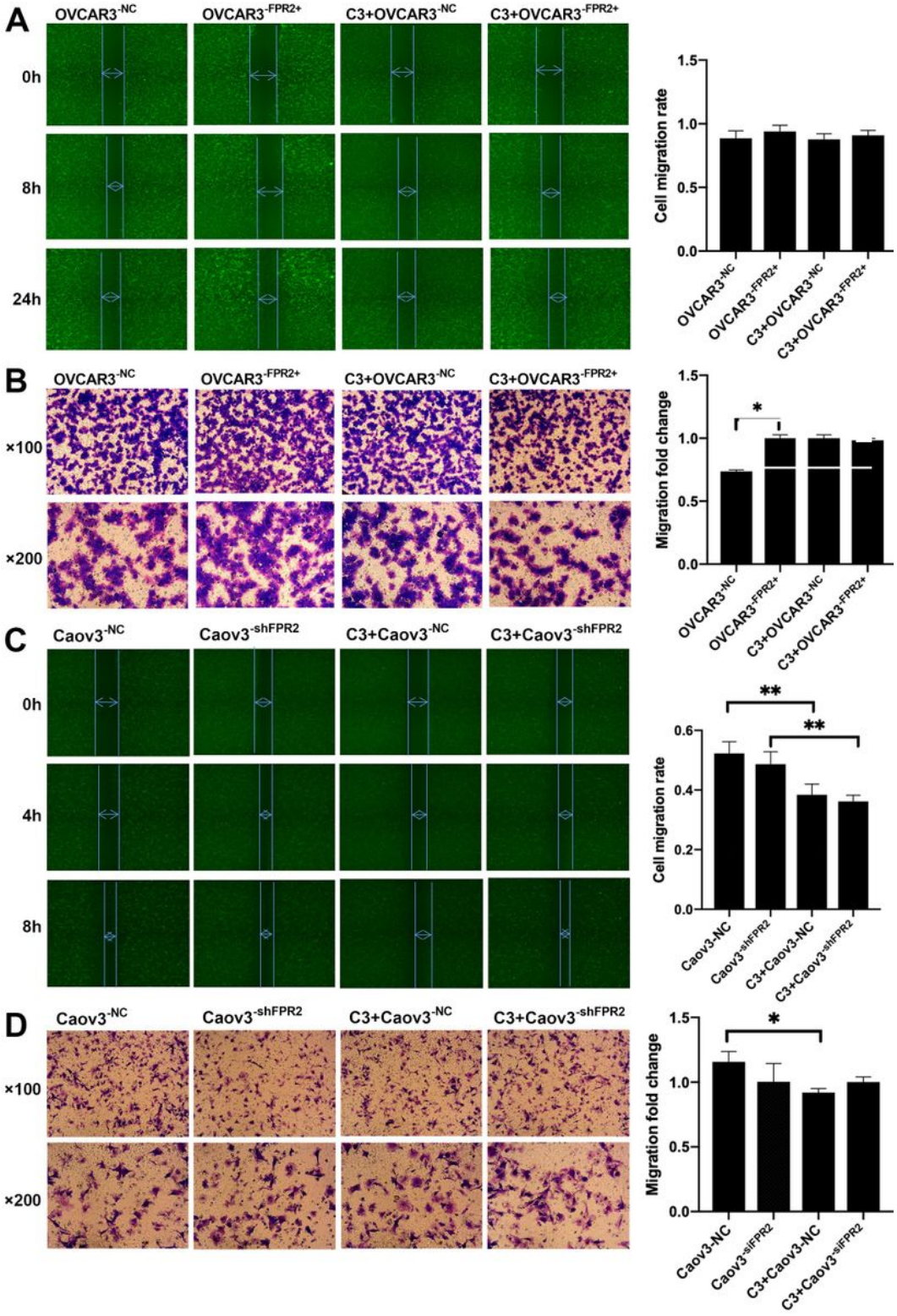

Figure 3

(A) The wound healing assay showed no significant difference of cell migration rate in each group. (B) The transwell assay showed that the number of transmembrane cells was evidently increased in

OVCAR3-FPR2+ cell lines than that in OVCAR3-NC cell lines (*P<0.05). (C) The wound healing assay showed cell migration rate was significantly decreased in C3+Caov3-NC cell lines than in Caov3-NC cell lines (**P<0.01). Compared to Caov3-shFPR2 cell lines, the cell migration rate was also significantly deceased in C3+Caov3-shFPR2 cell lines (**P<0.01). (D) The transwell assay showed that the number of transmembrane cells was significantly decreased in C3+Caov3-shFPR2 cell lines than that in Caov3-NC cell lines (*P<0.05).

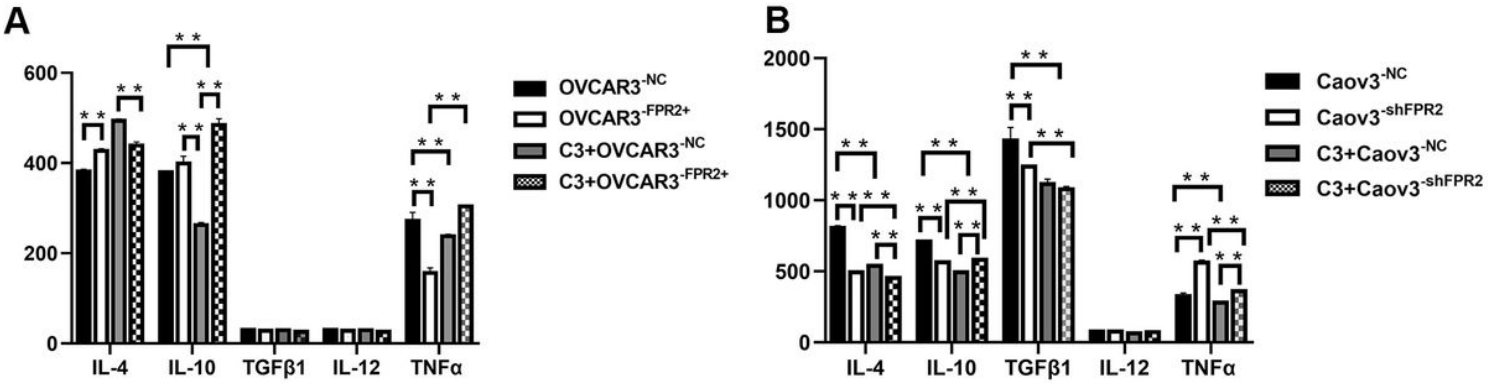

Figure 4

(A) IL-4 expression were significantly increased in OVCAR3-FPR2+ cell lines supernatant than in its control group (**P<0.01), and IL-10 expression was significantly decreased in C3+OVCAR3-NC cell lines than OVCAR3-NC cell lines(**P <0.01). TNFα expression was significantly decreased in OVCAR3-FPR2+ cell lines supernatant than in its control group (**P<0.01), and it was also significantly decreased in OVCAR3 cell lines when treated with C3 tranferase (**P<0.01). TGF-β1 and IL-12 expression showed no significant difference in each group (P >0.05). (B) IL-4, IL-10 and TGF-β1 expression were all significantly decreased in Caov3-shFPR2 cell lines supernatant than in the control group (**P<0.01), and C3 tranferase could evidently decrease secretion of IL-4, IL-10 and TGF-β1 in Caov3 cell lines (**P<0.01). TNFα expression was significantly increased in supernatant of Caov3-shFPR2 cell lines than its control group (**P<0.01), and C3 transferase would decrease TNFα secretion of Caov3 cell lines (**P<0.01). IL-12 expression had no significant difference between each group (P>0.05).

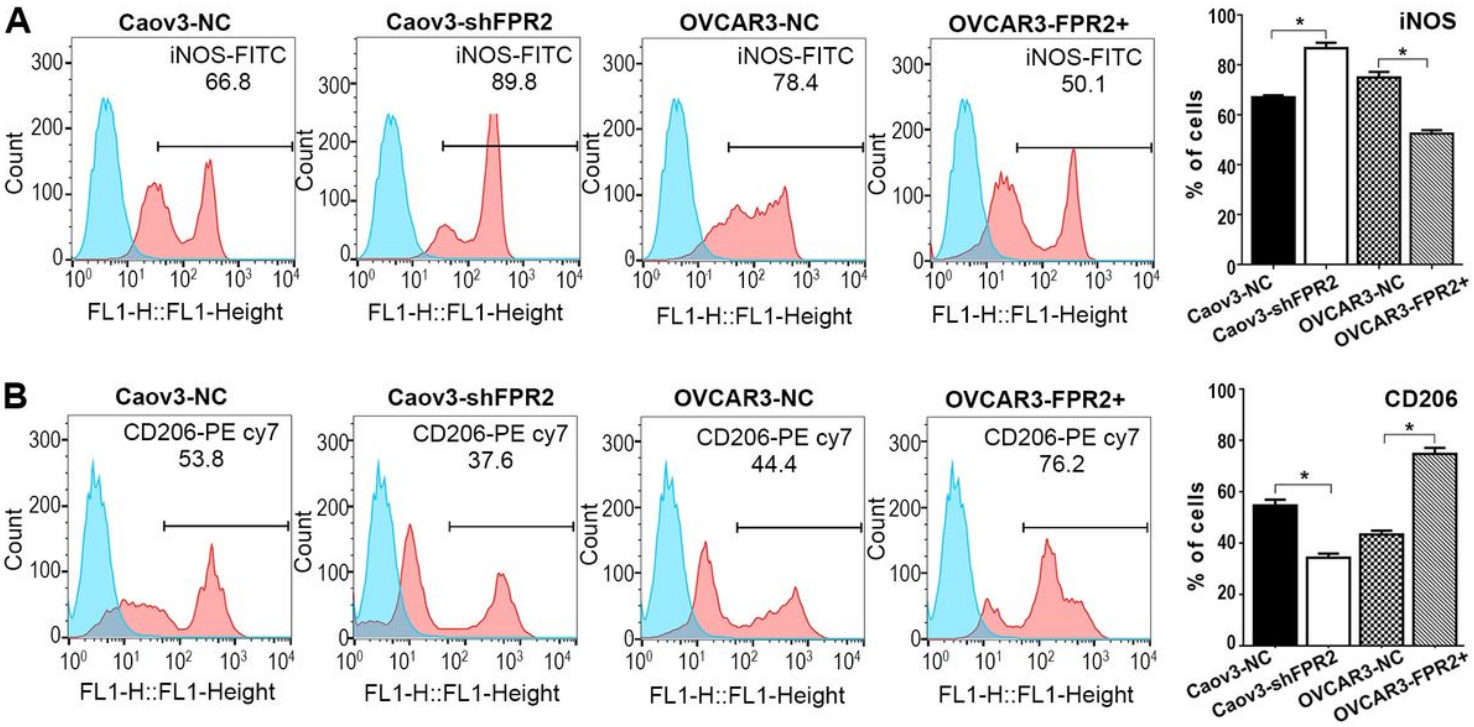

Figure 5

(A) The Flow cytometry results showed that iNOS expression was significantly increased in Caov3-shFPR2 group than that in the control group, which was significantly decreased in OVCAR3-FPR2+ group than that in the control group (*P□0.05) ; (B) CD206 expression was significantly increased in OVCAR3-FPR2+ group than that in the control group, whereas it was significantly decreased in Caov3-shFPR2 group than that in the control group (*P□0.05).

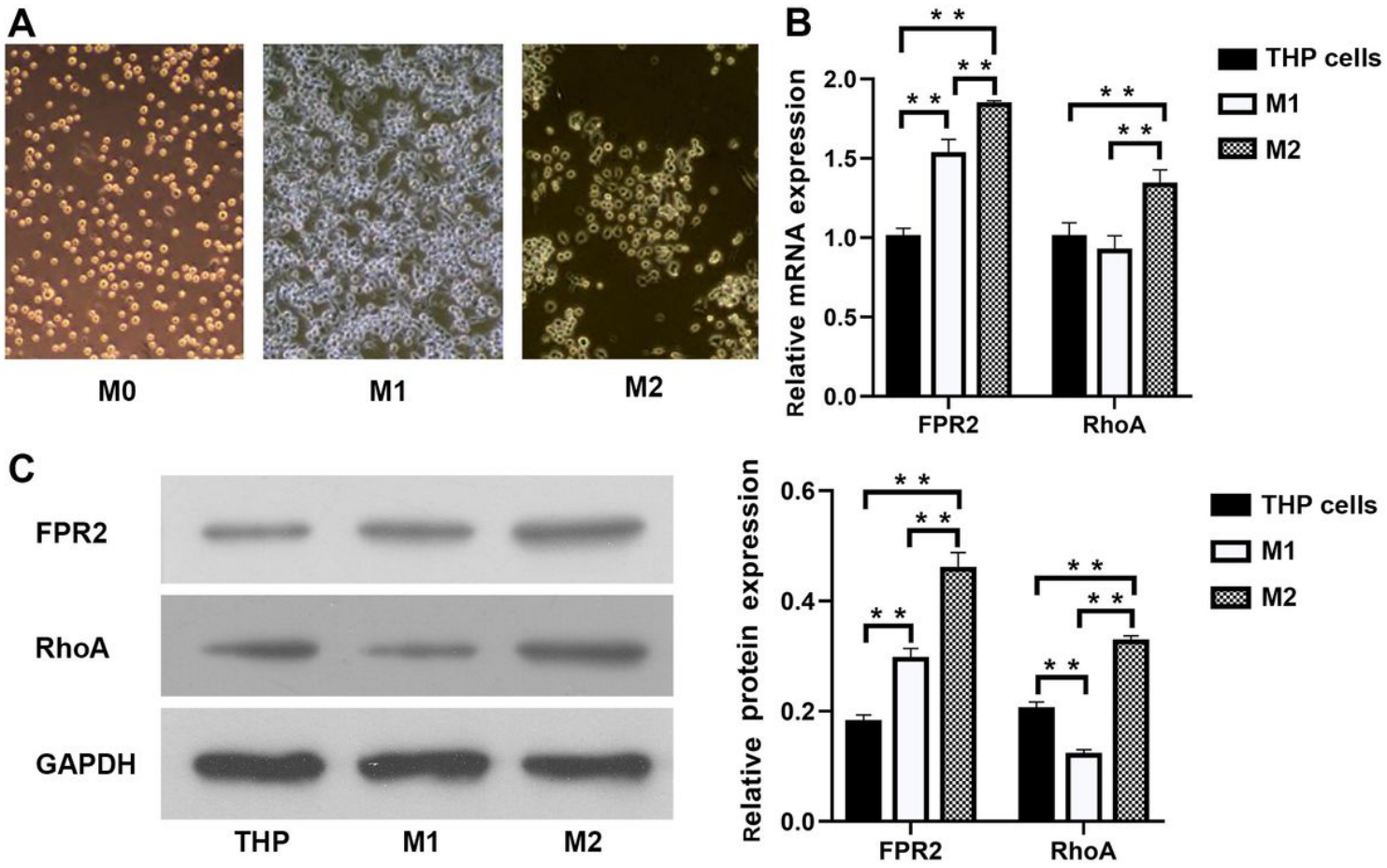

Figure 6

(A) M0, M1 and M2 macrophages. (B) RT-qPCR results showed that FPR2mRNA expression and RhoAmRNA expression were both significantly increased in M2 macrophages when compared to THP-1 cells and M1 macrophages (**P□0.01). (C) Western-blot results showed that relative FPR2 and RhoA protein expression were both evidently upregulated in M2 macrophages than in the THP-1 cells and M1 macrophages(**P□0.01), and expression of RhoA protein was significantly dicreased in M1 macrophages than in the THP-1 cells (**P□0.01).