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Based on the MAPK/NF-κB Pathway, Ezrin Regulates Macrophage M1/M2 Polarization in the Progression of Prostate Cancer and its Mechanism

Tengfei Zhang[1], Xu Lei[1], Tao Jiang[1], Zhixuan Deng[1], Botao Dong[1], Ning Yang[1,*]

[1]The Second Affiliated Hospital, Department of Urology, Hengyang Medical School, University of South China, 421001 Hengyang, Hunan, China
*Correspondence: yangning@usc.edu.cn (Ning Yang)
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Background: Ezrin exhibits aberrant expression across diverse cancer types and significantly contributes to cancer initiation and advancement. However, the precise mechanism by which Ezrin regulates M1/M2 macrophage polarization in prostate cancer (PCa) remains unclear. This study aimed to elucidate the role and mechanism of Ezrin in regulating M1/M2 macrophage polarization in PCa.

Methods: Initially, Ezrin levels were evaluated in transfected cells using real-time quantitative PCR (RT-qPCR), followed by Western blotting (WB) to evaluate Ezrin expression levels in PCa PC-3 cells overexpressing Ezrin and treated with the Mitogen-Activated Protein Kinase (MAPK) and nuclear factor kappa B (NF-κB) pathway inhibitors PD0325901 and BAY11-7082. Colony formation and Transwell assays were used to assess cell proliferation, migration, and invasion abilities. Additionally, its effects on levels of epithelial-mesenchymal transition (EMT)-associated markers (E-cadherin, ZO-1, Vimentin, Snail Slug, β-Catenin) and pathway-associated proteins were examined. Subsequently, transfected PC-3 cells were co-cultured with macrophages, and the expression of Cluster of Differentiation 206 (CD206) and CD86 in macrophages was assessed using flow cytometry and RT-qPCR. The expressions of M1/M2 markers (tumor necrosis factor (TNF)-α, interleukin (IL)-6, inducible nitric oxide synthase (iNOS), IL-10, Arg1) in macrophages were also detected by RT-qPCR. Finally, the impact of PD0325901 and BAY11-7082 on macrophage polarization after Ezrin overexpression was investigated.

Results: Ezrin expression decreased in the si-Ezrin group and increased in the oe-Ezrin group compared to the control group ($p < 0.05$). Overexpression of Ezrin heightened Ezrin expression, increased proliferative, migratory, and invasive potential of PC-3 cells, enhanced EMT transition ability, and upregulated pathway-related proteins compared to the control group ($p < 0.05$). However, expression of Ezrin decreased in the oe-Ezrin+PD0325901 group and oe-Ezrin+BAY11-7082 group compared to Ezrin overexpression alone, inhibiting the growth and migration capabilities of PC-3 cells, reducing the expression of EMT markers, and suppressing the activation of the MAPK/NF-κB pathway ($p < 0.05$). Compared to the control group, cells in the si-Ezrin group exhibited an increase in CD86, TNF-α, IL-6, and iNOS, meanwhile, those in the oe-Ezrin group displayed a rise in CD206, IL-10, and Arg1 ($p < 0.05$). Moreover, the addition of pathway inhibitors PD0325901 and BAY11-7082 resulted in reduced CD206, IL-10, and Arg1 expression, while enhancing CD86, TNF-α, IL-6, and iNOS expression compared to the oe-Ezrin group ($p < 0.05$).

Conclusion: Ezrin facilitates the proliferation and metastasis of PCa cells by activating the MAPK/NF-κB pathway. Additionally, Ezrin induces M2 polarization of macrophages, exacerbating the progression of PCa.

Keywords: Ezrin; the MAPK/NF-κB pathway; prostate cancer; growth and metastasis; EMT; macrophage polarization

Introduction

Prostate cancer (PCa) progression is a multifactorial process influenced by genetic, environmental, and lifestyle factors. Its incidence rises with age and exhibits notable regional variations. The incidence is higher in regions such as North America, Northern Europe, and Australia, while lower in Asian regions [1]. PCa development initiates with the malignant transformation of a single normal prostate cell. These transformed cells proliferate continuously, leading to tumor formation [2]. The regulation of cancer cell proliferation involves various biological and molecular factors, including cell cycle regulatory proteins, growth factors, and activation of signaling pathways. Dysregulated proliferation can result in tumor growth and spread [3]. Cancer cell migration is a pivotal step in PCa progression, encompassing cellular morphological alterations, adhesion, de-adhesion, and re-adhesion processes. Subsequently, invasion follows migration involving cancer cells breaching the basement membrane and tissue barriers, infiltrating adjacent healthy tissue, and establishing secondary tumor foci in distant sites [4].

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Ezrin is a member of the Ezrin-Radixin-Moesin (ERM) group of proteins, significantly contributing to various biological processes, including the maintenance of cell morphology, adhesion, migration, and signal transduction [5]. It interacts with tumor-related receptors on the cell membrane and cell adhesion proteins, facilitating the movement and infiltration of tumor cells, a process crucial for tumor dissemination and metastasis [6]. Chen *et al*. [7] reported that Ezrin overexpression enhances the migratory and invasive capabilities of PCa cells. Additionally, Xu *et al*. [8] and Li *et al*. [9] demonstrated that the Mitogen-Activated Protein Kinase (MAPK) and nuclear factor kappa B (NF-κB) signaling pathways in PCa cells contribute to the progression of epithelial-mesenchymal transition (EMT), thereby promoting enhanced invasion and metastasis of PCa cells. Epithelial-mesenchymal transition (EMT) significantly contributes to the malignant behavior of tumors by facilitating cancer cell invasion and metastasis [10].

EMT reduces intercellular adhesion among epithelial cells, enhancing their migratory and invasive capabilities, enabling cancer cells to breach the basement membrane and vasculature, and facilitating metastasis [11,12]. EMT also decreases the levels of cell adhesion molecules such as E-cadherin, weakening cell adhesion. Reduced expression of ZO-1 may disrupt the integrity of intercellular connections, promoting cell migration [13,14]. Elevated levels of Vimentin and Snail-Slug expression are often associated with cancer cell invasion and metastasis, serving as markers of the EMT process [15,16].

The polarization of macrophages entails phenotypic and functional alterations in response to various stimuli, leading to the differentiation into distinct subtypes, primarily M1 and M2 [17,18]. In PCa, macrophage polarization significantly influences the tumor microenvironment [19,20]. M1 macrophages secrete inflammatory mediators and exhibit anti-tumor effects by inducing tumor cell apoptosis and suppressing tumor progression. Conversely, M2 macrophages suppress immune cell activity, facilitating tumor evasion from immune surveillance [21,22]. Elevated Cluster of Differentiation 86 (CD86) expression in macrophages may elicit pro-inflammatory responses by releasing inflammatory mediators, triggering inflammation, attracting other immune cells, and exerting direct cytotoxic effects on tumor cells, thereby inhibiting tumor growth. This serves as a hallmark of M1 macrophages in the tumor microenvironment. Conversely, elevated CD206 expression in macrophages may contribute to anti-inflammatory responses and tissue repair by releasing anti-inflammatory factors, and it could also promote tumor progression, serving as a hallmark of M2 macrophages in the tumor microenvironment [23,24]. These alterations in surface markers reflect the polarization status of macrophages, which holds significant implications for understanding their functionality, interactions, and potential therapeutic strategies within the tumor microenvironment.

Table 1. Sequence of the transfection of si-*Ezrin* and oe-*Ezrin*.

Gene	Sequence (5′-3′)
si-*Ezrin*	F: GGAACUAUUGGAACUCUUUTT
	R: AAAGAGUUCCAAUAGUUCCTT
oe-*Ezrin*	F: ATGGAGGAGATCGGGGCCAG
	R: TCAGGGTGGGAGGGTCGGTG

In this study, we hypothesized that Ezrin could regulate the MAPK/NF-κB signaling pathway, promote epithelial-mesenchymal transition, instigate macrophage phenotypic shift from M1 to M2, and enhance the proliferation, migration, and invasion of PCa cells. This hypothesis is a foundation for developing personalized and precision cancer therapies, offering novel insights into identifying therapeutic targets to develop more effective clinical treatment strategies. However, it is imperative to acknowledge the limitations of the current study. While we observed the effects of Ezrin on regulating the MAPK/NF-κB pathway *in vitro*, the elucidation of its mechanisms of action in *in vivo* models remains incomplete. Future investigations should endeavor to validate these findings using animal models or clinical samples to gain a comprehensive understanding of the role of Ezrin in PCa progression.

Materials and Methods

Cell Culture

The mouse macrophage cell line RAW264.7 (CL-0190, Procell Life Science & Technology Co., Ltd., Wuhan, China) and the PCa cell line PC-3 (XY-XB-1383, Shanghai Hengya Biotechnology Co., Ltd., Shanghai, China) were cultured in RPMI1640 complete medium (11875119, Thermo Fisher Scientific Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, 10100147C; Thermo Fisher Scientific Inc., Waltham, MA, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (15140148; Thermo Fisher Scientific Inc., Waltham, MA, USA). Cells were maintained under controlled conditions with 5% CO_2 at 37 °C and passaged every 2–3 days. Upon reaching 80% confluence, PC-3 cells were subjected to Ezrin knockdown and overexpression treatments. For stable transfection of PC-3 cells with overexpressed Ezrin, MAPK/NF-κB inhibitors PD0325901 (10 nM, HY-10254, MedChem Express, Franklin Lake, NJ, USA) and BAY11-7082 (5 µM, 19542-67-7, MedChem Express, Franklin Lake, NJ, USA) were added to the culture medium. Subsequently, RAW264.7 cells were cultured for 24 hours in various conditions, including PBS, negative control (NC), si-Ezrin, oe-Ezrin-transfected, and medium from PC-3 cells treated with PD0325901 and BAY11-7082. All cells used in the experiment were subjected to STR identification and mycoplasma detection to confirm the absence of mycoplasma contamination. All procedures were conducted using aseptic techniques to prevent contamination of the cells.

Table 2. Primer sequence of the mRNA.

Genes	Forward primer	Reverse primer
Ezrin	GAGATCGCTGACGTGGATGC	TGGGTGCTTGTCTTCAGGGC
CD86	TGGGAAACACAGTCATCCAG	TGAGAGGAGAACAGGTGGAG
CD206	GCAACAGCATTTCCTGGAAC	CTGGAGAGACGCAGACAACA
TNF-α	CCCAGGGACCTCTCTCTAATC	TGGGAGTAGACAAGGTACAAC
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
iNOS	CCAAGGAGTCTCTGGAAAGTGG	GTTGTTGGCATGGTGAGTTAG
IL-10	GGACCTTGTTGCCTCAGCTTG	TCCACCTGCTCCACTGCCTTA
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

CD206, Cluster of Differentiation 206; *TNF-α*, tumor necrosis factor-α; *IL-6*, interleukin-6; *iNOS*, inducible nitric oxide synthase; *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

Cell Transfection

The Ezrin overexpression plasmid was cloned into the pSicoR lentiviral vector to establish Ezrin overexpression. 293T cells (CL-0005, Procell Life Science & Technology Co., Ltd., Wuhan, China) were employed to generate lentiviral particles. Subsequently, PC-3 cells were transduced with lentivirus and treated with puromycin for two weeks to establish the Ezrin overexpression cell line. The transfection efficiency was assessed using real-time quantitative PCR (RT-qPCR), and cells were harvested for further experiments.

The Ezrin sequence was cloned into the pcDNA3.1 vector to construct an overexpression plasmid. Cells were transfected using Lipofectamine® 3000 (L3000008, Thermo Fisher Scientific Inc., Waltham, MA, USA). Small interfering RNA (siRNA) targeting Ezrin and primers were obtained from Ibsbio (Shanghai, China), which also provided the negative control and other experimental conditions. The primer sequences are provided in Table 1.

Clone Formation Experiment

PC-3 cells were seeded in 6-well plates at a density of approximately 800 cells per well during the logarithmic growth phase. The cells were then incubated in a 37 °C culture chamber with 5% CO_2 for 10–15 days. Following the incubation, the culture medium was removed, and the cells were fixed using 4% paraformaldehyde (PFA) for 5 minutes. Subsequently, the cells were stained with crystal violet, and microscopic images were captured using a Optical microscope (N-SIM, Nikon, Tokyo, Japan). Colonies were quantified using Image J software (v1.8.0.112, NIH, Madison, WI, USA).

Transwell Assays

The invasive and migratory capabilities of PC-3 cells were assessed using Transwell assays with or without the presence of Matrigel (356234, BD Biosciences, Franklin Lake, NJ, USA). Cells were resuspended in a serum-free medium and then seeded at a density of 4×10^3 cells in the upper compartment of Transwell inserts. The lower chamber of the Transwell was filled with 600 μL of medium containing 10% FBS (CLS3395, Corning, Inc., Corning, NY, USA). Cell cultures were maintained for 24 hours at 37 °C in a 5% CO_2 environment.

Following the incubation, cells that had migrated to the lower chamber were fixed with 4% PFA (ml28498-5, Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) at room temperature for 10 minutes. Subsequently, cells were stained with Giemsa stain (ml28253-1, Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). Enumeration of migrated cells was conducted using a microscope (DP74; Olympus Corporation, Tokyo, Japan).

Flow Cytometry

Flow cytometric analysis was employed to investigate macrophage markers. Macrophages were detached using 0.25% EDTA-trypsin, washed with PBS, and resuspended in PBS. Subsequently, the cells were stained using either phycoerythrin (PE)-conjugated monoclonal antibodies (mAb) against F4/80 or fluorescein isothiocyanate (FITC)-conjugated mAb against CD86, and CD206, and incubated at room temperature for 30 minutes. Antibodies employed included F4/80-FITC (11-4801-81, Thermo Fisher Scientific Inc., Waltham, MA, USA), CD86-PE (MHCD8604, Thermo Fisher Scientific Inc., Waltham, MA, USA), and CD206-PE (12-2061-82, Thermo Fisher Scientific Inc., Waltham, MA, USA). Standard protocols for flow cytometry were followed, and antibody fluorescence was detected using the NovoCyte flow cytometer.

Western Blotting (WB) Assays

Cellular total proteins were extracted using RIPA lysis buffer (P0013B, Beyotime Institute of Biotechnology, Shanghai, China), and their concentrations were determined using the BCA protein assay kit (P0009, Beyotime, Shanghai, China). An equal amount of protein was loaded onto SDS-polyacrylamide gels and subsequently transferred onto nitrocellulose membranes (E0264, Millipore, Danvers, MA, USA). The membranes were then

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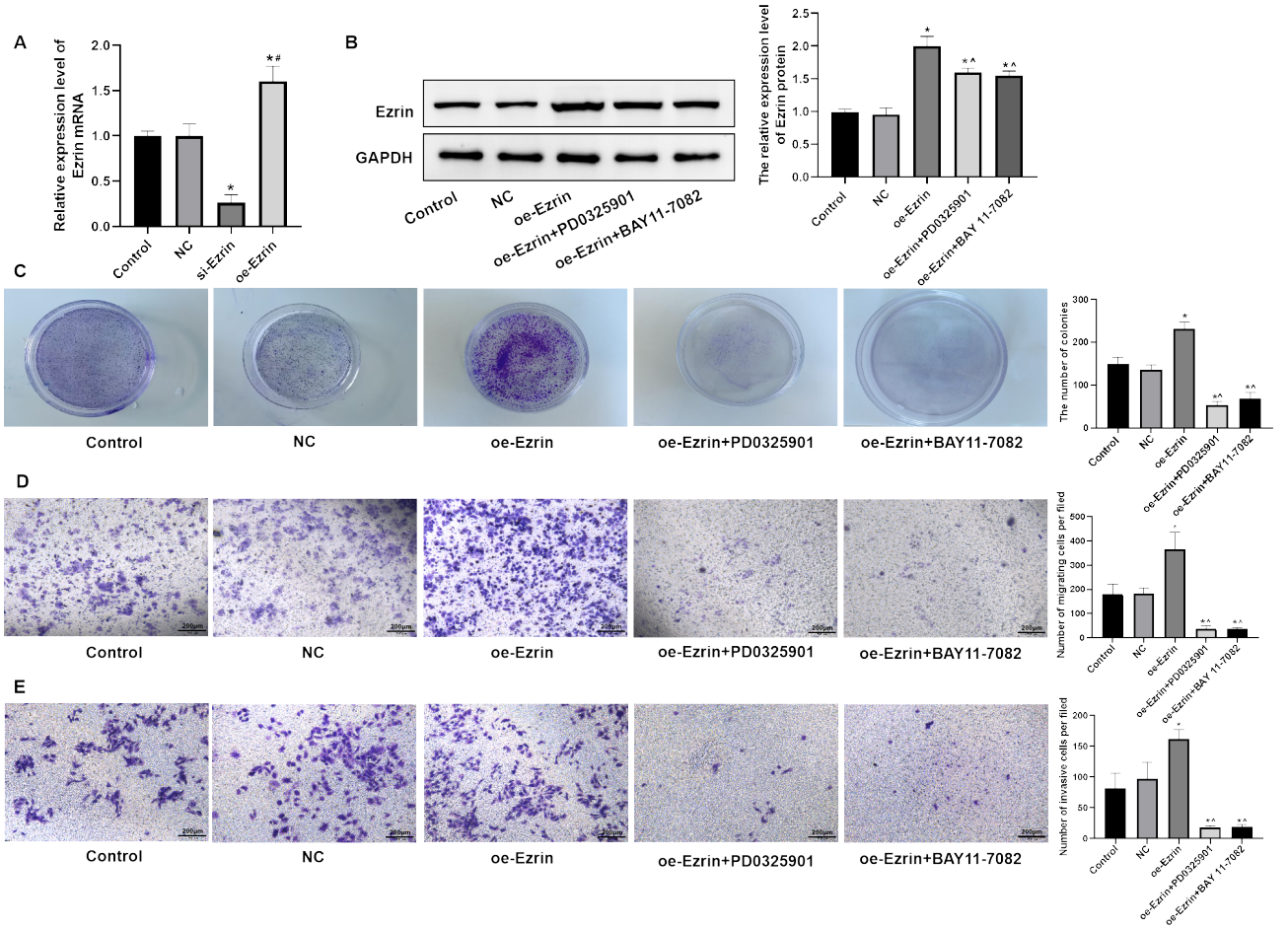

Fig. 1. Expression of Ezrin in PC-3 cells and its impact on growth and metastasis. (A) Evaluation of Ezrin expression in PC-3 cells post-transfection via real-time quantitative PCR (RT-qPCR). (B) Detection of Ezrin expression levels using Western blotting (WB). (C) Assessment of cell proliferation through plate cloning. (D) Evaluation of cell migration using the Transwell assay. (E) Detection of cell invasion through Transwell assay. Scale bar = 200 μm. n = 3. *$p < 0.05$ versus Control, ^$p < 0.05$ versus oe-Ezrin, #$p < 0.05$ versus si-Ezrin.

blocked using 5% skim milk at room temperature for 1 hour. Following blocking, the membranes were incubated overnight at 4 °C with primary antibodies, including Ezrin (1:1000, ab79256, Abcam, Cambridge, UK), MAP kinase kinase 1 (MEK1) (1:1000, ab307509, Abcam, Cambridge, UK), phosphorylated (p-) extracellular signal-regulated kinase (p-ERK1/2) (1:10,000, ab201015, Abcam, Cambridge, UK), ERK1/2 (1:10,000, ab17942, Abcam, Cambridge, UK), phosphorylated inhibitor of kappa B alpha (p-IκBα) (1:10,000, ab109393, Abcam, Cambridge, UK), IκBα (1:10,000, ab32132, Abcam, Cambridge, UK), p-P65 (1:10,000, ab32536, Abcam, Cambridge, UK), P65 (1:10,000, ab27792, Abcam, Cambridge, UK), E-cadherin (1:10,000, ab212059, Abcam, Cambridge, UK), ZO-1 (1:10,000, ab307799, Abcam, Cambridge, UK), Vimentin (1:1000, ab137321, Abcam, Cambridge, UK), Snail Slug (1:10,000, ab18017, Abcam, Cambridge, UK), and β-Catenin (1:1000, ab32095, Abcam, Cambridge, UK).

After primary antibody incubation, the membranes were probed with the secondary antibody (Goat Anti-Mouse IgG H & L) (1:10,000, Abcam, Cambridge, UK) at room temperature for 1 hour. Following a thorough washing step, protein signals were visualized using the BeyoECL Plus detection kit (P0018S, Beyotime, Shanghai, China). Finally, the relative expression levels of proteins were quantified using Image J software (v1.8.0.112, NIH, Madison, WI, USA).

RT-qPCR

RNA was isolated from cells using TRIzol reagent (15596026CN, Invitrogen, Carlsbad, CA, USA) and converted into cDNA using the PrimeScript RT kit (RR014A, Takara, Dalian, China). The synthesized cDNA was then combined with specific primers, and PCR reactions were conducted using SYBR Premix Ex TaqII (TaKaRa) in the PCR system. The mRNA expression levels of *Ezrin*, *CD86*, *CD206*, tumor necrosis factor (*TNF*)-α, in-

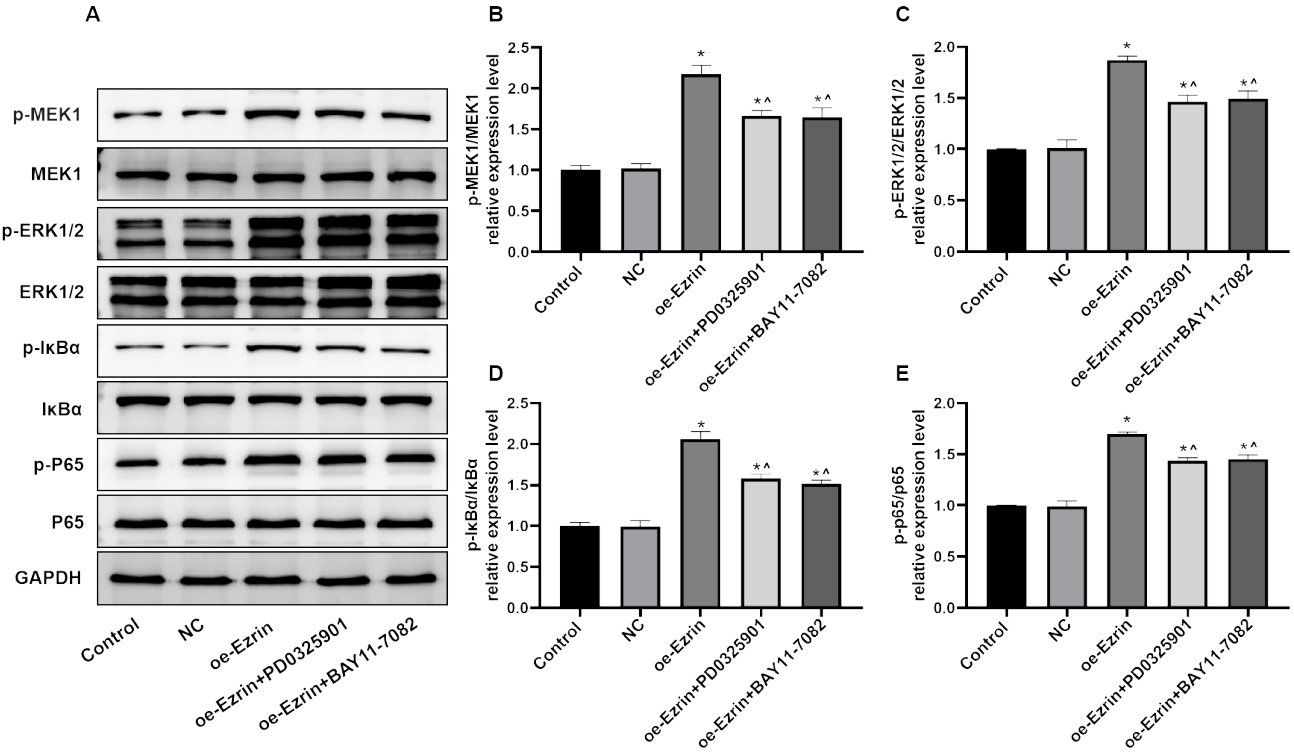

Fig. 2. Effect of Ezrin on the Mitogen-Activated Protein Kinase (MAPK)/nuclear factor kappa B (NF-κB) pathway in PC-3 cells. (A–E) Expression levels of MAPK/NF-κB pathway proteins (MEK1, ERK1/2, IκBα, P65). n = 3. *$p < 0.05$ versus control, ^$p < 0.05$ versus oe-Ezrin. MEK1, MAP kinase kinase 1; ERK, extracellular signal-regulated kinase; IκBα, inhibitor of kappa B alpha.

terleukin (*IL*)-*6*, inducible nitric oxide synthase (*iNOS*), *IL-10*, and *Arg1* were evaluated utilizing the $2^{-\Delta\Delta Ct}$ method, with Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH)* serving as the internal reference during analysis. Primer sequences are provided in Table 2.

Statistical Analysis

Mean values with their respective standard deviations (SD) were utilized to present the data. Data analysis was conducted using GraphPad Prism 9 (Dotmatics, Boston, MA, USA). Group differences in statistical significance were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons to determine statistical significance at a *p*-value < 0.05.

Results

Ezrin Expression in PCa Cell Lines and its Impact on Cell Growth and Migration

In PC-3 cells derived from prostate cancer, Ezrin mRNA levels demonstrated a decrease in the si-Ezrin group and an increase in the oe-Ezrin group compared to the control group. Additionally, the oe-Ezrin group exhibited a higher Ezrin mRNA level than the si-Ezrin group (Fig. 1A). Subsequent examination of Ezrin expression in PC-3 cells overexpressing Ezrin was conducted after treatment with MAPK and NF-κB inhibitors PD0325901 and BAY11-7082, along with an assessment of their effects on cell growth and migration.

Relative to the control group, the expression level of Ezrin was elevated in the oe-Ezrin group, oe-Ezrin+PD0325901 group, and oe-Ezrin+BAY11-7082 group. However, compared to the oe-Ezrin group, Ezrin expression was decreased in the oe-Ezrin+PD0325901 group and the oe-Ezrin+BAY11-7082 group (Fig. 1B). Moreover, the oe-Ezrin group exhibited significantly enhanced cellular proliferation, migration, and invasion compared to the control group. Conversely, cell growth and migration abilities were notably inhibited in the oe-Ezrin+PD0325901 and the oe-Ezrin+BAY 11-7082 groups. Furthermore, compared to the oe-Ezrin group, the oe-Ezrin+PD0325901 and oe-Ezrin+BAY 11-7082 groups showed significant reductions in cell growth and migration abilities (Fig. 1C–E) ($p < 0.05$).

Influence of Ezrin on the MAPK/NF-κB Pathway in PCa Cells

The findings indicated that cells from the oe-Ezrin group, oe-Ezrin+PD0325901 group, and oe-Ezrin+BAY 11-7082 group exhibited an upward trend in the expressions of proteins associated with the MAPK/NF-κB pathway (MEK1, ERK1/2, IκBα, P65) compared to those in the control group. Conversely, compared to the oe-Ezrin group,

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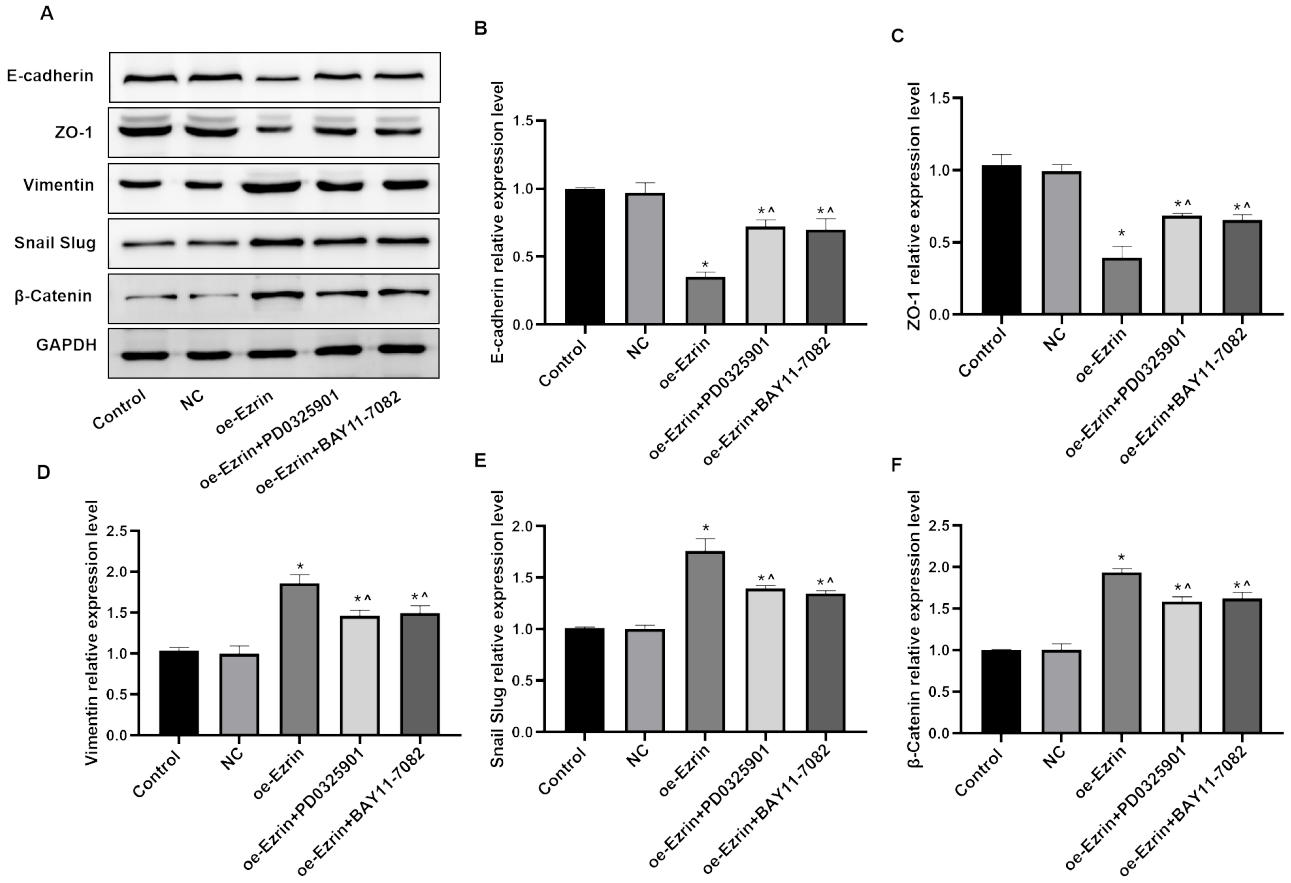

Fig. 3. Effect of Ezrin on epithelial-mesenchymal transition (EMT) in PC-3 cells. (A–F) Detection of EMT-related protein levels (E-cadherin, ZO-1, Vimentin, Snail Slug, and β-Catenin) using Western blotting (WB). n = 3. *$p < 0.05$ versus control, ^$p < 0.05$ versus oe-Ezrin.

the expression levels of proteins related to the MAPK/NF-κB pathway in cells from the oe-Ezrin+PD0325901 group and oe-Ezrin+BAY 11-7082 group showed a significant decrease (Fig. 2A–E) ($p < 0.05$).

Influence of Ezrin on EMT-related Marker Expression in PCa Cells

Comparative analysis with the control group revealed a consistent decrease in the levels of E-cadherin and ZO-1 proteins across cells from the oe-Ezrin group, oe-Ezrin+PD0325901 group, and oe-Ezrin+BAY 11-7082 group. Concurrently, there was an upregulation in the expression levels of Vimentin, Snail Slug, and β-Catenin, indicating an increased degree of EMT transition. Notably, cells from the oe-Ezrin+PD0325901 group and oe-Ezrin+BAY 11-7082 group demonstrated a notable reduction in EMT transition compared to the oe-Ezrin group (Fig. 3A–F) ($p < 0.05$).

Effects of Ezrin Silencing and Overexpression on M1/M2 Polarization of Macrophages

To explore the influence of Ezrin on macrophage polarization, PC-3 cells were subjected to Ezrin knockdown and overexpression treatments, followed by co-culturing with the RAW264.7 macrophage cell line. Flow cytometry and RT-qPCR were employed to assess the expressions of CD86 and CD206 in RAW264.7 cells. The findings revealed an upregulation of CD86 expression in the si-Ezrin group compared to the control group, accompanied by a decrease in CD206 expression ($p < 0.05$). Conversely, in the oe-Ezrin group, CD206 expression increased, while CD86 expression decreased ($p < 0.05$) (Fig. 4A–F). To further validate the phenotypes of these macrophages, RT-qPCR was utilized to evaluate the expression levels of typical M1 markers (*TNF-α*, *IL-6*, *iNOS*) and M2 markers (*IL-10*, *Arg1*).

Compared to the control group, the si-Ezrin group exhibited elevated expressions of TNF-α, IL-6, and iNOS, alongside reduced expressions of IL-10 and Arg1 ($p < 0.05$), indicating an M1 phenotype. Conversely, the oe-Ezrin group demonstrated increased expressions of IL-10 and Arg1, coupled with decreased levels of TNF-α, IL-6, and iNOS ($p < 0.05$), suggesting an M2 phenotype (Fig. 4G–K).

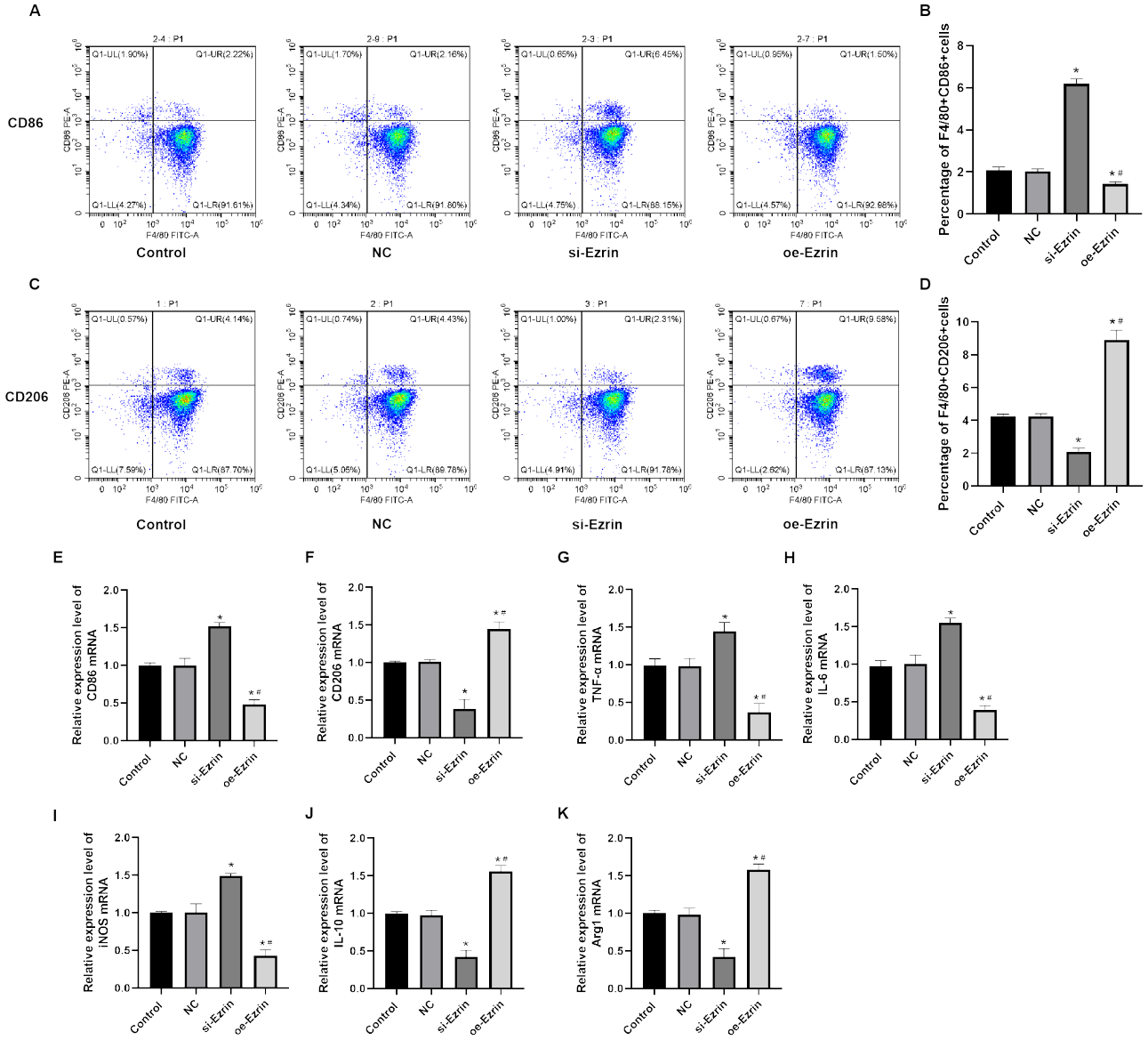

Fig. 4. The effect of Ezrin on M1/M2 polarization of macrophages co-cultured with PC-3 cells. (A,B) Assessment of CD86 expression in macrophages using flow cytometry. (C,D) Detection of CD206 expression in macrophages using flow cytometry. (E,F) Evaluation of *CD86* and *CD206* mRNA expression in macrophages by RT-qPCR. (G–K) Measurement of inflammatory factor expression levels (*TNF-α*, *IL-6*, *iNOS*, *IL-10*, and *Arg1*) in cells via RT-qPCR. n = 3. *$p < 0.05$ versus control, #$p < 0.05$ versus si-Ezrin.

Effects of Overexpression of Ezrin on M1/M2 Polarization of Macrophages via the MAPK/NF-κB Pathway

To investigate whether the impact of overexpressed Ezrin in PCa cells on macrophage M1/M2 polarization is linked to the expression of the MAPK/NF-κB pathway, we co-cultured PCa cells overexpressing Ezrin, and those overexpressing Ezrin treated with PD0325901 and BAY 11-7082, with macrophages. Compared to the control group, a significant increase in CD206, IL-10, and Arg1 expression levels was observed in cells from the oe-Ezrin group, oe-Ezrin+PD0325901 group, and oe-Ezrin+BAY 11-7082 group. Conversely, CD86, TNF-α, IL-6, and iNOS expression levels exhibited a notable decrease, indicating a shift of macrophages towards the M2 phenotype ($p < 0.05$).

Compared to the oe-Ezrin group, the expression levels of CD206, IL-10, and Arg1 decreased in the oe-Ezrin+PD0325901 group and oe-Ezrin+BAY 11-7082 group, while CD86, TNF-α, IL-6, and iNOS exhibited elevated expression, indicating a trend towards M1 polarization of macrophages (Fig. 5A–K) ($p < 0.05$).

Discussion

Prostate cancer (PCa) typically originates in the small glandular follicles of the prostate and initially remains localized. However, over time, it may infiltrate surround-

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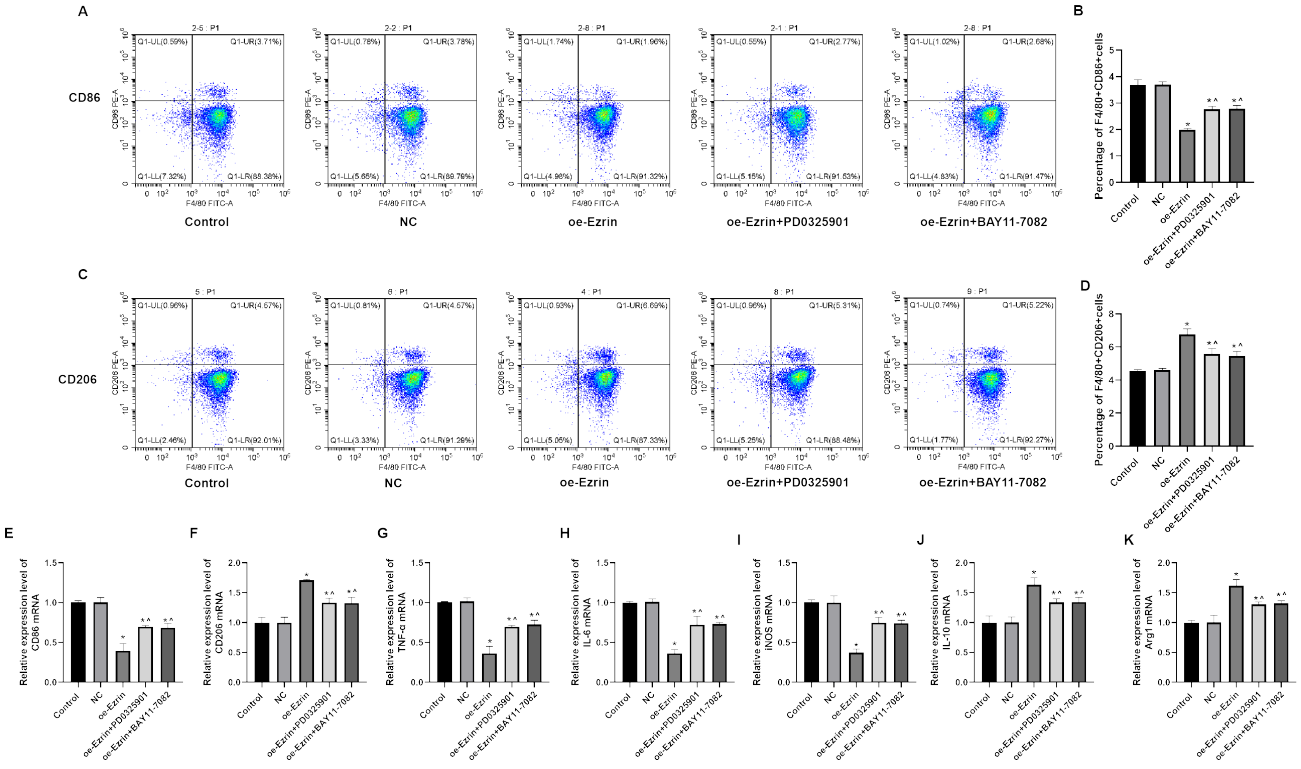

Fig. 5. The effect of Ezrin overexpression on M1/M2 polarization of macrophages co-cultured with PC-3 cells via the MAPK/NF-κB pathway. (A,B) Assessment of CD86 expression in macrophages using flow cytometry. (C,D) Detection of CD206 expression in macrophages using flow cytometry. (E,F) Evaluation of CD86 and CD206 mRNA expression in macrophages by RT-qPCR. (G–K) Measurement of inflammatory factor expression levels (TNF-α, IL-6, iNOS, IL-10, and Arg1) in cells via RT-qPCR. n = 3. $*p < 0.05$ versus control, $\hat{}p < 0.05$ versus oe-Ezrin.

ing tissues, ultimately evolving into a highly metastatic tumor [25]. The progression and metastatic growth of PCa significantly contribute to patient mortality. While early-stage PCa often responds well to treatment, some patients may experience recurrence and metastasis following therapy [26]. Therefore, in-depth research into the molecular mechanisms of PCa and its associated signaling pathways is essential for a better understanding of the development of the disease and the exploration of new treatment strategies.

In PCa, aberrant activation of the MAPK/NF-κB pathway is strongly associated with cancer initiation and progression. MEK1 (also known as MAP2K1), a kinase in the MAPK pathway, is activated by external stimuli and primarily activates ERK1/2 through phosphorylation, leading to its activation. Activated ERK1/2 moves into the nucleus and regulates gene transcription, thereby modulating cell growth and migration. The NF-κB pathway is typically initiated by various stimuli, such as inflammatory factors, cytokines, and oxidative stress, resulting in its activation. These stimuli induce the phosphorylation and subsequent degradation of IκB proteins, liberating NF-κB (typically the P65/P50 dimer), which enters the nucleus and initiates gene transcription [27,28]. Activation of the MAPK/NF-κB pathway influences tumor development by altering the polarization status of macrophages towards M1/M2 phenotypes [29,30]. M2-polarized macrophages are typically associated with tumor advancement and spread, promoting tumor growth and metastasis.

Our findings indicate that Ezrin levels significantly decreased in the si-Ezrin group while increasing in the oe-Ezrin group compared to the control group in PCa PC-3 cells. Further investigations revealed that in the oe-Ezrin group, there was a notable enhancement in cell proliferation, migration, and invasion abilities, whereas these capabilities were significantly inhibited in the oe-Ezrin+PD0325901 group and oe-Ezrin+BAY 11-7082 group. Moreover, the expression levels of MAPK/NF-κB pathway-related proteins in the oe-Ezrin, oe-Ezrin+PD0325901, and oe-Ezrin+BAY 11-7082 groups exhibited an increasing trend. However, compared to the oe-Ezrin group, the expression levels significantly decreased in the oe-Ezrin+PD0325901 and oe-Ezrin+BAY 11-7082 groups.

Additionally, significant changes were observed in the expression levels of EMT-related proteins in the oe-Ezrin, oe-Ezrin+PD0325901, and oe-Ezrin+BAY 11-7082 groups compared to the control group, indicating an increase in the degree of EMT transition. Regarding macrophages, the si-

Ezrin group exhibited M1 characteristics, whereas the oe-Ezrin group showed M2 characteristics. Moreover, the oe-Ezrin+PD0325901 and oe-Ezrin+BAY 11-7082 groups exhibited a trend towards M1 polarization in macrophages, while the oe-Ezrin group exhibited a trend towards M2 polarization.

In summary, our study elucidates the significant role of Ezrin in PCa and the pivotal role of the MAPK/NF-κB pathway in PCa, primarily in modulating its effects on macrophage M1/M2 polarization and EMT transition processes. These findings provide valuable insights into the molecular mechanisms underlying PCa and offer potential avenues for therapeutic interventions. While our research highlights the role of Ezrin in PCa, certain limitations remain, including the lack of detailed elucidation of molecular mechanisms and clinical validation of therapeutic potential. Future studies should focus on addressing these aspects to gain a comprehensive understanding of the role of Ezrin in cancer progression and explore its potential as a therapeutic target.

Conclusion

Our research findings demonstrate that Ezrin is highly expressed in PCa, suggesting its potential to activate the MAPK/NF-κB signaling pathway, induce EMT, and drive macrophage polarization towards the M2 phenotype. These mechanisms collectively promote the growth and metastasis of PCa, further accelerating its progression.

Availability of Data and Materials

The datas used and/or analyzed during the current study are available from the corresponding author.

Author Contributions

TFZ and XL contributed equally to the conception and design of the research study, performed experiments, analyzed and interpreted the data, and drafted the manuscript. TJ performed experiments, analyzed and interpreted the data. ZXD provided technical support in the acquisition of data, performed statistical analysis, and contributed to the interpretation of the data. BTD provided expertise in the field of study and contributed to the interpretation of the data. NY provided guidance on the design of the research study and contributed to the interpretation of the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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