



Epigallocatechin-3-gallate inhibits the formation of neutrophil extracellular traps and suppresses the migration and invasion of colon cancer cells by regulating STAT3/CXCL8 pathway

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Abstract

Colon cancer is a common malignant tumor of the digestive tract. Tea catechin exerts anti-tumor effects in colon cancer. This work aimed to determine the functions of epigallocatechin-3-gallate (EGCG), one of the main active components of Tea catechins, in the progression of colon cancer. In this work, enzyme-linked immune-sorbent assay, quantitative real-time PCR and western blotting was utilized to examine the levels of IL-1 β , TNF- α , STAT3, p-STAT3 and CXCL8 in colon cancer patients and healthy controls. Compared with healthy controls, the levels of IL-1 β and TNF- α were significantly increased in the peripheral blood of colon cancer patients, and the expression of STAT3, p-STAT3 and CXCL8 was elevated in the neutrophils derived from colon cancer patients. Moreover, neutrophils were treated with phorbol ester (PMA) or DNase I to induce or impede the formation of neutrophil extracellular traps (NETs). Both STAT3 overexpression and PMA treatment promoted the expression of CXCL8, myeloperoxidase (MPO) and citrullinated histone H3 (H3Cit) in the colon cancer-derived neutrophils, indicating that STAT3 overexpression facilitated the formation of NETs. STAT3 deficiency suppressed the formation of NETs, which consistent with the results of DNase I treatment. Transwell assay was utilized to detect the migration and invasion of colon cancer cell line SW480. EGCG treatment suppressed the formation of NETs and the expression of STAT3 and CXCL8 in the colon cancer-derived neutrophils, and then inhibited the migration and invasion of SW480 cells. In conclusion, this work demonstrated that EGCG inhibited the formation of NETs and subsequent suppressed the migration and invasion of colon cancer cells by regulating STAT3/CXCL8 signalling pathway. Thus, this study suggests that EGCG may become a potential drug for colon cancer therapy.

Keywords Epigallocatechin-3-gallate · STAT3 · CXCL8 · Neutrophil extracellular traps · Migration · Invasion · Colon cancer

Introduction

Colon cancer is a malignant tumor that originates from the intestinal mucosal epithelium, and its incidence is increasing in recent years. Colon cancer ranks third in terms of incidence

and second in terms of mortality around the world [1]. It is difficult to diagnose colon cancer at an early stage. At present, comprehensive treatment based on surgery is the mainly treatment method of colon cancer. Chemotherapy shows high toxicity and low sensitivity, which reduces the quality of life of colon cancer patients [2]. Moreover, colon cancer is prone to metastases and the patients with a high risk of recurrence after surgery [3, 4]. The 5-year survival rate after surgery is only about 60% [5]. Therefore, a comprehensive understanding of the molecular mechanisms of colon cancer is of great significance for formulating colon cancer treatment strategies.

Neutrophils are important inflammatory cells, and the infiltration of neutrophils reflects the local inflammatory state of the tumor [6]. In tumor microenvironment, tumor cells enhance the inflammatory properties of neutrophils, and then neutrophil extracellular traps (NETs) generated

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from the activated neutrophils [7]. Recent studies have found that the neutrophils in tumor microenvironment can promote tumor progression through affecting the formation of NETs, release of ROS, and secretion of tumor-promoting cytokines and chemokines [8–10]. The discovery of NETs is a new direction in neutrophil biology, and its role in tumor progression is of great significance.

Interleukin 8, also known as CXCL8, is an effective chemokine for neutrophils [11, 12]. Previous study has confirmed that CXCL8 is highly expressed in the tumor microenvironment and promote the growth and metastasis of colon cancer [13]. CXCL8 overexpression accelerates the epithelial-mesenchymal transition and malignant phenotypes of colon cancer cells through regulating PI3K/AKT/NF- κ B signalling pathway [14]. Additionally, interleukin 22 induces the autocrine expression of CXCL8 in colon cancer cells by activating signal transducer and activator of transcription 3 (STAT3), thereby enhancing the chemotherapy resistance of tumor cells [15]. Down-regulation of STAT3 inhibits the expression of CXCL8 in colon cancer cells [16]. These findings have suggested that STAT3 may be involved in the progression of colon cancer through regulating CXCL8. However, whether STAT3 can regulate the formation of NETs via CXCL8, and thus to affect the development of colon cancer still remains unclear.

Tea catechins are a group of substances with antioxidant activity, and they are also the mainly functional compounds in tea [17]. Tea catechins exert anti-tumor effect in various cancers, including colon cancer [18, 19]. Epigallocatechin-3-gallate (EGCG) is one of the main active components of Tea catechins. The study of Tang et al. has demonstrated that EGCG suppresses the malignant phenotypes of pancreatic cancer cells by interfering with the STAT3 signalling pathway [20]. EGCG inhibits the proliferation, migration and invasion of colon cancer cells by suppressing the expression of STAT3 [21]. Thus, this work attempted to investigate whether EGCG can inhibit the formation of NETs by inhibiting the STAT3/CXCL8 pathway, and thus to inhibit the migration and invasion of colon cancer cells.

Materials and methods

Participants

Colon cancer patients ($N=20$) and healthy volunteers ($N=20$) were recruited from Gaoxin Branch Of The First Affiliated Hospital Of Nanchang University. Peripheral blood samples were obtained from the colon cancer patients

and healthy volunteers. The written informed consents were obtained from each participant. The protocol was carried out following the Declaration of Helsinki, and was approved by the Ethics Committee of Gaoxin Branch Of The First Affiliated Hospital Of Nanchang University (YLK[2022]16).

Isolation of polymorphonuclear neutrophils

Primary neutrophils were isolated from the peripheral blood samples of the colon cancer patients and healthy volunteers using Human peripheral blood neutrophil isolation kit (P9040; Solarbio, Beijing, China). In brief, peripheral blood samples were added onto the gradient interface formed by reagent C and reagent A, and were centrifuged at 1000 g for 30 min. The neutrophils in the lower layer were carefully collected by a straw, and were washed with phosphate buffer saline (PBS) for 2 times. The neutrophils were resuspended in RPMI-1640 medium (SH30027.01; HyClone, Logan, Utah, USA) for further use.

Cell culture and treatment

Neutrophils and human colon cancer cell line SW480 (ATCC, Manassas, VA, USA) were cultured in RPMI-1640 medium at 37 °C and 5% CO₂. The medium supplemented with 10% fetal bovine serum (FBS; SV30208.01; HyClone) and 1% penicillin/streptomycin (P7630; Solarbio, Beijing, China). Neutrophils were treated with 100 nM phorbol ester (PMA; 16,561–29-8; Sigma-Aldrich, St. Louis, MO, USA) for 4 h to induce the formation of NETs. Neutrophils were treated with 100 U/mL Deoxyribonuclease I (DNase I; 11284932,001; Sigma-Aldrich) for 4 h to inhibit the formation of NETs. In addition, neutrophils were treated with different concentrations of EGCG (0, 5, 10, 25, 50 μ M) for 48 h. EGCG was purchased from the Sigma-Aldrich (989-51-5).

For co-culture, neutrophils were seeded into the lower chamber of 24-well Transwell plates (Corning, NY, USA), and SW480 cells were seeded into the upper chamber with semipermeable inserts. After 48 h of cell culture, neutrophils and SW480 cells were collected for further analysis.

Cell transfection

The pcDNA3.1 vector containing full length of STAT3 (STAT3-OE) was constructed for STAT3 overexpression. The specific small interference RNA (siRNA) targeting STAT3 (si-STAT3; 5'-GGGACCUGGUGUGAA UUAUdTdT-3') was used to silence STAT3. The empty

pcDNA3.1 (Vector) and scrambled siRNA (si-NC; 5'-UUC UCCGAACGUGUCACGUTT-3') served as negative control (NC). The plasmids were synthesized by GeneChem (Shanghai, China). Neutrophils were transfected with above plasmids or siRNA utilizing Lipofectamine 2000 Transfection Reagent (11668019; Invitrogen, Carlsbad, CA, USA). Neutrophils were collected after 48 h of transfection for further study.

Enzyme-linked immune-sorbent assay (ELISA)

The levels of IL-1 β , TNF- α and CXCL8 in the serum of colon cancer patients and healthy controls, and the levels of CXCL8 in the cell culture supernatant of neutrophils were examined by ELISA kit. The diluted standards for each cytokine were respectively prepared in according to the Human IL-1 β ELISA kit (QK201; R&D Systems, Minneapolis, MN, USA), Human TNF- α ELISA kit (ml064303; Milbio, Shanghai, China) and Human CXCL8 ELISA kit (D8000C; R&D Systems), and all ELISA assays were carried out according to the manufacturer's introductions. The absorbance value at 450 nm of samples was detected spectrophotometrically on a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA), and the concentrations of the samples were determined according to the respective standard curve. The levels of myeloperoxidase (MPO)-DNA and citrullinated histone H3 (H3Cit)-DNA in the serum of participants were assessed by ELISA as previous protocol described [22]. In brief, a 96-well plate was coated with anti-MPO (ab232939; Abcam, Cambridge, United Kingdom) or anti-H3Cit antibody (PA5-16672; Thermo Fisher Scientific) overnight, and then blocked with 1% BSA in PBS. The plasma samples were added into the 96-well plate and then incubated with anti-DNA antibody (Cell Death ELISAPLUS, 11774425001; Roche, Basel, Switzerland) for 1 h. Finally, the absorbance value at 405 nm of samples was detected spectrophotometrically on a microplate reader, and the concentrations of the samples were determined according to the respective standard curve.

Quantitative real-time PCR (qRT-PCR)

Neutrophils were treated with TRIzol reagent (15,596,018; Invitrogen), and then total RNA was extracted. In brief, cells were collected and resuspended in 1 ml of TRIzol reagent for 5 min. Next, 200 μ l of chloroform was added into the cell suspension for shaking 10 s, and the mixture was centrifuged at 12,000 \times g for 5 min under 4 °C environment. After that, cell supernatant was collected, and were mixed with 500 μ l of isopropanol, which was stable for 30 min at

room temperature. Above mixture was centrifuged again at 12,000 \times g for 10 min under 4 °C environment, and the cell supernatant was removed. After washing with 75% ethanol, the sediment was dissolved in DEPC H₂O for next step. The integrity of RNA was measured by 1.5% agarose gel electrophoresis. Subsequently, total RNA was served as template to synthesize complementary DNA, and then PCR reaction was carried out applying TransScript® Two-Step RT-PCR SuperMix (TransGen Biotech, Beijing, China). The primer sequences (3'–5') were listed as follows: STAT3: forward-CAGCAGCTTGACACACGGTA and reverse-AAACACCAAAGTGGCATGTGA; CXCL8: forward-TCTGCTAGC CAGGATCCACA and reverse-TGCTTCCACATGCTCTCA CA; GAPDH: forward-TGCACCACCAACTGCTTAGC and reverse-GGCATGGACTGTGGTCATGAG. GAPDH served as loading control. The relative expression of mRNAs was quantified using 2^{- $\Delta\Delta$ CT} method.

Western blotting

Neutrophils were treated with RIPA Lysis Buffer (89,901; Thermo Fisher Scientific) to extract total protein. BCA Protein Assay Kit (P0012S; Beyotime, Shanghai, China) was applied to detect the concentrations of protein samples. The equal mass (25 μ g) and volume (20 μ l) of protein samples were separated by the 10% SDS-PAGE, and then proteins were transferred onto a nitrocellulose membrane. After blocking with 5% non-fat milk, the membranes were incubated with primary antibodies, including anti-STAT3 (ab68153; 1:2000 dilution; Abcam), anti-p-STAT3 (ab267373; 1:1000 dilution; Abcam), anti-CXCL8 (ab289967; 1:1000 dilution; Abcam), anti-MPO (ab208670; 1:1000 dilution; Abcam), anti-H3Cit (ab281584; 1:10,000 dilution; Abcam) or anti-GAPDH (ab9485; 1:2500 dilution; Abcam), at 4 °C overnight, and then were incubated with horseradish peroxidase-conjugated second antibody (1:10,000 dilution; Abcam). GAPDH served as loading control. The immunoreactive bands were detected applying BeyoECL Moon kit (P0018FS; Beyotime) and then analyzed by Image J software.

Transwell migration and invasion assay

A 24-well Transwell insert system (Corning) was used to detect cell migration and invasion of SW480 cells. For migration assay, SW480 cells (5×10^4) were seeded into the top chamber of the insert. For invasion assay, SW480 cells (8×10^4) were seeded into the top chamber of the insert-coated with 1 mg/mL Matrigel (BD Biosciences, Billerica, MA, USA). The upper chamber was added with serum-free

RPMI-1640 medium, and the lower chamber was added with the RPMI-1640 medium containing 10% FBS. After 24 h of incubation, the migrated and invaded cancer cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. The migrated and invaded cells were counted and photographed using an inverted microscope (Olympus, Tokyo, Japan).

Statistical analysis

Each experiment was carried out for 3 times. The measurement data were expressed as mean \pm standard deviation. Graph Pad Prism 5 (Graph Pad Software Inc, San Diego, CA) was used for statistical analysis. Two-tailed Student's *t* test was used to analyze the statistical difference between

two independent groups. One-way ANOVA was applied to analyze the statistical difference among multiple groups. The difference was considered as statistically significant when *P* less than 0.05.

Results

STAT3 and CXCL8 were up-regulated in colon cancer patients

To determine the functional role of STAT3 and CXCL8 in colon cancer, the levels of inflammatory factors, STAT3 and CXCL8 in colon cancer patients were detected. ELISA results revealed that the levels of IL-1 β , TNF- α and CXCL8

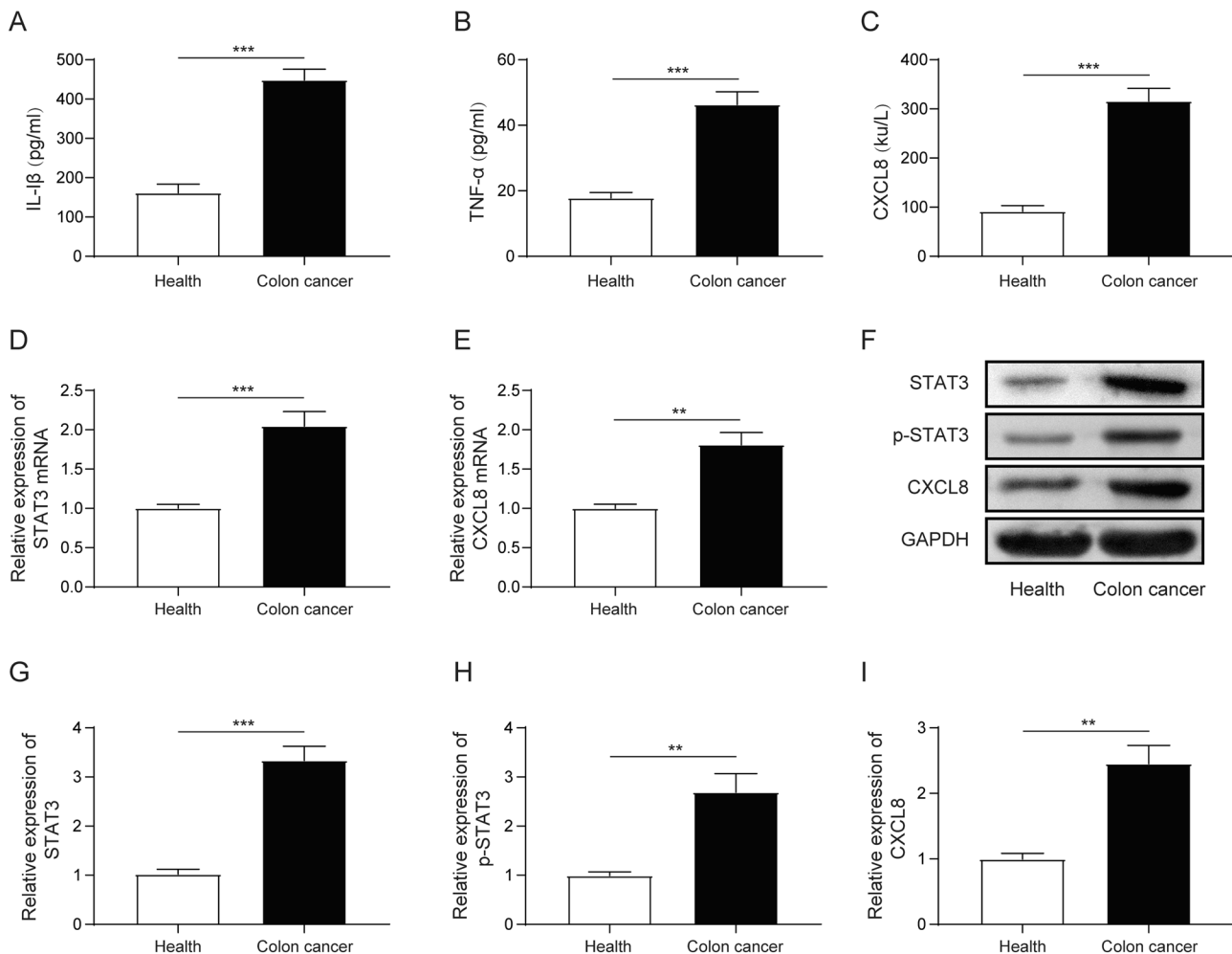


Fig. 1 STAT3 and CXCL8 were up-regulated in colon cancer patients. The levels of IL-1 β (A), TNF- α (B) and CXCL8 (C) in the peripheral blood of colon cancer patients and healthy controls were detected by ELISA. The mRNA expression of STAT3 (D) and CXCL8 (E) in the neutrophils derived from colon cancer patients and healthy controls was examined by qRT-PCR. (F) The protein expres-

sion of STAT3 (G), p-STAT3 (H) and CXCL8 (I) in the neutrophils derived from colon cancer patients and healthy controls was examined by western blotting. The relative expression levels of STAT3, p-STAT3 and CXCL8 were normalized to GAPDH. ***P* < 0.01, ****P* < 0.001.

were increased in the peripheral blood specimens of colon cancer patient when contrasted to healthy controls (Fig. 1A–C). Then, the expression of STAT3, p-STAT3 and CXCL8 was detected by qRT-PCR and western blotting. Compared with healthy controls, the mRNA expression of STAT3 and CXCL8 was significantly increased in the neutrophils derived from colon cancer patients (Fig. 1D–E). The protein expression of STAT3, p-STAT3 and CXCL8 was higher in the neutrophils derived from colon cancer patients than that in the healthy controls (Fig. 1F–I). All these data indicated that STAT3 and CXCL8 were up-regulated in colon cancer patients.

STAT3 overexpression increased CXCL8 production and the formation of NETs in the colon cancer-derived neutrophils

To explore the mechanism of STAT3 in regulating the formation of NETs, STAT3 was overexpressed in the colon cancer-derived neutrophils. The results of qRT-PCR uncovered that the transfection of STAT3 overexpression vector caused an up-regulation of STAT3 mRNA and CXCL8 mRNA in the colon cancer-derived neutrophils. Meantime, the mRNA expression of STAT3 and CXCL8 was increased in the colon cancer-derived neutrophils following PMA treatment (Fig. 2A–B). Western blotting data revealed that both STAT3 overexpression and PMA treatment elevated the protein expression of STAT3, p-STAT3, CXCL8, and NET markers (MPO and H3Cit) in the colon cancer-derived neutrophils (Fig. 2C–H). The production of CXCL8 in the colon cancer-derived neutrophils were detected by ELISA. The results showed that the level of CXCL8 in the colon cancer-derived neutrophils was enhanced by STAT3 overexpression and PMA treatment (Fig. 2I). Moreover, the levels of MPO-DNA and H3Cit-DNA in the peripheral blood specimens of colon cancer patients were examined by ELISA. The levels of MPO-DNA and H3Cit-DNA were higher in the peripheral blood specimens of colon cancer patient than that in healthy controls (Fig. 2J–K). Thus, these data suggested that STAT3 overexpression promoted the production of CXCL8 and the formation of NETs in colon cancer-derived neutrophils.

STAT3 silencing suppressed the production of CXCL8 and the formation of NETs in the colon cancer-derived neutrophils

To determine the influence of STAT3 deficiency to the formation of NETs, STAT3 was silenced using the specific siRNA in colon cancer-derived neutrophils. The data

obtained from qRT-PCR showed that both STAT3 silencing and DNase I treatment markedly suppressed the mRNA expression of STAT3 and CXCL8 in the colon cancer-derived neutrophils (Fig. 3A–B). The protein expression of STAT3, p-STAT3, CXCL8, MPO and H3Cit in the colon cancer-derived neutrophils was inhibited following STAT3 deficiency and DNase I treatment (Fig. 3C–H). ELISA results revealed that the production of CXCL8 was inhibited in the colon cancer-derived neutrophils following transfection of si-STAT3 or DNase I treatment (Fig. 3I). These findings indicated that STAT3 deficiency suppressed the production of CXCL8 and the formation of NETs in colon cancer-derived neutrophils.

EGCG treatment suppressed the expression of STAT3 and CXCL8 in colon cancer-derived neutrophils

The influence of EGCG treatment to the expression of STAT3 and p-STAT3 in the colon cancer-derived neutrophils was determined by qRT-PCR and western blotting. As shown in Fig. 4A, STAT3 mRNA expression was down-regulated in the colon cancer-derived neutrophils following EGCG treatment in a concentration-dependent manner. The protein expression of STAT3 and p-STAT3 in the colon cancer-derived neutrophils also was inhibited by EGCG (10, 25, 50 μM) treatment, although at different extent (Fig. 4B–D). EGCG at 10, 25, 50 μM dosages also decreased the mRNA and protein expression of CXCL8, and suppressed the production of CXCL8 in the colon cancer-derived neutrophils (Fig. 4E–H). Therefore, EGCG treatment suppressed the expression of STAT3 and CXCL8 in colon cancer-derived neutrophils.

EGCG treatment suppressed the migration and invasion of SW480 cells via targeting STAT3/CXCL8 in a co-culture system of SW480 and neutrophils

SW480 cells were co-cultured with the EGCG-treated neutrophils. Colon cancer-derived neutrophils were planted into the lower chamber of the Transwell plates, and were administrated with EGCG alone or in combination with PMA or STAT3 overexpression vector. EGCG treatment suppressed STAT3 expression, but the expression of it was significantly upregulated following transfection of the STAT3 overexpression vector (Fig. 5A). The level of CXCL8 in the neutrophils was detected by ELISA, and the data showed that EGCG treatment significantly inhibited the production of CXCL8 in the neutrophils. EGCG treatment-mediated the inhibition

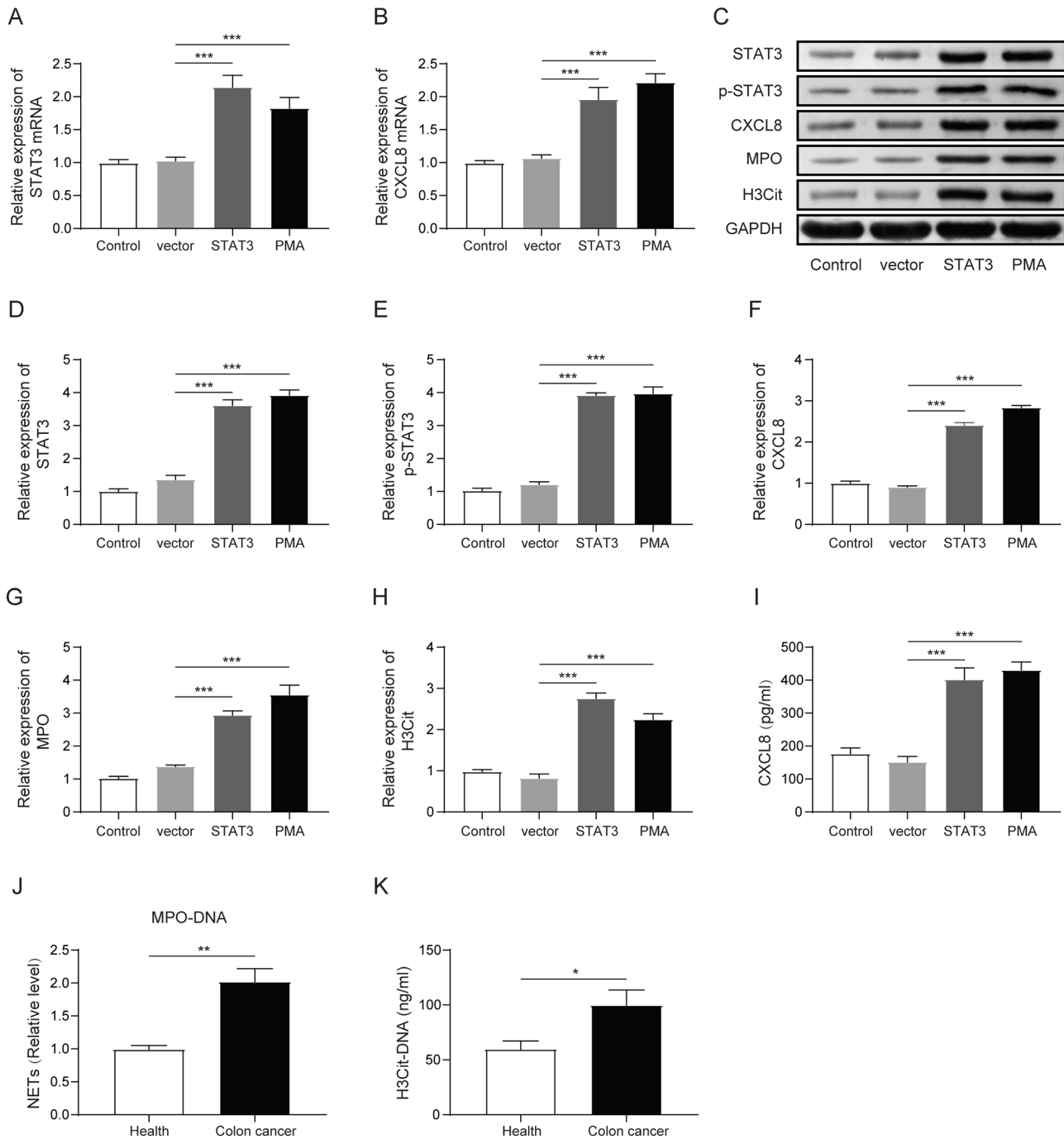


Fig. 2 STAT3 overexpression promoted the production of CXCL8 and the formation of NETs in colon cancer-derived neutrophils. Colon cancer-derived neutrophils were transfected with STAT3-OE, Vector, or treated with PMA. Normal colon cancer-derived neutrophils served as control. The mRNA expression of STAT3 (**A**) and CXCL8 (**B**) in the neutrophils was examined by qRT-PCR. **C** The protein expression of STAT3 (**D**), p-STAT3 (**E**), CXCL8 (**F**), MPO

(**G**) and H3Cit (**H**) in the neutrophils was examined by western blotting. The relative expression levels of STAT3, p-STAT3, CXCL8, MPO and H3Cit were normalized to GAPDH. **I** The production of CXCL8 in the neutrophils was assessed by ELISA. The levels of MPO-DNA (**J**) and H3Cit-DNA (**K**) in the peripheral blood of colon cancer patients and healthy controls were detected by ELISA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

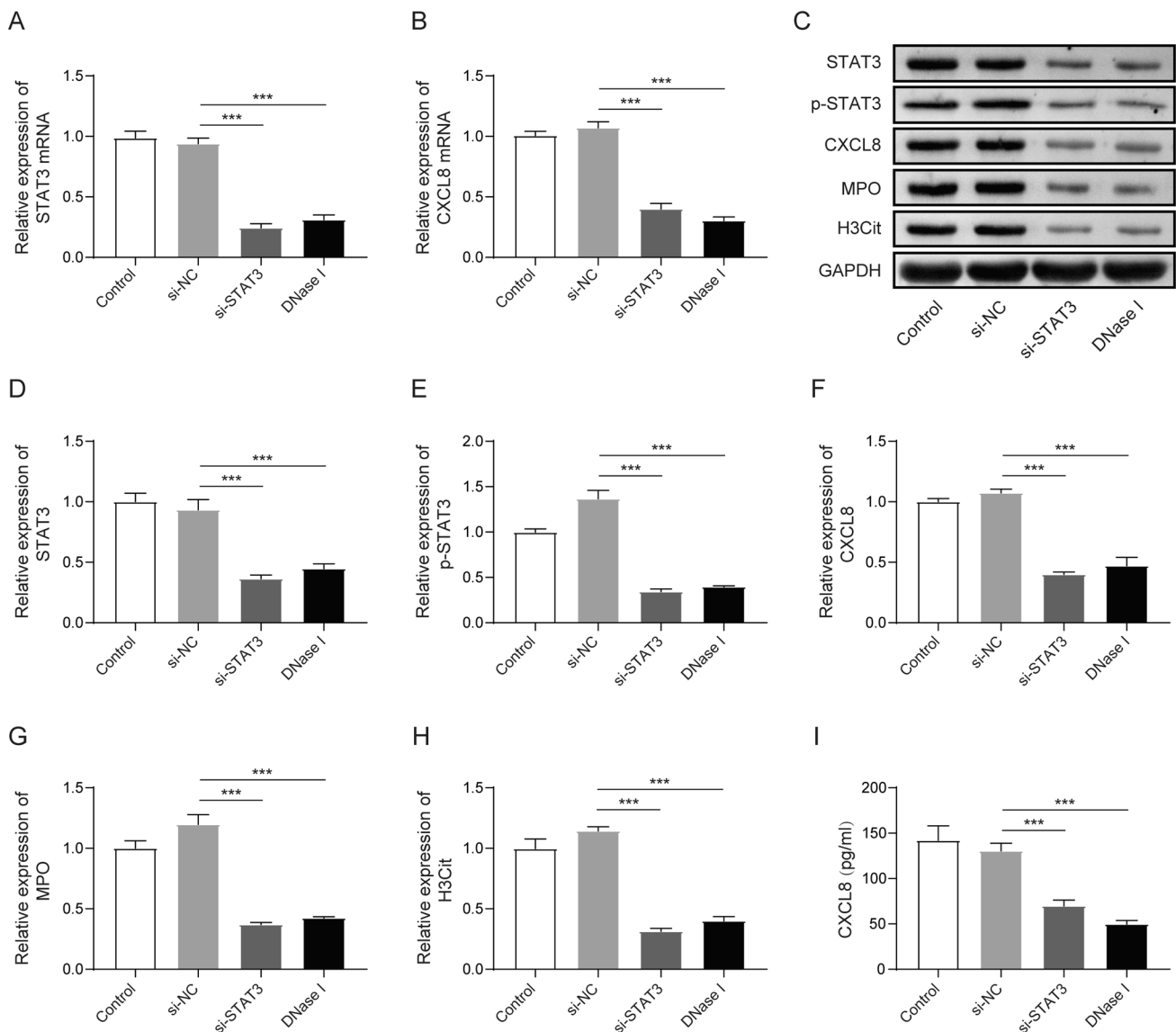


Fig. 3 STAT3 deficiency suppressed the production of CXCL8 and the formation of NETs in colon cancer-derived neutrophils. Colon cancer-derived neutrophils were transfected with Si-STAT3, Si-NC, or treated with DNase I. Normal colon cancer-derived neutrophils served as control. The mRNA expression of STAT3 (A) and CXCL8 (B) in the neutrophils was examined by qRT-PCR. C The protein

expression of STAT3 (D), p-STAT3 (E), CXCL8 (F), MPO (G) and H3Cit (H) in the neutrophils was examined by western blotting. The relative expression levels of STAT3, p-STAT3, CXCL8, MPO and H3Cit were normalized to GAPDH. I The production of CXCL8 in the neutrophils was assessed by ELISA. *** $P < 0.001$

to CXCL8 was abrogated by STAT3 overexpression and PMA treatment (Fig. 5B). Western blotting was performed to examine the expression of NET markers in the neutrophils. EGCG treatment suppressed the protein expression of MPO and H3Cit in the neutrophils, which was abolished by STAT3 overexpression and PMA treatment (Fig. 5C). Furthermore, the impact of EGCG treatment on the migration

and invasion of SW480 cells was detected by Transwell assay. As shown in Fig. 5D-E, the migration and invasion of SW480 cells was strengthened after co-culture with the colon cancer-derived neutrophils, whereas EGCG-treated neutrophils limited the migration and invasion of SW480 cells. STAT3 overexpression and PMA treatment could impair the inhibition of EGCG to the neutrophil-mediated

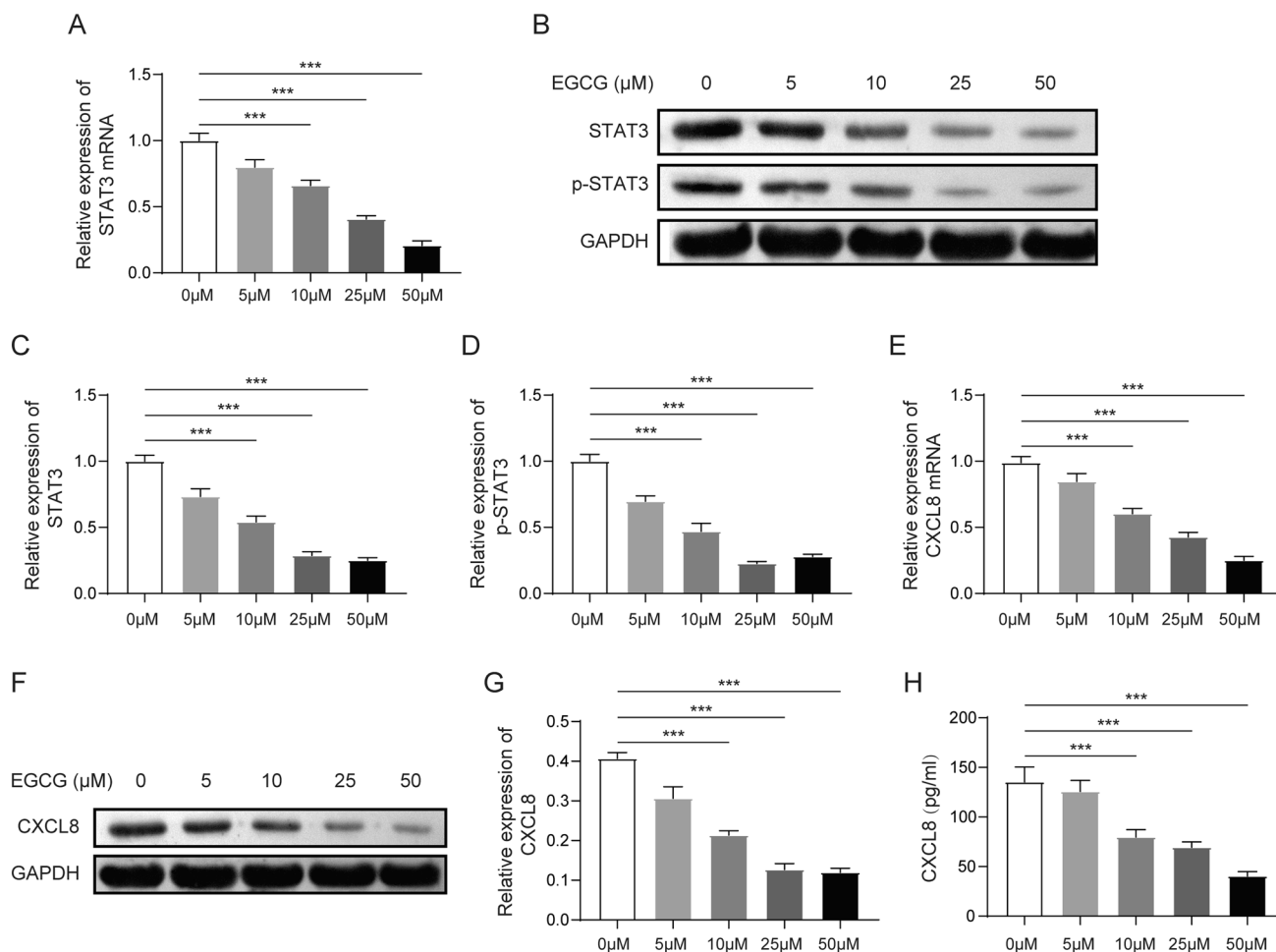


Fig. 4 EGCG treatment suppressed the expression of STAT3 and CXCL8 in colon cancer-derived neutrophils. Colon cancer-derived neutrophils were treated with different concentrations of EGCG (0, 5, 10, 25, 50 μM). **A** QRT-PCR was utilized to detect the mRNA expression of STAT3 in the neutrophils. **B** Western blotting was utilized to assess the protein expression of STAT3 **C** and p-STAT3

D in the neutrophils. The relative expression levels of STAT3 and p-STAT3 were normalized to GAPDH. QRT-PCR (**E**) and western blotting (**F**, **G**) was used to examine the mRNA and protein expression of CXCL8 in the neutrophils. **H** The production of CXCL8 in the neutrophils was assessed by ELISA. *** $P < 0.001$

migration and invasion of SW480 cells. Taken together, EGCG treatment suppressed the production of CXCL8 in colon cancer-derived neutrophils and thus to inhibit the migration and invasion of SW480 cells, which was abrogated by STAT3 overexpression.

Discussion

Colon cancer progression is a complex process involving multiple links, especially inflammatory response [23]. In this work, our data showed that the levels of inflammatory factors, IL-1β and TNF-α, were significantly increased in the peripheral blood of colon cancer patients when contrasted to healthy controls. STAT3 and CXCL8 were upregulated in

the neutrophils derived from colon cancer patients. STAT3 overexpression promoted the production of CXCL8 and the formation of NETs in the colon cancer-derived neutrophils. Moreover, EGCG treatment suppressed the formation of NETs and the expression of STAT3 and CXCL8 in the colon cancer-derived neutrophils, and then inhibited the migration and invasion of SW480 cells. All these findings demonstrated that EGCG inhibited the formation of NETs and subsequently suppressed the migration and invasion of colon cancer cells by regulating STAT3/CXCL8 signalling pathway.

STAT3 could regulate the biological behaviors of tumor cells and immune cells by mediating the extracellular signals of inflammatory mediators. It is an indispensable key molecule in the process of chronic inflammation that promoting tumorigenesis and tumor-related inflammation [24, 25]. A growing body of evidences have confirmed the functional role of STAT3 in colon cancer. A previous study has found that aianthone inhibits the proliferation and migration and accelerate apoptosis and cell cycle arrest of colon cancer cells through regulating JAK/STAT3 signalling pathway [26]. Auranofin in combination with the Wnt/ β -catenin pathway inhibitor ICG-001 administration could suppress the phosphorylation of STAT3, thereby inhibit the proliferation and metastasis of colon cancer [27]. Cucurbitacin inhibits the polarization of macrophages to M2 phenotype and the proliferation and migration of colon cancer cells by suppressing JAK2/STAT3 signalling pathway [28]. In this work, we also confirmed the carcinogenesis of STAT3 in colon cancer. STAT3 was highly expressed in the neutrophils derived from colon cancer patients. Moreover, STAT3 overexpression promoted the formation of NETs, and then enhanced the migration and invasion of colon cancer cells. Whereas, the formation of NETs can be suppressed following STAT3 silencing.

CXCL8 is a typical chemokine of the CXC family, which mainly produced by neutrophils, monocytes, macrophages, T cells, epithelial cells and endothelial cells. As one of the most important members of the Glu-Leu-Arg (ELR) chemokine family, CXCL8 plays an important role in mediating inflammatory responses [29]. CXCL8 is related to the occurrence and development of various cancers, especially colorectal cancer and its liver

metastasis [30]. Shen et al. have revealed that CXCL8 overexpression accelerates colon cancer progression by inducing epithelial-mesenchymal transition via PI3K/AKT/NF- κ B signalling axis [14]. The miR-20a inhibits CXCL8 function by interacting with it in stromal fibroblasts, thereby influencing tumor latency of colon cancer [31]. CXCL8 is highly expressed in the tumor tissues of colon cancer patients, which is one of the chief biomarkers for colon cancer [32]. Consistently, the secretion and expression of CXCL8 was increased in the colon cancer patients. The expression of CXCL8 was enhanced by STAT3 overexpression, and then participated in promoting the formation of NETs and the migration and invasion of colon cancer cells.

EGCG exists various biological activities such as antibacterial, anti-inflammatory, cancer prevention, and prevention of cardiovascular and cerebrovascular diseases [33]. It has been reported that EGCG enhances the production of ROS and promotes CTR1 expression by regulating ERK1/2/NEAT1 signalling pathway, which contributes to elevate the cisplatin sensitivity in non-small-cell lung cancer cells [34]. EGCG and doxorubicin exert synergistic anti-cancer effect in bladder cancer by regulating NF- κ B/MDM2/p53 signalling pathway [35]. EGCG could promote the cell apoptosis and inhibit the migration and invasion of colon cancer cells by suppressing STAT3 expression [21]. Consistently, EGCG inhibited the expression of STAT3 and CXCL8 in the colon cancer-derived neutrophils. Moreover, EGCG inhibited the formation of NETs, and further suppressed the migration and invasion of colon cancer cells by regulating STAT3/CXCL8 signalling pathway.

Certainly, this article also has some shortcomings. This work only initially explored the function role of EGCG in colon cancer through in vitro experiments. In further works, a xenograft mouse model should be constructed, and the influence of EGCG on the growth of colon cancer should be explored in vivo.

In conclusion, this work demonstrated that EGCG inhibited the formation of NETs, and subsequently suppressed the migration and invasion of colon cancer cells by regulating STAT3/CXCL8 signalling pathway. Thus, this work suggests that EGCG may become a potential drug for colon cancer treatment.

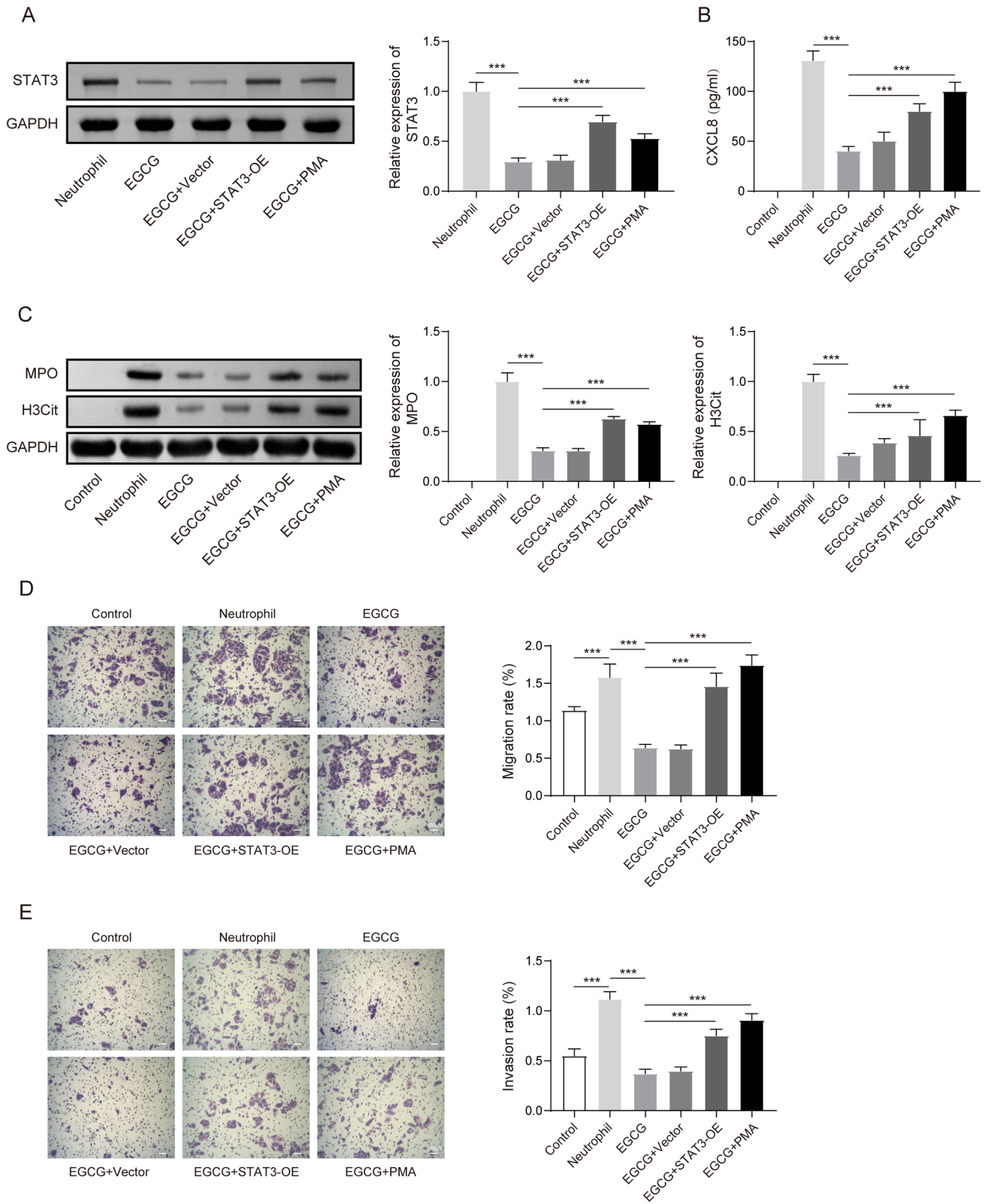


Fig. 5 EGCG treatment suppressed the production of CXCL8 in neutrophils and thus to inhibit the migration and invasion of SW480 cells via targeting STAT3/CXCL8 signaling. Colon cancer-derived neutrophils were treated with 25 μ M EGCG in associated with STAT3-OE and Vector transfection or PMA treatment, and then were co-cultured with SW480 cells. Normal SW480 cells served as control. **A** The protein expression of STAT3 in neutrophils was measured by western blotting. The relative expression level of STAT3 was normalized to GAPDH. **B** The production of CXCL8 in the neutrophils was assessed by ELISA. **C** The protein expression of MPO and H3Cit in the neutrophils was examined by western blotting. The relative expression levels of MPO and H3Cit were normalized to GAPDH. Transwell assay was utilized to measure the migration (**C**) and invasion (**D**) of SW480 cells. *** $P < 0.001$

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interests The authors declare that they have no conflict of interest.

Ethics approval Colon cancer patients ($N=20$) and healthy volunteers ($N=20$) were recruited from Gaoxin Branch Of The First Affiliated Hospital Of Nanchang University. The protocol was carried out following the Declaration of Helsinki, and approved by the Ethics Committee of Gaoxin Branch Of The First Affiliated Hospital Of Nanchang University (YLK[2022]16).

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