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EGF promotes HIF-1α expression in colorectal cancer cells and tumor metastasis by regulating phosphorylation of STAT3

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Abstract. – OBJECTIVE: Hypoxia-inducible factor 1α (HIF-1α) functions importantly in the development of colorectal cancer. HIF-1α is induced by some cytokines and growth factors and is also regulated by another kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways. Meanwhile, inhibiting HIF-1α expression can inhibit the development of colorectal cancer. The aim of this study was to explore the effect of epidermal growth factor (EGF) on the activation of signal transducer and activator of transcription 3 (STAT3) in human colorectal cancer cells SW480. In addition, the underlying mechanism of the STAT3 signaling pathway in regulating HIF-1α and further affecting tumorigenesis and metastasis was investigated.

MATERIALS AND METHODS: Immunofluorescence and Western blotting were used to detect the activation of STAT3 by EGF in human colorectal cancer cells SW480. SW480 cells were transfected with STAT3 siRNA or treated with STAT3 inhibitor Niclosamide, and then stimulated with EGF to change the expressions of STAT3 and p-STAT3. The expression level of HIF-1α mRNA in SW480 cells was detected by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). In addition, transwell assay and tumor formation experiments were performed to validate whether STAT3 and HIF-1α affected SW480 through EGF.

RESULTS: STAT3 was not activated in SW480 cells *in vitro*. EGF induced STAT3 activation and enhanced its phosphorylation level, so that it shuttled into the nucleus. Phosphorylated activation of STAT3 was a necessary condition for EGF to induce HIF-1α up-regulation. Both HIF-1α and EGF-induced phosphorylation of STAT3 could significantly promote the proliferation and metastasis of SW480, and enhance tumorigenesis.

CONCLUSIONS: In SW480 cells, EGF regulated HIF-1α through the STAT3 phosphorylation pathway, eventually promoting the occurrence and metastasis of colorectal cancer.

Key Words
STAT3, HIF-1α, Colorectal cancer, EGF.

Introduction

Colorectal cancer is the third most common type of cancer that causes human death. Although colorectal cancer can be treated by surgical resection, radiotherapy and chemotherapy, the prognosis of patients is still poor[1]. In recent years, with the development of small molecule targeted drugs, the survival time of patients with colorectal cancer has been significantly prolonged. Meanwhile, the quality of their life has been improved. Clinical studies have shown that the effective rate of EGFR tyrosine kinase inhibitors (TKIs) therapy in patients with a positive mutation in colorectal cancer receptor (EGFR) is 70%-80% (TKIs). However, the effective rate is only about 10%-20% in patients with wild-type EGFR, suggesting that TKIs treatment still has certain limitations[2-4]. Even in patients with effective EGFR-TKI initial treatment, acquired resistance will eventually occur with the prolongation of the treatment[5].

Signal transducer and activator of transcription 3 (STAT3) is a key protein involved in signal transduction and transcriptional activation. Studies have showed that the sustained activation and expression of STAT3 are associated with the occurrence, development and prognosis of multiple malignant tumors. STAT3 can regulate various abnormalities involved in the transcription factor of tumor signaling pathways, such as anti-apoptotic gene B-cell lymphoma 2 (Bcl-2) and cell cycle control genes of Myc and cyclin D1. This may eventually induce tumor cell proliferation and inhibit cell apoptosis[6,7]. Therefore, STAT3 is recognized as one of the most important oncogenes. Hypoxia-inducible factor 1α (HIF-1α) is the initiating factor for the changes of tumor biological characteristics under hypoxic conditions. Its expression and stability are closely related to the biological characteristics and clinical features of tumors[8]. Previous studies have observed that

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the expression of HIF-1α is associated with the prognosis, recurrence, metastasis and tumor aggressiveness of colorectal cancer[9]. STAT3, which is a costimulatory factor of HIF-1α, can activate downstream target genes of HIF-1α (such as vascular endothelial growth factor) and drive HIF-1α-dependent tumorigenesis under hypoxic condition[10-12]. However, the effects of STAT3 and HIF-1α expression in colorectal cancer induced by EGF remain elusive. The aim of this study was to explore the inducing effect of EGF on STAT3 and HIF-1α in colorectal cancer cells, thereby providing new suggestions for the treatment of colorectal cancer.

Materials and Methods

Cell Culture

Human colorectal cancer cell line SW480 was purchased from the Cell Bank of Chinese Academy of Sciences' Type Culture Collection Committee (Shanghai, China). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) complete medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 100 IUg/mL penicillin, 100 μg/mL streptomycin at 37°C in a 5% CO_2 incubator. Antibodies against EGF, EGFR, HIF-1α, STAT3 and p-STAT3 were purchased from Abcam (Cambridge, MA, USA). STAT3 inhibitor Niclosamide and HIF-1α inhibitor 2-Methoxyestradiol were purchased from Selleck (Houston, TX, USA).

Immunofluorescence Assay

SW480 cells were cultured in slides for 24 h. After stretching and attaching to the slides, the cells were washed with phosphate-buffered saline (PBS) and cultured with medium containing 0.1% FBS overnight. On the next day, the medium was replaced by complete medium supplemented with 100 ng/mL EGF and 10% FBS. After 1 h, the cells were rinsed with PBS and fixed with 4% paraformaldehyde for 10 min. Then, they were treated with 0.25% Triton X-100 for permeabilization and blocked with 5% FBS for 1 h. Subsequently, the cells were incubated with primary antibody against STAT3 or p-STAT3 overnight. On the next day, the cells were incubated with FITC-labeled secondary antibody for 2 h. After propidium iodide (PI) cell nuclear staining, the cells were observed under a fluorescence microscope and photographed.

Transfection of STAT3 siRNA

STAT3 siRNA sequence was 5'-CCUGAGUUGAAUUAUCAGCUU-3'. When the confluency of SW480 cells was up to 80%-90%, they were transfected with STAT3 siRNA according to the instructions of Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA). Silencing efficiency was verified by Western blot after 72 h of transfection.

Western Blot Assay

After the treatment, the nuclear protein/cytoplasmic protein and total protein in cells were extracted according to the kit instructions. Briefly, 50 μg of protein sample from each group was separated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% skim milk for 30 min, the membrane was incubated with primary antibody against STAT3 or p-STAT3 (diluted 1:1000) at 4°C overnight. β-actin was used as an internal reference. Then the membranes were washed with Tris-Buffered Saline and Tween 20 (TBST) and incubated with the corresponding secondary antibody at room temperature for 2 h. Protein bands were observed by enhancedchemiluminescence (ECL) (Thermo-Fisher Scientific, Waltham, MA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Cells were collected, and total RNA was extracted in one step using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary Deoxyribose Nucleic Acid (cDNA) was synthesized from 500 ng of total RNA, and the template was synthesized according to the instructions of RT-PCR kit (TaKaRa, Otsu, Shiga, Japan). Primer sequences used in this study were as follows: HIF-1α (f: 5'-CTTTCTTGGAAACGAGTGAAAGG-3'; r: 5'-TGAGTAATTCTTCACCCTGCAG-3'), β-actin (f: 5'-AAATCGTGCGTGACATTAA-3'; r: 5'-CTCGTCATACTCCTGCTTG-3'). QRT-PCR results were obtained from an ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA).

Transwell Assay

Cells in the experimental group or the control group were re-suspended in 600 μL of serum-free medium and cultured in the upper chamber of transwell. Meanwhile, 600 μL of complete medium was added to the lower chamber. After incubation at 37°C for 24 hours, cells in the

upper chamber were gently removed with a cotton swab. The cells on the surface of the lower chamber were stained with 0.1% crystal violet and photographed under a microscope.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA) and GraphPad 6 software (La Jolla, CA, USA) were used for all statistical analyses. Data were expressed by mean ± SD. One-way analysis of variance (ANOVA) was used to compare the differences among different groups, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

EGF Promoted STAT3 Activation in Colorectal Cancer Cells SW480

To determine whether EGF could promote STAT3 activation *in vitro*, SW480 cells were first treated with EGF. Subsequently, immunofluorescence staining was performed to detect STAT3 activation. Under fluorescence microscopy, we found that activated STAT3 (p-STAT3) in untreated SW480 cells was mainly located in the cytoplasm, and the level of activation was very low. After EGF stimulation, STAT3 in SW480 cells partially shuttled into the nucleus and was activated at a high level (Figure 1A). Meanwhile, no significant change was found in STAT3 expression in the cytoplasm of SW480 cells after EGF stimulation (Figure 1B). Western blot analysis demonstrated that p-STAT3 was lowly expressed in untreated SW480 nuclei, whereas was highly expressed in the nucleus after EGF treatment (Figure 1C). However, STAT3 was highly expressed in the cytoplasm before and after EGF stimulation, showing no significant difference (Figure 1D). Thus, EGF could increase the level of nuclear p-STAT3 and phosphorylation of STAT3.

Phosphorylation of STAT3 Induced by EGF Enhanced HIF-1α Expression

To further investigate the effect of STAT3 on SW480, STAT3-siRNA was transfected in cells to

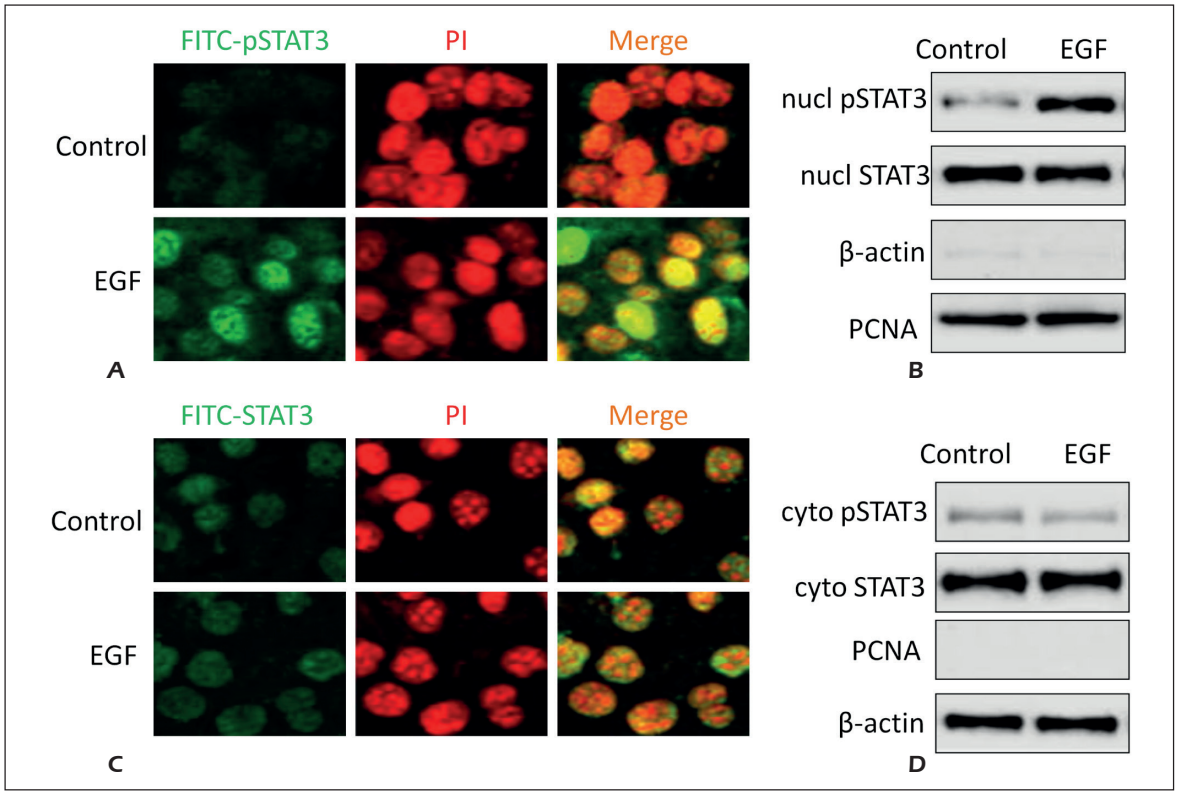

Figure 1. EGF promoted the increase of nuclear and phosphorylation of STAT3. **A**, Immunofluorescence was used to detect the expression of pSTAT3 in SW480 cells before and after 10 ng/ml EGF treatment. **B**, Western blot was used to detect the expression of STAT3 and pSTAT3 in the nucleus before and after EGF treatment. **C**, Immunofluorescence was used to detect the expression of STAT3 in SW480 cells before and after 10 ng/ul EGF treatment. **D,** Western blot was used to detect the expression of STAT3 and pSTAT3 in cytoplasm of SW480 cells before and after EGF treatment.

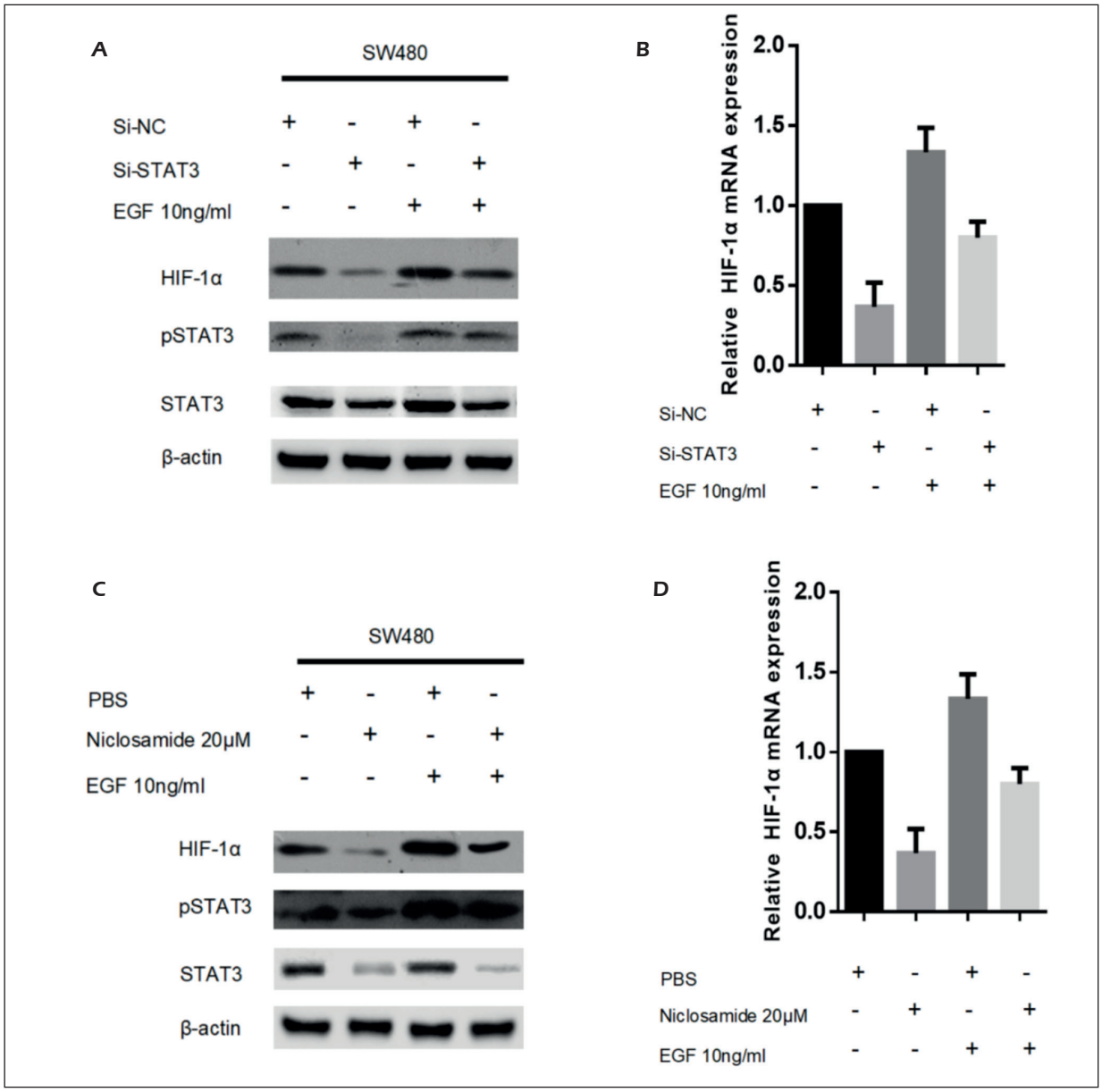

Figure 2. Phosphorylation of STAT3 induced by EGF enhance the mRNA expression of HIF-1α. **A**, 10 ng/mL EGF and STAT3 siRNA were transfected into SW480 cells simultaneously. Western blot was used to detect the protein expressions of STAT3 and pSTAT. **B**, After EGF and STAT3 silencing, HIF-1α mRNA expression was detected by RT-PCR. **C**, 10 ng/mL EGF and 20 μM Niclosamide were transfected into SW480 cells simultaneously. Western blot was used to detect the protein expression levels of STAT3 and p-STAT3. **D**, After EGF and Niclosamide administration, HIF-1α mRNA expression was detected by RT-PCR.

knockdown STAT3. Western blot analysis showed that the protein expression of STAT3 in cells transfected with STAT3-siRNA was significantly decreased when compared with that of the negative control group ($p<0.05$). This indicated that STAT3-siRNA could significantly block STAT3 expression. After EGF stimulation, si-STAT3 exerted no effect on STAT3 expression, but significantly promoted p-STAT3 expression (Figure 2A). On the other hand, qRT-PCR results revealed that the mRNA expression of HIF-1α in cells transfected with si-STAT3 was significantly lower than that of the negative control group ($p<0.05$). Although EGF remarkably increased HIF-1α mRNA expression, this effect could be attenuated by si-STAT3 transfection (Figure 2B). Besides, SW480 cells were treated with STAT3 inhibitor Niclosamide. Western blot indicated that Niclosamide had no significant effect on STAT3 expression. However, it markedly inhibited the phosphorylation of

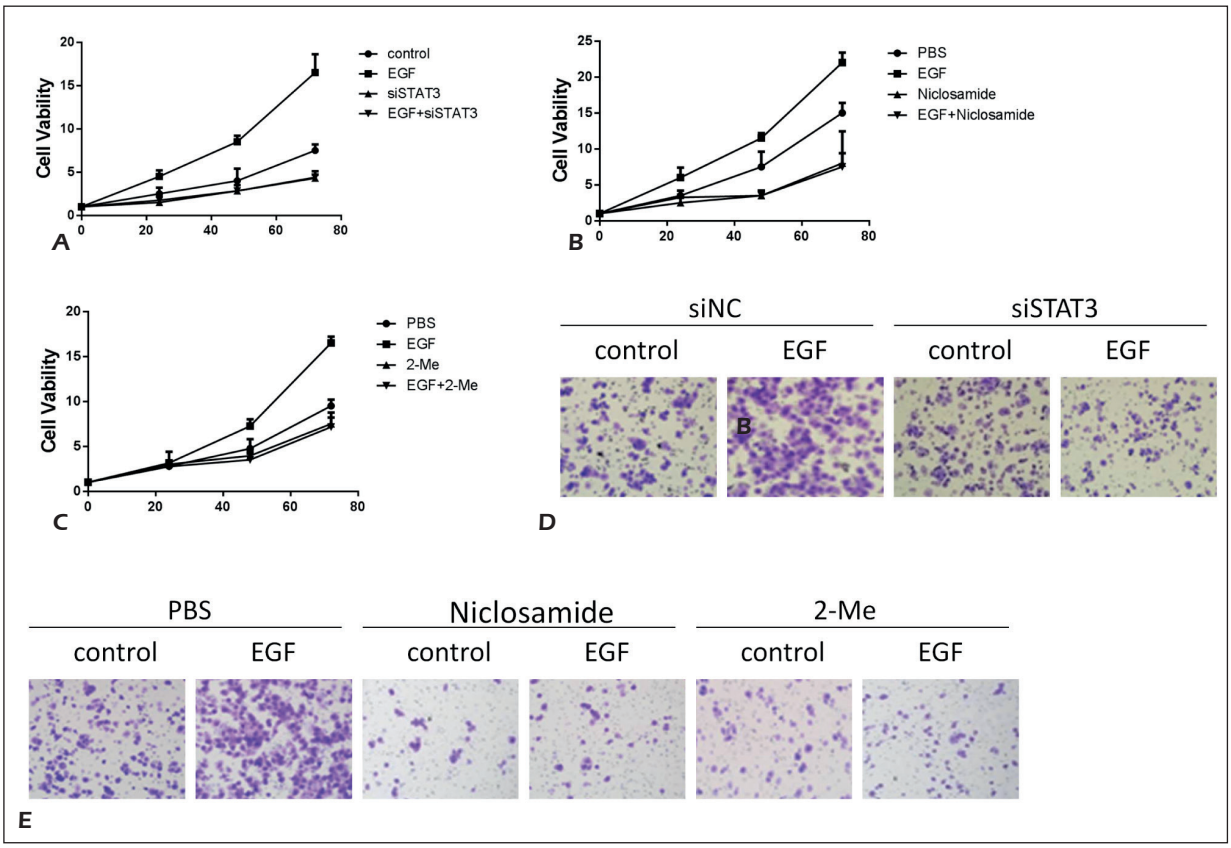

Figure 3. EGF-induced proliferation and invasion of colorectal cancer was dependent on STAT3 and HIF-1α. **A**, SW480 cells were simultaneously treated with 10 ng/ml EGF and STAT3 siRNA. The proliferation rate of the experimental group and the control group was examined, respectively. **B**, SW480 cells were simultaneously treated with 10 ng/ml EGF and 20 μM Niclosamide. The proliferation rate of the experimental group and the control group was examined, respectively. **C**, SW480 cells were simultaneously treated with 10 ng/ml EGF and 60 μM 2-Methoxyestradiol. The proliferation rate of the experimental group and the control group was examined, respectively. **D**, transwell assay was performed to detect the effect of EGF and STAT3-siRNA on SW480 cell invasion. **E**, transwell assay was performed to detect the effects of EGF, Niclosamide, 2-Methoxyestradiol on SW480 cell invasion.

STAT3. After treatment with Niclosamide, the activation of STAT3 by EGF was also significantly weakened ($p<0.05$) (Figure 2C). In addition, the effect of EGF on up-regulating the mRNA expression of HIF-1α was remarkably attenuated by Niclosamide ($p<0.05$) (Figure 2D). The above results proved that p-STAT3 had a function in regulating HIF-1α expression in SW480 cells.

EGF-Induced Proliferation and Invasion of Colorectal Cancer was Depended on STAT3 and HIF-1α

Previous results have shown that EGF promotes the proliferation of SW480 cells. However, after STAT3 knock-down, EGF-induced cell proliferation was significantly attenuated (Figure 3A). After treatment with STAT3 inhibitor Niclosamide, the proliferation of EGF-induced SW480 cells was also weakened (Figure 3B). 2-Methoxyestradiol is one of the specific inhibitors of HIF-1α. After inhibition of HIF-1α by 2-Methoxyestradiol, EGF-induced SW480 cell proliferation was remarkably attenuated as well (Figure 3C). Subsequently, we explored whether the effect of EGF on cell migration was dependent on STAT3 using transwell assay. The results showed that STAT3-siRNA transfection could significantly inhibit the promoting effect of EGF on the invasion of SW480 cells (Figure 3D). At the same time, the administration of Niclosamide and 2-Methoxyestradiol in SW480 cells also significantly inhibited the promoting effect of EGF (Figure 3E). These results indicated that EGF promoted the proliferation and invasion of SW480 cells through the phosphorylation of STAT3 and activation of HIF-1α.

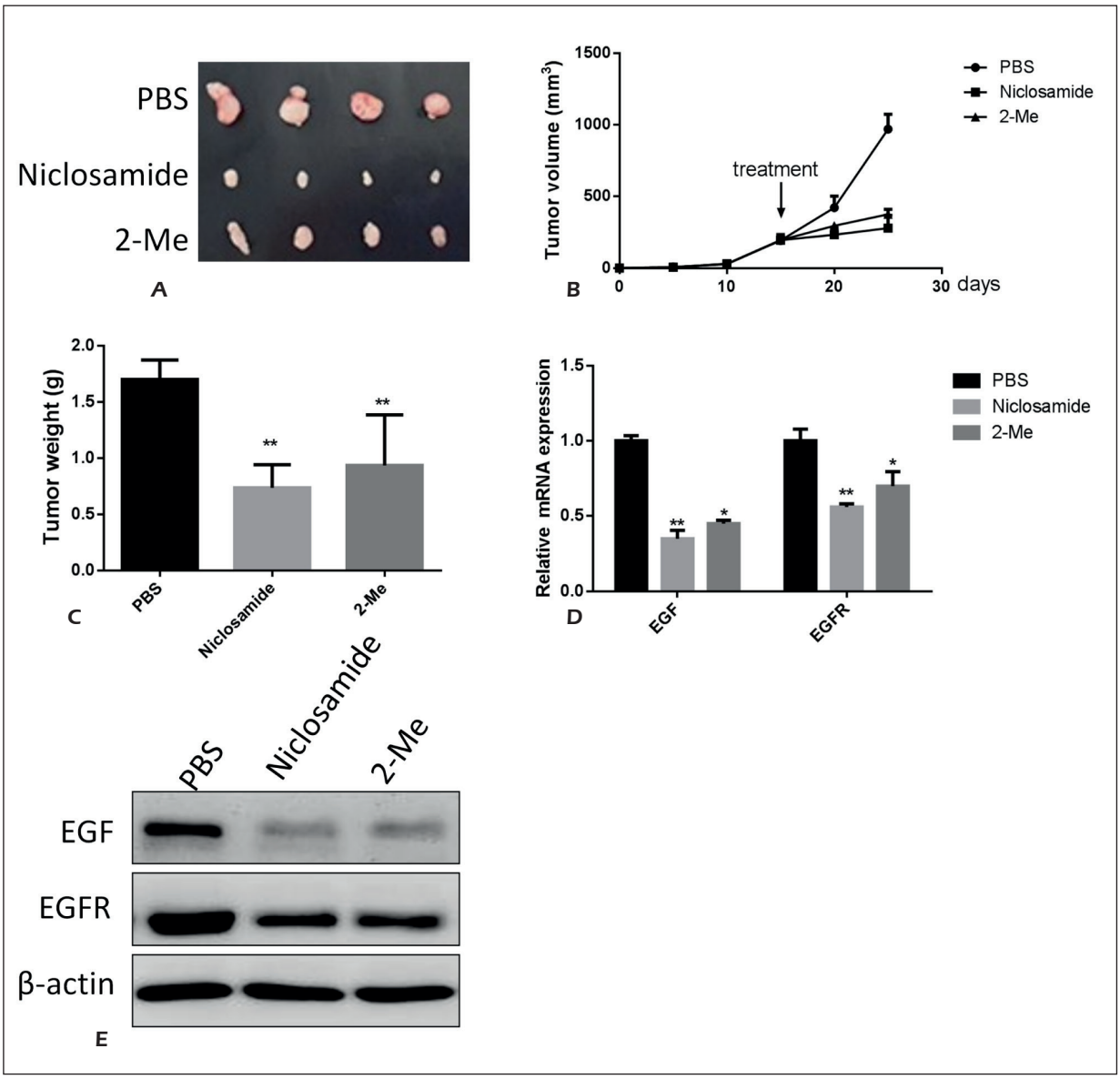

Figure 4. STAT3 and HIF-1α promoted tumorigenesis of colorectal cancer and increased EGF expression. **A**, The same number of SW480 cells were implanted subcutaneously in nude mice. Niclosamide and 2-Methoxyestradiol were injected 15 days later. Tumors were photographed after 25 days. **B**, Tumor growth curve. **C**, Weight statistics of tumors after 25 days. **D**, RT-PCR was used to detect the mRNA expressions of EGF and EGFR in the above tumor tissues. E, Western blot was applied to detect the protein expressions of EGF and EGFR in the above tumor tissues.

The Promoting Effect of STAT3 and HIF-1α on the Tumorigenesis of Colorectal Cancer was Associated with EGF-EGFR In vivo

To further explore the effect of EGF on tumorigenesis *in vivo*, we implanted SW480 cells subcutaneously in nude mice. When tumors were formed to the same size, mice in different groups were injected through tail veins with PBS, Niclosamide, and 2-Methoxyestradiol, respectively. It was found that Niclosamide and 2-Methoxyestradiol significantly inhibited tumor formation and the proliferation of SW480 cells (Figure 4A). Meanwhile, the tumor size and weight were significantly less than those of control PBS mice (Figure 4B and 4C). Then the tumors were removed, and the mRNA and protein expressions were detected. QRT-PCR (Figure 4D) and Western blot (Figure 4E) showed that both the mRNA and protein expressions

of EGF and EGFR in tumor tissues treated with Niclosamide and 2-Methoxyestradiol were significantly decreased. Therefore, the inhibition of STAT3 phosphorylation and HIF-1α expression could suppress the tumorigenesis of colorectal cancer. Moreover, the tumor-promoting effects of STAT3 and HIF-1α were closely related to EGF.

Discussion

Studies have shown that abnormal activation of STAT3 exists in many malignancies, including leukemia, breast and prostate cancer[11,13]. Phosphorylation occurs when STAT3 is activated, which then forms a dimer and shuttles into the nucleus to combine with a homologous DNA-binding region. This may induce transcriptional activation[14]. STAT3 tyrosine phosphorylation has been observed in colorectal cancer[6]. Among the seven members of the STAT family, STAT3 and STAT5 have been most widely researched. STAT3 locates in the cytoplasm and can be stimulated by cytokines or growth factors. Under the action of tyrosine kinases, especially just another kinase (JAK) kinases, tyrosine residues of the carboxyl-terminal domain are phosphorylated to activate STAT3[15]. Phosphorylated STAT3 forms homologous or heterologous dimers and shuttles into the nucleus to recognize and bind to the response element of target gene-specific promoter[16]. The inhibitor Niclosamide can suppress DNA replication and selectively reduce the phosphorylation level of STAT3. However, it has no significant inhibitory effect on the activation of other homologs, such as STAT1 and STAT5[17]. RNA interference (RNAi) technology belongs to post-transcriptional gene silencing and it is the most commonly used tool for the research of gene function. Therefore, siRNA and inhibitor Niclosamide act at different levels. STAT3 functions through phosphorylation and de-phosphorylation. The use of siRNA alone cannot fully explain the function of STAT3. Besides, the inhibitor Niclosamide can only inhibit the phosphorylation active form of STAT3. Consequently, the above two ways were combined to inhibit STAT3 expression and phosphorylation, to further explain the regulation of phosphorylated STAT3 on HIF-1α expression.

EGF is an important cytokine that promotes cell proliferation, adhesion and metastasis. After several trials and experiments, we determined that 10 ng/mL of EGF could significantly enhance the invasion and metastasis of SW480 cells. HIF-1α is a transcription factor widely found in mammals and humans under hypoxic condition. During the growth of malignant tumors, excessive tissue hyperplasia can induce local severe hypoxia, eventually leading to an imbalance between energy supply and energy consumption[18]. HIF-1α enhances energy supply by up-regulating the expression of EGF and glycolytic enzymes, indicating that HIF-1α is closely related to tumorigenesis[19]. In this study, we also revealed a positive correlation between HIF-1α and EGF. Our findings suggested a promoting effect of EGF on tumor proliferation. Studies have shown that STAT3 is able to regulate a variety of HIF-1α-related abnormal tumor signaling pathways involved, thereby inducing tumor cell proliferation and blocking cell apoptosis[12]. Therefore, STAT3 gene is recognized as one of the most important oncogenes. Studies have also indicated that STAT3 can directly bind to the promoter of HIF-1α, thereby regulating the expression of HIF-1α. In this study, we found that there was a correlation between STAT3 and EGF expression. Meanwhile, phosphorylated STAT3 could enhance the invasive and migratory abilities of colorectal cancer cells. Thus, blocking the STAT3 signaling pathway could inhibit multiple signaling pathway-mediated tumorigenesis and prevent tumor growth and metastasis. Meanwhile, pSTAT3 and HIF-1α might be effective targets for inhibiting the expression of EGF in colorectal cancer.

Conclusions

In the present study, we found that EGF enhanced the phosphorylation level of STAT3 in colorectal cancer cell line SW480 and elevated the expression of HIF-1α. Furthermore, it ultimately promoted tumorigenesis and tumor invasive ability of colorectal cancer cells.

Acknowledgements

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Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Su WB, Liu ZY. MiR-431 inhibits colorectal cancer cell invasion via repressing CUL4B. Eur Rev Med Pharmacol Sci 2018; 22: 3047-3052.
2) Sartore-Bianchi A, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, Di Nicolantonio F, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 2009; 69: 1851-1857.
3) Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di Nicolantonio F, Gambacorta M, Siena S, Bardelli A. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol 2005; 6: 279-286.
4) Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. Cancer Discov 2014; 4: 1269-1280.
5) Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, Valtorta E, Schiavo R, Buscarino M, Siravegna G, Bencardino K, Cercek A, Chen CT, Veronese S, Zanon C, Sartore-Bianchi A, Gambacorta M, Gallicchio M, Vakiani E, Boscaro V, Medico E, Weiser M, Siena S, Di Nicolantonio F, Solit D, Bardelli A. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012; 486: 532-536.
6) Kusaba T, Nakayama T, Yamazumi K, Yakata Y, Yoshizaki A, Inoue K, Nagayasu T, Sekine I. Activation of STAT3 is a marker of poor prognosis in human colorectal cancer. Oncol Rep 2006; 15: 1445-1451.
7) Kryczek I, Lin Y, Nagarsheth N, Peng D, Zhao L, Zhao E, Vatan L, Szeliga W, Dou Y, Owens S, Zgodzinski W, Majewski M, Wallner G, Fang J, Huang E, Zou W. IL-22(+)CD4(+) T cells promote colorectal cancer stemness via STAT3 transcription factor activation and induction of the methyltransferase DOT1L. Immunity 2014; 40: 772-784.
8) Han J, Xi Q, Meng Q, Liu J, Zhang Y, Han Y, Zhuang Q, Jiang Y, Ding Q, Wu G. Interleukin-6 promotes tumor progression in colitis-associated colorectal cancer through HIF-1alpha regulation. Oncol Lett 2016; 12: 4665-4670.
9) Roberts DL, Williams KJ, Cowen RL, Barathova M, Eustace AJ, Brittain-Dissont S, Tilby MJ, Pearson DG, Ottley CJ, Stratford IJ, Dive C. Contribution of HIF-1 and drug penetrance to oxaliplatin resistance in hypoxic colorectal cancer cells. Br J Cancer 2009; 101: 1290-1297.
10) Dodd KM, Yang J, Shen MH, Sampson JR, Tee AR. mTORC1 drives HIF-1alpha and VEGF-A signalling via multiple mechanisms involving 4E-BP1, S6K1 and STAT3. Oncogene 2015; 34: 2239-2250.
11) Reddy KR, Guan Y, Qin G, Zhou Z, Jing N. Combined treatment targeting HIF-1alpha and Stat3 is a potent strategy for prostate cancer therapy. Prostate 2011; 71: 1796-1809.
12) Shen W, Li HL, Liu L, Cheng JX. Expression levels of PTEN, HIF-1alpha, and VEGF as prognostic factors in ovarian cancer. Eur Rev Med Pharmacol Sci 2017; 21: 2596-2603.
13) Zhou J, Wulfkuhle J, Zhang H, Gu P, Yang Y, Deng J, Margolick JB, Liotta LA, Petricoin ER, Zhang Y. Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proc Natl Acad Sci U S A 2007; 104: 16158-16163.
14) Bui QT, Im JH, Jeong SB, Kim YM, Lim SC, Kim B, Kang KW. Essential role of Notch4/STAT3 signaling in epithelial-mesenchymal transition of tamoxifen-resistant human breast cancer. Cancer Lett 2017; 390: 115-125.
15) Kotha A, Sekharam M, Cilenti L, Siddiquee K, Khaled A, Zervos AS, Carter B, Turkson J, Jove R. Resveratrol inhibits Src and Stat3 signaling and induces the apoptosis of malignant cells containing activated Stat3 protein. Mol Cancer Ther 2006; 5: 621-629.
16) Lee TL, Yeh J, Van Waes C, Chen Z. Epigenetic modification of SOCS-1 differentially regulates STAT3 activation in response to interleukin-6 receptor and epidermal growth factor receptor signaling through JAK and/or MEK in head and neck squamous cell carcinomas. Mol Cancer Ther 2006; 5: 8-19.
17) You S, Li R, Park D, Xie M, Sica GL, Cao Y, Xiao ZQ, Deng X. Disruption of STAT3 by niclosamide reverses radioresistance of human lung cancer. Mol Cancer Ther 2014; 13: 606-616.
18) Jeong YJ, Cho HJ, Magae J, Lee IK, Park KG, Chang YC. Ascofuranone suppresses EGF-induced HIF-1alpha protein synthesis by inhibition of the Akt/mTOR/p70S6K pathway in MDA-MB-231 breast cancer cells. Toxicol Appl Pharmacol 2013; 273: 542-550.
19) Yeom CJ, Goto Y, Zhu Y, Hiraoka M, Harada H. Microenvironments and cellular characteristics in the micro tumor cords of malignant solid tumors. Int J Mol Sci 2012; 13: 13949-13965.