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Research

HOXB8 mediates resistance to cetuximab in colorectal cancer cells through activation of the STAT3 pathway

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Abstract
Homeobox B8 (HOXB8) belongs to the HOX family and was essential to the development of colorectal carcinoma. Among the prevalent monoclonal antibodies for treating RAS/BRAF wild-type metastatic colorectal cancer (mCRC) patients, cetuximab stands out, but resistance to cetuximab frequently arises in targeted treatments. Currently, the role of HOXB8 in cetuximab-resistant mCRC remains unclear. By comparing drug-sensitive cell lines (SW48) with drug-resistant cell lines (HCT116, CACO2), we discovered that HOXB8 was substantially expressed in cetuximab-resistant cell lines, and furthermore, in drug-resistant cell lines (HCT116, CACO2), HOXB8 knockdown increased the cytotoxicity of cetuximab via blocking the signal transducer and activator of transcription 3 (STAT3) signaling pathway. Conversely, the excessive expression of HOXB8 reduced the growth suppression in SW48 cells caused by cetuximab by triggering the STAT3 signaling pathway. Conclusively, we conclude that HOXB8 has played an essential role in cetuximab-resistant mCRC and that treating HOXB8 specifically may be a useful treatment approach for certain cetuximab-resistant mCRC patients.

1 Introduction

Globally, colorectal cancer ranks among the most common types of malignant tumors; according to research, it will rank second and third globally in terms of incidence and fatality in 2022 [1]. Upon initial diagnosis, approximately 23% of colorectal cancer patients show metastases, so systemic chemotherapy continues to play a vital function in colorectal cancer treatment, despite the increasing surgical skill of physicians. The introduction of fluorouracil-based chemotherapy regimens has resulted in a significant prolongation of median survival in patients with mCRC, while the combined use of the molecularly targeted biologic cetuximab has extended median survival to 30 months in sick people with KRAS/NRAS/BRAF wild-type mCRC [2]. The signaling route of the epidermal growth factor receptor (EGFR) holds a pivotal position in the advancement of colorectal cancer, and cetuximab inhibits EGFR by concentrating on the extracellular structural domain (ECD) [3], thereby exerting an inhibitory effect on tumor cells. However, cetuximab treatment isn't effective for all patients; indeed, a mere 10% of those with non-specific chemotherapy who have refractory metastatic colorectal cancer show positive responses to cetuximab alone [4]. Due to primary resistance, patients with cancer-causing mutations

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in the RAS gene family—particularly KRAS and NRAS—do not profit from anti-EGFR treatment [5, 6], and even if initial therapy is effective, acquired resistance develops within 3–18 months [7]. Currently, scholars have proposed a number of primary or acquired resistance mechanisms in RAS wild-type (WT) colorectal cancer [8], such as genomic alterations of downstream regulators of the EGFR signaling pathway (such as RAS, BRAF, and PIK3CA), mutations in the extracellular domain of the EGFR, and triggers of other RTKs (receptor tyrosine kinases) such as ERBB2 or MET and their signaling pathways, etc. [6, 9, 10]. However, the principle of cetuximab resistance in chemotherapy-refractory mCRC patients is still incompletely understood; therefore, exploring the underlying molecular mechanisms of cetuximab resistance is of great significance and will provide new therapeutic ideas for cetuximab-resistant mCRC patients.

Homeobox genes were first identified in Drosophila melanogaster, which encode homologous proteins that play a major role as regulatory transcription factors in the developing embryo [11]. Recent research has shown that the HOX gene has a strong impact on the development, invasion, and metastasis of many cancers, such as colorectal, prostate, lung, breast, gastric, renal cancers, etc. [12–14]. HOXB8 is a member of the HOX family [15] and has been found to be overexpressed in different developmental stages of colorectal cancers, including precancerous polyps stages [16]. Our previous study has demonstrated that HOXB8 overexpression significantly activates p-STAT3, whereas silencing HOXB8 decreases p-STAT3 expression, thereby inhibiting the multiplication and metastasis of CRC cells [17].

Proteins known as signal transducer and activator of transcription (STAT) belong to the cytoplasmic transcription factor group, with the mammalian STAT family encompassing STAT1, STAT3, STAT4, STAT5a, STAT5b, and STAT6. Among them, the STAT3 signaling pathway plays a significant part in tumor formation, metastasis, and drug resistance [18]. Lo et al.'s [19] study showed that inhibition of STAT3 enhances the efficacy of anti-EGFR drugs, which is consistent with our finding that HOXB8 knockdown improves cellular drug sensitivity by down-regulating p-STAT3 expression, further confirming that STAT3 plays a critical role in drug resistance. Haura et al. [20] demonstrated that sustained STAT3 phosphorylation in NSCLC patients may lead to primary tumor resistance to EGFR inhibitors. In light of the aforementioned research, we postulated that HOXB8 could be a crucial component of the cetuximab resistance mechanism. HOXB8 may render colorectal cancer cells resistant to cetuximab by activating the STAT3 pathway.

In the current investigation, we discovered that cetuximab-resistant colorectal cancer cells have elevated expression of HOXB8 and p-STAT3. HOXB8 knockdown made CRC cells more susceptible to cetuximab therapy by decreasing the expression of p-STAT3, conversely, overexpression of HOXB8 had the opposite impact. By reducing the expression of p-STAT3, HOXB8 knockdown dramatically reduced cell proliferation in cetuximab-resistant colorectal cancer cells. This discovery suggests HOXB8 might offer a promising avenue for managing cetuximab-resistant colorectal cancer treatments.

2 Methods

2.1 Cell culture

Human cell lines for colorectal cancer HCT-116, CACO-2, and SW48 were provided from the Chinese Academy of Sciences (CAS) Cell Bank (Shanghai, China). HCT-116 was kept in RPMI-1640 medium that was added with 100 U/ml of penicillin/streptomycin (Solarbio, Beijing, China) and 10% fetal bovine serum (Gibco, Eggenstein, Eggenstein). The same supplements were used to sustain SW48 and CACO-2 cells in DMEM. Each cell was kept in an incubator set at 37°C, containing 5% CO_2. Cells were harvested and propagated using 0.25% trypsin when 80–100% confluence was achieved for subsequent experiments.

2.2 Reagents and antibodies

Cetuximab was obtained from Merck KGaA, Germany. PCR primers were purchased from Generay Biotechnology (Shanghai, China). The antibody HOXB8 was provided by Affinity Biosciences. Phosphorylated STAT3 (p-STAT3) antibody, STAT3 antibody, and GAPDH antibody were obtained from Proteintech Group (Chicago, USA). Takara (Shiga, Japan) provided a quantitative real-time PCR (qRT-PCR) kit. The TRIzol reagent was purchased from Thermo Fisher (Waltham, USA).

2.3 Cell proliferation assay

Cell viability was evaluated by MTT analysis. According to pharmacokinetics, the maximum in vivo plasma concentration Cmax Mean (SD) = 306 (63) μg/ml when cetuximab administration occurred at a dose of 500 mg/m^2 every 14 days [21]. HCT-116, CACO-2, and SW48 cells were inoculated in 96-well plates (HCT-116, CACO-2, and SW48 require 6000, 6000, and 4000 cells per well, respectively) overnight, and after that subjected to various cetuximab concentrations (cetuximab concentrations for HCT-116, CACO-2 cell lines were: 0, 10, 50, 100, 200, 400, 800, 1000, 2000 μg/ml, and cetuximab concentration for SW48 cell line: 0, 10, 20, 40, 80, 100, 150, 200, 300 μg/ml) for 48 h. Following the addition of the MTT reagent to each well, the mixture had been kept at 37°C with 5% CO_2. Post 4 h, the culture media was thrown away, and then each well received 150 μl of Dimethyl sulfoxide (DMSO) to dissolve the formaldehyde dimethyl sulfoxide. Using a Multi-function Enzyme Labeler iD3 (MD, USA), the absorbance of OD 490 nm was determined.

2.4 Colony formation test

Spread at a density of 1000 per well on 6-well plates, three cell lines were then cultured for the whole night. After treatment with varying doses of cetuximab (0, 10, 20, 40, 80, 100 μg/ml) for 48 h, the cells were continued to be seeded at 37°C in a 5% CO_2 incubator for 7–10 days. Using 4% paraformaldehyde, the cells were fixed for 10 min, and then the colonies were stained with 0.1% crystal violet at ambient temperature for 20 min. Image J software was used for calculating the colonies.

2.5 Wound healing test

Cell migration and motility were evaluated through the wound healing assay. In 6-well plates, HCT-116, CACO-2, and SW48 were cultivated as confluent monolayers. Applying a 10 μl pipette tip, the cells were gently scraped, and 1× PBS was used to rinse them in order to eliminate any debris. Cell migration was assessed after treatment with different concentrations of cetuximab (0, 10, 50, 100, 200, 300 μg/ml) for 48 h, and changes in migration size were observed with an inverted microscope.

2.6 Transfection

GenePharma (Shanghai, China) provided the negative control siRNA (NC-siRNA) and the HOXB8-targeting siRNA. To knock down HOXB8 in the HCT-116, CACO-2 cell line, LipoRNAi (Beyotime, China) was used to transduce the HOXB8 siRNA, which was transfected into cells. The effectiveness of transfection was assessed by protein blotting analysis and qRT-PCR. The following sequences made up the negative control siRNA and HOXB8 siRNA: siHOXB8-1 (sense 5′-GUUCCUAUUUAAUCCCUAUTT-3′, antisense 5′-AUAGGGAUUAAAUAGGAACTT-3′); siHOXB8-2 (sense 5′-GCAAUUUCUACGGCUACGATT-3′, antisense 5′-UCGUAGCCGUAGAAAUUGCTT-3′); negative control siRNA (sense 5′-UCUUUCCGAACGUGUCACGUTT-3′, antisense 5′-ACGUGACACGUUCGGAGAATT-3′).

2.7 Building cell lines overexpressing HOXB8

Using the HOXB8 overexpression vector, while employing an empty lentiviral vector as control, we successfully established the SW48 overexpressing HOXB8. To generate lentiviral particles, the HOXB8 overexpression vector, the pVSV-G vector, and the pGag/Pol vector were transfected into the carrier 293T cells with Lipofectamine™ 3000 reagent in accordance with the manufacturer's protocols (Invitrogen). The virus was gathered post-48 h, and the stable HOXB8 overexpression cell line was established by infecting the SW48 cells with 10 μg/mL polybrene. In 8 h, the culture medium was switched. The efficiency of transfection was assessed using Western blotting and qRT-PCR techniques.

2.8 Western blot

Using RIPA lysis buffer, total proteins were recovered from the cells, and the Bradford process was used to calculate protein concentration. Equal quantities of protein (80 μg) were collected by 10% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and shifted to PVDF membranes (Merck Millipore, Germany). The membranes were sealed for 1.5 h at ambient temperature using 5% skim milk, and then they were treated for an overnight period at 4°C with primary

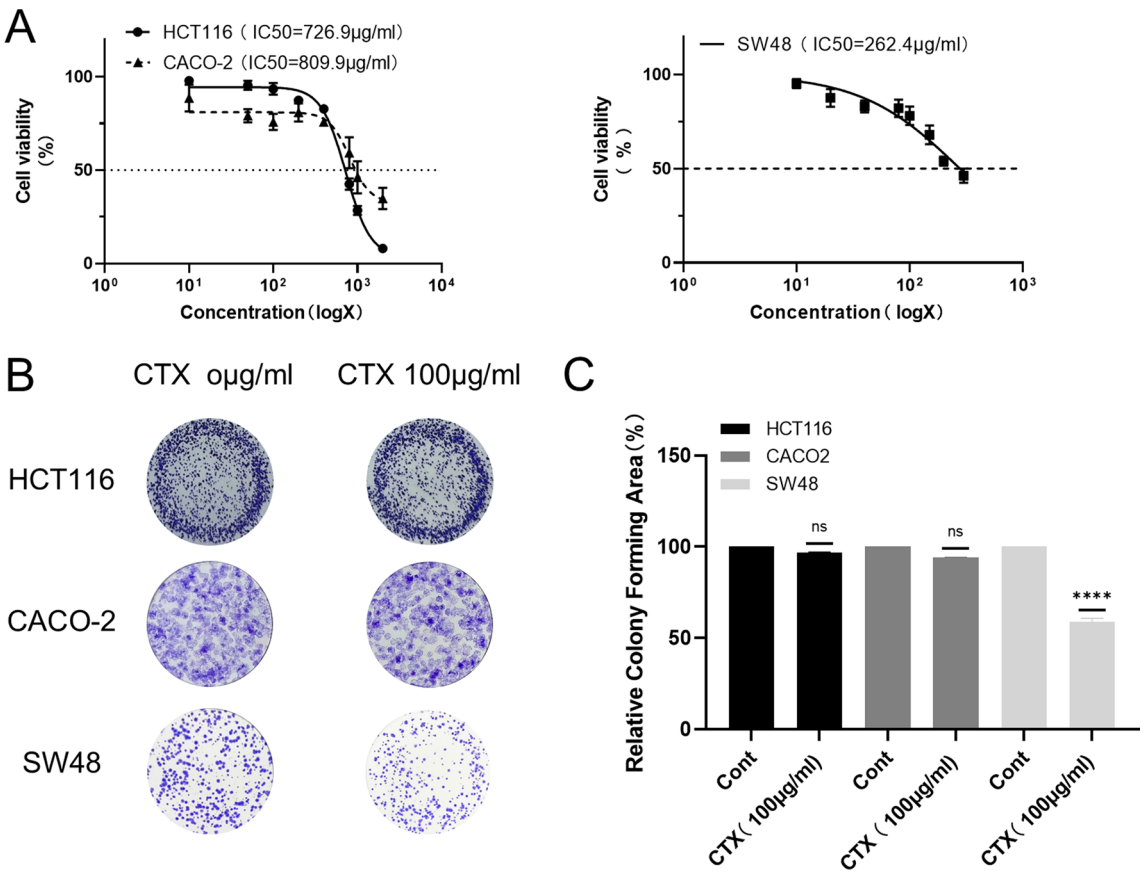


Fig. 1 Three types of cells respond differently to cetuximab **A** SW48, HCT116, and CACO2 cells were handled with varying dosages of cetuximab (0, 10, 50, 100, 200, 400, 800, 1000, and 2000 μg/ml) for 48 h, and the MTT test was used to determine cell viability. The circle indicates HCT 116, the triangle indicates CACO2, and the box indicates SW48, relative cell viability at different concentrations of cetuximab. **B**, **C** Colony formation assays were performed in three cell types after treatment with 100 μg/ml cetuximab. **** $p < 0.0001$ and ns (not significant), cetuximab (100 μg/ml) vs. control

antibodies (HOXB8 1:1000), p-STAT3 (dilution 1:1000), STAT3 (dilution 1:1000), and GAPDH (dilution 1:1000). Following TBST washing, the membranes were incubated at ambient temperature for 1 h with secondary antibodies labeled with horseradish peroxidase (HRP). Protein strips were observed using an Enhanced Chemiluminescence (ECL) development solution (Bio-Rad, Hercules, CA, USA).

2.9 Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from cells was separated using the TRIzol reagent. The integrity and amount of RNA were evaluated using NanoDropOne (Thermo Fisher Scientific, USA). The PrimeScript™ RT Master Mix (Takara, Shiga, Japan) facilitated the reverse transcription of total RNA into cDNA. RT-PCR was then performed on a Mastercyker ep realplex from Eppendorf, Germany, using TB Green Premix Ex taq™ II reagent. $2^{-\Delta\Delta CT}$ method (Livak and Schmitgen 2002) was used to standardize the relative expression of mRNA levels. Below is a list of primers used in qRT-PCR analysis: HOXB8 (F: 5′-TAAGCGGCGATTCGAGGTAT-3′, R: 5′-TGTTTCTCCAGCTCCTG-3′); GAPDH (F: 5′-TCAAGGCTGAGAACGGGAAG-3′, R: 5′-GACTCCACGACGTACTCAGC-3′).

2.10 Statistical analysis

The data was shown as the mean SD derived from three separate tests. All data were examined using the *t* test or one-way analysis of variance (ANOVA) between various groups using the software programs SPSS 21.0 and GraphPad Prism 8.0. Variations were deemed to hold statistical significance when the *p* value fell below 0.05.

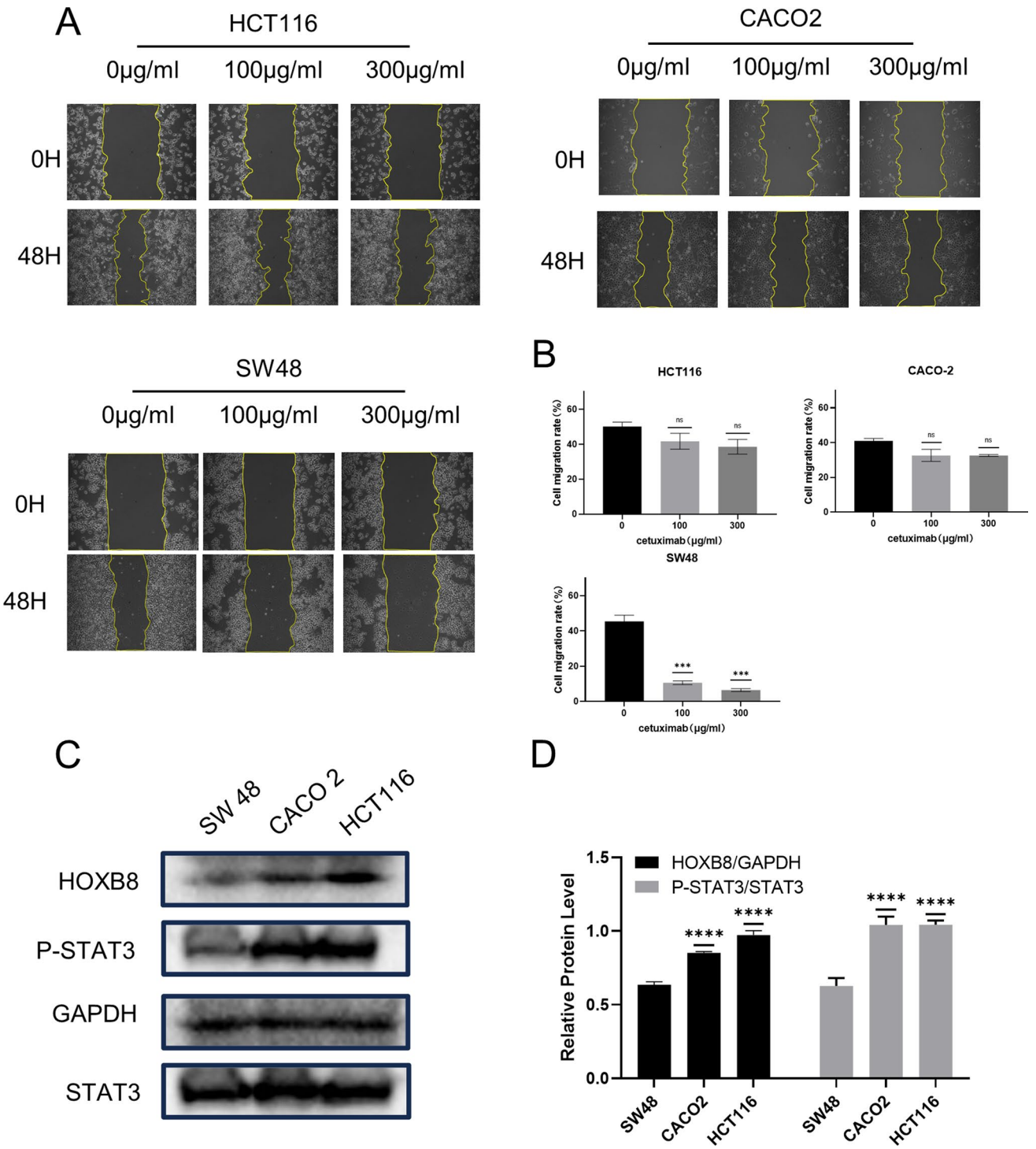


Fig. 2 HOXB8 expression was up-regulated in cetuximab-resistant cells **A**, **B** In three kinds of cells, cetuximab dramatically reduced SW48 cells' capacity to migrate and invade, and had no significant effect on HCT116 and CACO2 cells. *** $p < 0.001$, cetuximab experimental group in contrast with the control group; **C**, **D** The evaluation of HOXB8, p-STAT3, GAPDH, and STAT3 levels in three cells was conducted using protein blotting analysis. **** $p < 0.0001$, CACO2 HCT116 compared with SW48, respectively

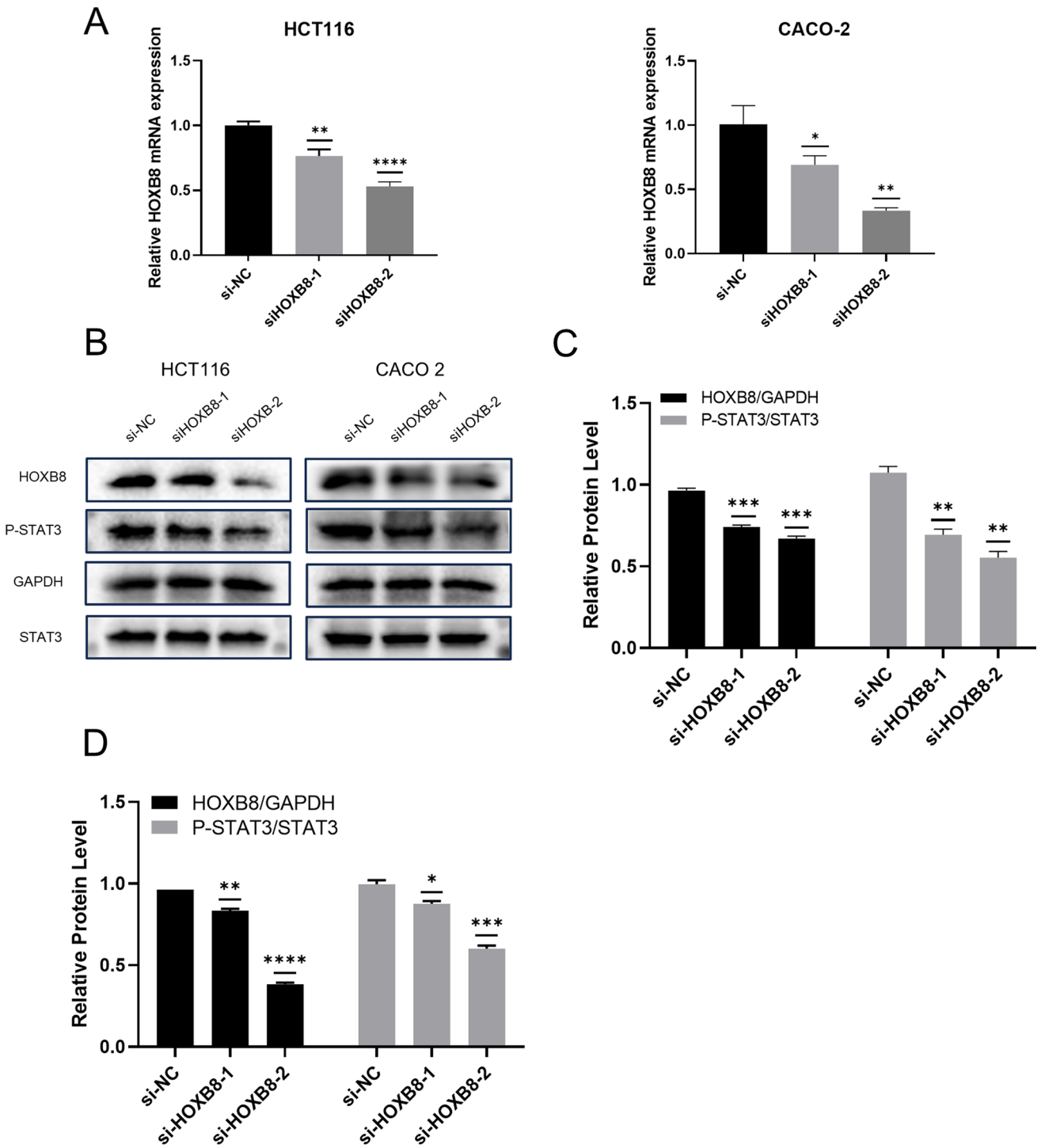


Fig. 3 Silencing HOXB8 inhibits the STAT3 pathway **A** The expression levels of HOXB8 mRNA were quantified through qRT-PCR 24 h following the introduction of three siRNAs into CACO2 and HCT116 cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, siHOXB8-1 or siHOXB 8–2 relative to si-NC. **B**–**D** After silencing HOXB8 in CACO2 and HCT116 cells for 24 h, HOXB8, GAPDH, STAT3, and p-STAT3 protein expression was determined by western blot. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, siHOXB8-1 or siHOXB8-2 relative to si-NC

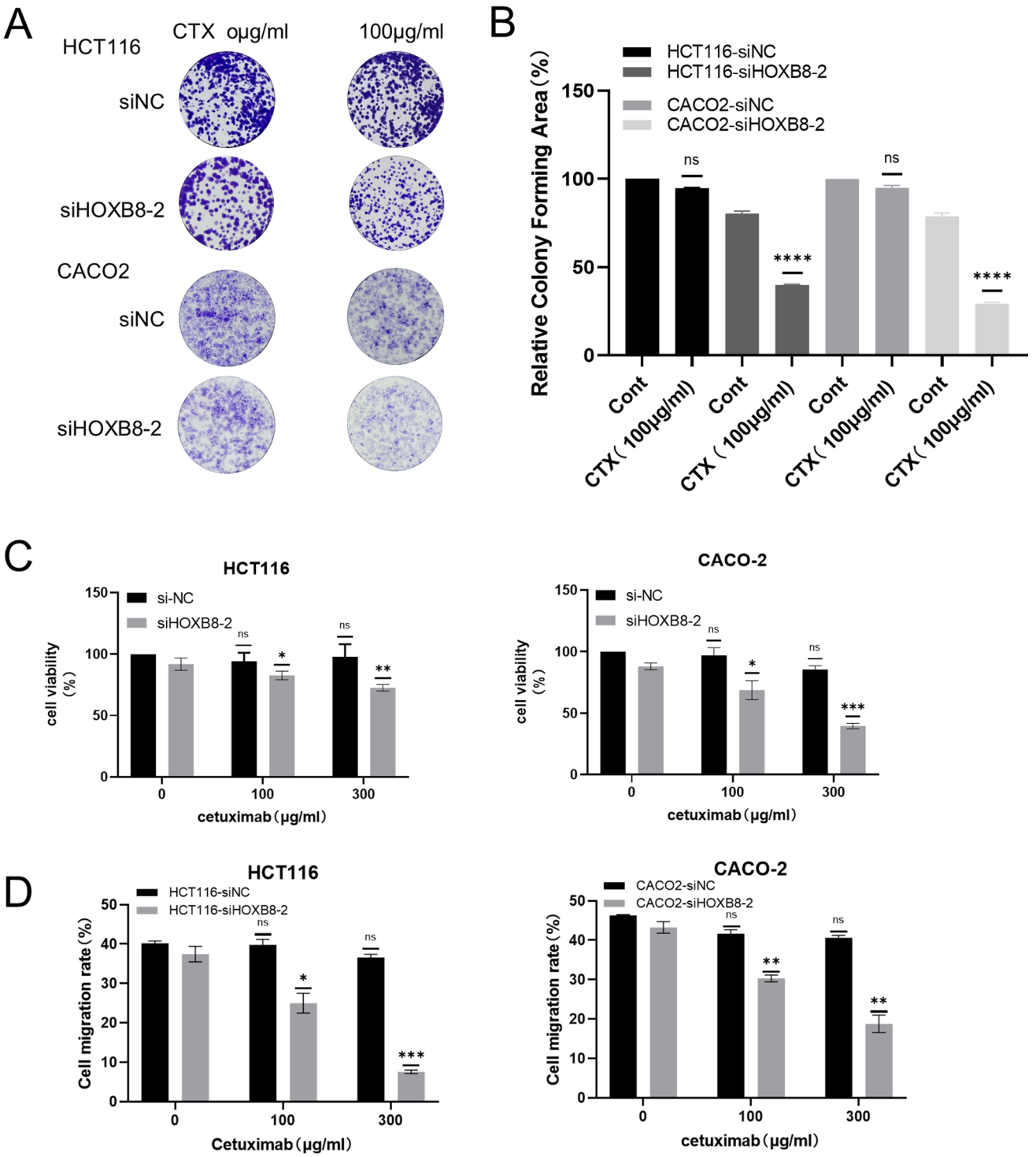


Fig. 4 By inhibiting STAT3 signaling, HOXB8 knockdown increases the susceptibility of HCT116 and CACO2 cells to cetuximab (**A-B**) HCT116 and CACO2 cells with knockdown of HOXB8 were handled with varying dosages of cetuximab (0, 100 μg/ml). The colony formation area was evaluated and analyzed using ImageJ software. **** $p < 0.0001$ and ns (not significant) for siHOXB8-2 relative to siNC. **C** HCT116 and CACO2 cells with knockdown of HOXB8 were delivered with diverse levels of cetuximab (0, 100, and 300 μg/ml), and after a 24-h period, the MTT test was used to determine the cells' relative vitality. **D** HCT116 and CACO2 cells with knockdown of HOXB8 were dealed with distinct concentrations of cetuximab (0, 100, 300 μg/ml), and their cell migration ability was determined. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and ns, cetuximab experimental group relative to control group

Fig. 5 HOXB8 overexpression reduces cetuximab sensitivity in SW48 cell lines **A**–**C** Following HOXB8 overexpression in SW48 cells, mRNA ▶ and protein levels were assessed using qRT-PCR and protein blotting techniques. ** $p < 0.01$, OE group relative to EV group. **D** SW48 cells overexpressing HOXB8 as well as control cells were dealed with diverse levels of cetuximab (0, 100, and 300 µg/ml). The relative cell viability was determined using the MTT test. An experiment for wound healing was performed to evaluate their capacity for cell migration. *** $p < 0.001$, ** $p <$ Lo0.01 and ns (not significant) for cetuximab experimental group relative to control group. **E**, **F** SW48 cells overexpressing HOXB8 were dealed with varying dosages of cetuximab (0, 100 µg/ml). The colony formation area was evaluated and analyzed using ImageJ software. **** $p < 0.0001$ and ns (not significant), cetuximab experimental group relative to control group

These methods ensured that we were able to systematically evaluate the function of HOXB8 in cetuximab resistance in colorectal cancer cells.

3 Results

3.1 Cetuximab-resistant cell lines have significant levels of HOXB8 expression

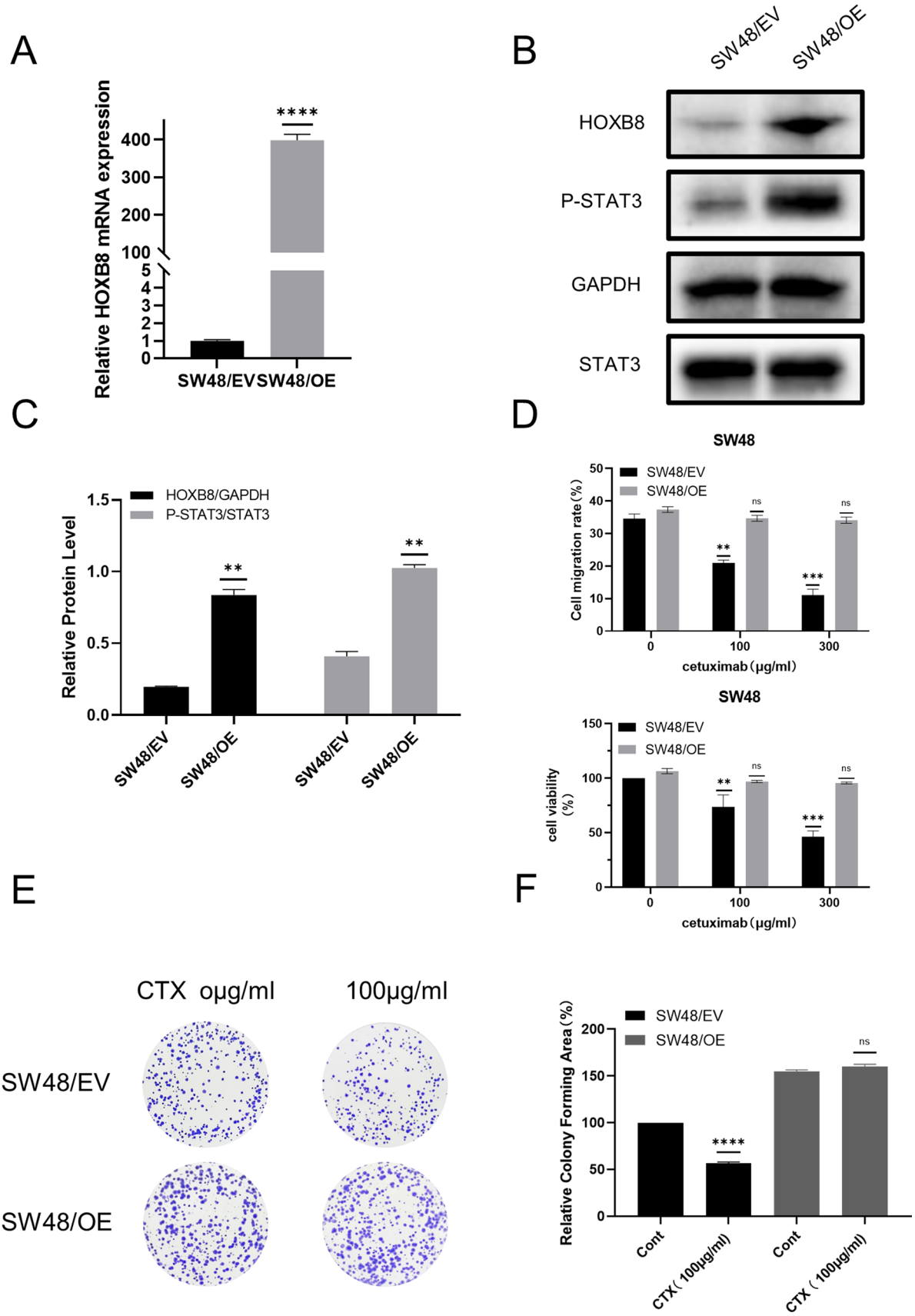
KRAS-mutated CRC cell lines are resistant to cetuximab due to primary resistance [7] and only about 50% of KRAS-wild-type cells are effective against cetuximab [2]. Consequently, we chose three specific intestinal cancer cell lines—HCT-116 (KRAS-MUT), CACO-2 (KRAS-WT), and SW48 (KRAS-WT)—and conducted MTT assays on these cells following their treatment with different levels of cetuximab. The findings demonstrated that cetuximab dose-dependently suppressed the growth of SW48 cells with an IC50 value of 262.4 µg/ml. Meanwhile, HCT116 and CACO2 cells showed significant resistance to cetuximab (IC50 > 306 µg/ml) (Fig. 1A). This shows that SW48 cells are more sensitive to cetuximab, whereas HCT116 and CACO2 cells show significant resistance. The colony formation experiment demonstrated that cetuximab administration greatly suppressed the capacity of SW48 cells to form colonies while having no effect on HCT-116 or CACO2 cells (Fig. 1B, C). Furthermore, the wound healing assay confirmed that treatment with cetuximab markedly reduced the migratory ability of SW48 cells and had almost no effect on the migratory ability of HCT-116 and CACO2 cells (Fig. 2A, B). These findings imply that HCT-116 and CACO2 cells are resistant to cetuximab. Additionally, we detected and compared the three cells' expressions of p-STAT3 and HOXB8. HOXB8 and p-STAT3 expression was significantly elevated in HCT116 and CACO2 cells in comparison with SW48 cells (Fig. 2C, D), suggesting that these proteins are participate in cetuximab resistance in colorectal tumor cells.

3.2 Knockdown of HOXB8 improved the cetuximab sensitivity in CACO2 and HCT116 cells

To examine the impact of HOXB8 on cetuximab-mediated growth suppression in colorectal cancer cells, we used two separate siRNAs to knock down HOXB8 in HCT116 and CACO2 cells (Fig. 3A). The knockdown efficacy has been verified by qRT-PCR and protein blotting testing. Following siRNA transfection, there was a substantial reduction in HOXB8 mRNA and protein levels. Crucially, the suppression of HOXB8 markedly reduced the levels of p-STAT3 in HCT116 and CACO2 cells (Fig. 3B–D). Reducing HOXB8 levels amplified cetuximab's suppressive impact on colon cancer cells' growth, movement, and ability to form colonies (Fig. 4A–D), indicating that colon cancer cells' cetuximab sensitization is tightly related to the HOXB8-STAT3 axis.

3.3 HOXB8 overexpression decreases sensitivity of SW48 cells to cetuximab

To learn more about whether HOXB8 contributes much to the resistance of colon cancer cells to cetuximab, we established the SW48 cell line that stably overexpresses HOXB8. When comparing SW48/HOXB8 to equivalent control cells, there was a substantial increase of HOXB8 mRNA and protein expression (Fig. 5A–C). Subsequent research revealed that overexpressing HOXB8 enhanced the proliferation of colorectal cancer cells and considerably reduced cetuximab's growth-inhibiting impact in SW48 cells (Fig. 5D). Similarly, overexpressing HOXB8 improved SW48 cells' capacity to form colonies and reversed the inhibition of colony-forming ability of SW48 cells by cetuximab (Fig. 5E, F). Furthermore, the excessive expression of HOXB8 diminished the suppressive impact of cetuximab on the migration of SW48 cells (Fig. 5D). As expected, HOXB8 overexpression significantly induced p-STAT3 expression, suggesting a momentous function of the HOXB8-STAT3 axis in cetuximab resistance in colorectal cancer.


A
Relative HOXB8 mRNA expression
****
SW48/EV
SW48/OE
B
SW48/EV
SW48/OE
HOXB8
P-STAT3
GAPDH
STAT3
C
HOXB8/GAPDH
P-STAT3/STAT3
Relative Protein Level
D
SW48
SW48/EV
SW48/OE
Cell migration rate(%)
cetuximab(μg/ml)
SW48
cell viability (%)
E
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Relative Colony Forming Area (%)
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CTX(100μg/ml)

4 Discussion

EGFR is a tyrosine kinase receptor (RTK), which is expressed in a wide range of cancers and 60%-80% of colorectal cancers and has a significant part in the growth of tumors [22]. Resistance to anti-EGFR monoclonal antibodies can be characterized as primary and acquired resistance mechanisms, which largely focus on the MEK-ERK and PIK3CA-AKT signaling pathways. The intrinsic ATPase activity of the RAS gene is impaired by point mutations in exon 2 codons 12 and 13 of the KRAS gene, which results in persistent activation of the MAPK pathway against EGFR inhibition [6]. Furthermore, it has been demonstrated that, in a small percentage of patients, KRAS amplification is the cause of cetuximab resistance, which is linked to a low response to anti-EGFR treatment [23, 24]. For individuals with colorectal cancer, the BRAF V600E mutation is an indicator of a bad prognosis [25], and by circulating tumor DNA (ctDNA) research, the BRAF mutation has also been introduced as a cause of the first-time anti-EGFR mechanism of acquired resistance in patients responding to treatment [26]. Activating mutations in PIK3CA and loss of PTEN have been shown to be the causes of primary resistance to cetuximab in the PIK3CA/AKT/mTOR signaling pathway [27, 28]. As a member of the ErbB family, HER2 amplification can generate resistance to EGFR antibodies by bypassing stimulation of the MEK-ERK signaling pathway regardless of EGFR signaling [29]. Anyway, the reasons for cetuximab resistance in colorectal cancer are still partially unknown, and in this research, we proved that HOXB8 was strongly linked to cetuximab resistance in colon cancer cells through activation of the STAT3 pathway. Significantly, by blocking STAT3 signaling, HOXB8 silencing was able to increase the inhibitory impact of cetuximab in colorectal cancer cells, indicating a crucial role for HOXB8 in primary drug-resistant colorectal cancer cells.

A range of cytokines and growth factor receptors, such as the epidermal growth factor receptor, activate the transcription factor STAT3 [30, 31]. Continuous activation of the STAT3 pathway has been detected in colon cancer, breast cancer, head and neck squamous carcinoma, and other malignancies in which anti-EGFR drugs are clinically applied [32], and phosphorylated STAT3 conveys signals from the EGFR to the nucleus, where it begins to transcribe a variety of pro-carcinogenic genes [30, 33]. Research has shown that p-STAT3 may be a useful predictor of how well anti-EGFR treatment works for patients with colorectal cancer [34]. Furthermore, co-targeting the STAT3 pathway may be a useful therapeutic approach since it enhances the anti-tumor activity of cetuximab in EGFR inhibitor-resistant models of squamous head and neck and bladder cancers [35]. Based on this, we postulated that the HOXB8-STAT3 axis might participate in the development of cetuximab resistance in colorectal cells. As expected, HOXB8 and p-STAT3 expression rose in cetuximab-resistant colon cancer cells, whereas both were decreased in cetuximab-sensitive colorectal cancer cells. Silencing or overexpression of HOXB8 both resulted in changes in the transcript level of p-STAT3 in colorectal neoplasms cells, thereby affecting the cellular response to cetuximab.

Finally, our research demonstrated the important function of HOXB8 in cetuximab-resistant colorectal cancer cells, and these results offer compelling evidence in favor of using HOXB8 as an innovative target for the medical therapy of colorectal cancer that is resistant to drugs, and future studies could further explore the prospects for the clinical application of HOXB8 inhibitors. Of course there are still many shortcomings in this study, such as the specific mechanism of HOXB8-mediated STAT3 phosphorylation was not thoroughly investigated, and animal experiments were not carried out for validation, etc., which need to be further investigated.

Author contributions RC and STL were in charge of the study hypothesis and design. YNL and HL conducted the experiments, collected the data, and authored the manuscript. ZSJ conducted the experiments and analyzed the data. QZ performed the statistical analysis. Every author reviewed and sanctioned the final version of the manuscript.

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Data availability Data is provided within the manuscript or supplementary information files.

Code availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63.
2. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. JAMA. 2021;325(7):669–85.
3. Schirripa M, Lenz HJ. Overcoming resistance to anti-EGFR therapy—where do we stand? Nat Rev Gastroenterol Hepatol. 2016;13(5):258–9.
4. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004;351(4):337–45.
5. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Can Res. 2006;66(8):3992–5.
6. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010;11(8):753–62.
7. Misale S, Di Nicolantonio F, Sartore-Bianchi A, et al. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. Cancer Discov. 2014;4(11):1269–80.
8. Parseghian CM, Napolitano S, Loree JM, et al. Mechanisms of innate and acquired resistance to anti-EGFR therapy: a review of current knowledge with a focus on rechallenge therapies. Clin Cancer Res. 2019;25(23):6899–908.
9. Bardelli A, Corso S, Bertotti A, et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov. 2013;3(6):658–73.
10. Bertotti A, Migliardi G, Galimi F, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1(6):508–23.
11. Lewis EB. A gene complex controlling segmentation in Drosophila. Nature. 1978;276(5688):565–70.
12. Haria D, Naora H. Homeobox gene deregulation: impact on the hallmarks of cancer. Cancer Hallm. 2013;1:67–76.
13. Hur H, Lee JY, Yun HJ, et al. Analysis of HOX gene expression patterns in human breast cancer. Mol Biotechnol. 2014;56:64–71.
14. Ding WJ, Zhou M, Chen MM, et al. HOXB8 promotes tumor metastasis and the epithelial–mesenchymal transition via ZEB2 targets in gastric cancer. J Cancer Res Clin Oncol. 2017;143:385–97.
15. Wang GG, Calvo KR, Pasillas MP, et al. Quantitative production of macrophages or neutrophils ex vivo using conditional Hoxb8. Nat Methods. 2006;3(4):287–93.
16. Vider BZ, Zimber A, Hirsch D, et al. Human colorectal carcinogenesis is associated with deregulation of homeobox gene expression. Biochem Biophys Res Commun. 1997;232(3):742–8.
17. Wang T, Lin F, Sun X, et al. HOXB8 enhances the proliferation and metastasis of colorectal cancer cells by promoting EMT via STAT3 activation. Cancer Cell Int. 2019;19:1–12.
18. Zou S, Tong Q, Liu B, et al. Targeting STAT3 in cancer immunotherapy. Mol Cancer. 2020;19:1–19.
19. Lo HW, Cao X, Zhu H, et al. Constitutively activated STAT3 frequently coexpresses with epidermal growth factor receptor in high-grade gliomas and targeting STAT3 sensitizes them to Iressa and alkylators. Clin Cancer Res. 2008;14(19):6042–54.
20. Haura EB, Sommers E, Song L, et al. A pilot study of preoperative gefitinib for early-stage lung cancer to assess intratumor drug concentration and pathways mediating primary resistance. J Thorac Oncol. 2010;5(11):1806–14.
21. Tabernero J, Ciardiello F, Rivera F, et al. Cetuximab administered once every second week to patients with metastatic colorectal cancer: a two-part pharmacokinetic/pharmacodynamic phase I dose-escalation study. Ann Oncol. 2010;21(7):1537–45.
22. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005;5(5):341–54.
23. Valtorta E, Misale S, Sartore-Bianchi A, et al. KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. Int J Cancer. 2013;133(5):1259–65.
24. Mekenkamp LJM, Tol J, Dijkstra JR, et al. Beyond KRAS mutation status: influence of KRAS copy number status and microRNAs on clinical outcome to cetuximab in metastatic colorectal cancer patients. BMC Cancer. 2012;12:1–11.
25. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol. 2008;26(35):5705–12.
26. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early-and late-stage human malignancies. Sci Transl Med. 2014;6(224):224ra24.
27. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Can Res. 2009;69(5):1851–7.
28. Seymour MT, Brown SR, Middleton G, et al. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. Lancet Oncol. 2013;14(8):749–59.
29. Yonesaka K, Zejnullahu K, Okamoto I, et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci Transl Med. 2011;3(99):99ra86.
30. Bromberg JF, Wrzeszczynska MH, Devgan G, et al. Stat3 as an oncogene. Cell. 1999;98(3):295–303.

31. Zhong Z, Wen Z, Darnell JE Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science. 1994;264(5155):95–8.
32. Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. Clin Cancer Res. 2002;8(4):945–54.
33. Darnell JE Jr. Validating Stat3 in cancer therapy. Nat Med. 2005;11(6):595–6.
34. Dobi E, Monnien F, Kim S, et al. Impact of STAT3 phosphorylation on the clinical effectiveness of anti-EGFR-based therapy in patients with metastatic colorectal cancer. Clin Colorectal Cancer. 2013;12(1):28–36.
35. Sen M, Joyce S, Panahandeh M, et al. Targeting Stat3 abrogates EGFR inhibitor resistance in cancer. Clin Cancer Res. 2012;18(18):4986–96.