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EGFR/STAT3 signaling mediates the upregulation of CD47 in HPV-positive cervical cancer by activating p65 and exosome transporter RAB31

Xiaoping KE[1,#], Li LI[1,#], Qi YAN[1], Xianjing WANG[2,*], Ping LIU[2,*]

[1]Department of Obstetrics and Gynecology, Yangpu Hospital, School of Medicine, Tongji University, Shanghai, China; [2]Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

**Correspondence: 9756060@qq.com; lpingdoc01@163.com*
#Contributed equally to this work.

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In cervical cancer, the regulatory mechanisms of CD47 and its enrichment in exosomes have not been fully elucidated. In this study, we aim to explore the mechanisms of how EGFR/STAT3 signaling regulates CD47 expression in cervical cancer, and whether p65 or RAB31 is involved in this process. RNA interference was used to knock down the fragment of HPV in cervical cancer cells. EGFR and RAB31 phosphorylation were detected by western blot, and exosomal CD47 was determined by ELISA. EGFR or STAT3 was then knocked down and western blot was used to detect the expression or phosphorylation of EGFR, STAT3, p65, CD47, RAB31, and its related proteins. ChIP-qPCR was used to investigate p65 enrichment in the CD47 promoter region. Our results showed that HPV fragment knockdown reduced the phosphorylation of EGFR and RAB31, and exosomal CD47 expression. Knocking down EGFR inhibited phosphorylation of STAT3 and p65, and expression of RAB31-related proteins. Phosphorylated p65 was bound to the P3 and P7 promoter regions of CD47. EGFR/STAT3 signaling upregulated CD47 expression by phosphorylating p65 and enhanced exosomal CD47 by activated RAB31. Thus, a dual regulatory role of EGFR/STAT3 signaling on CD47 expression and secretion involving transcriptional factor p65 and exosome transporter RAB31 was demonstrated.

Key words: cervical cancer; CD47; HPV; EGFR; STAT3

Cervical cancer is a prevalent malignancy among women, posing a significant threat to the global female population's health. Its onset is intricately linked to persistent infection with high-risk human papillomavirus (HR-HPV) [1, 2]. The oncogenic impact of HR-HPV primarily stems from the activities of numerous oncoproteins encoded within its genome, notably the pivotal functions of E6 and E7. These proteins play a crucial role in the initiation and progression of cervical cancer by targeting the tumor suppressor protein p53 for degradation, thereby disrupting the functions of retinoblastoma protein and transcription factor E2F [1, 3]. In recent years, by targeting programmed cell death protein-1 (PD-1)/programmed cell death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), immunological checkpoint drugs and related immunotherapies have gradually become some of the most effective clinical treatments for cancers [4, 5].

CD47 is a key "do not eat me" signaling molecule of the innate immune system. It is highly expressed in almost all human malignant solid and hematological tumors and is associated with tumor progression. It is therefore an important therapeutic target for cancers [6]. Exosomes are subcellular vesicle-like structures secreted by many cell types in the body in the endosomal sorting complex required for transport-dependent or -independent processes [7–10]. Active molecules, such as proteins, lipids, and miRNAs, contained in the exosomes enter the recipient cells after exosomes are internalized by them, thereby exerting their regulatory effect on recipient cell behavior. Preclinical studies and clinical trials on drugs targeting CD47 and Fc fusion proteins are actively being conducted [11]. Although CD47 can be used as a target for cervical cancer treatment, the regulatory mechanisms of CD47 expression in cancer cells and its enrichment in cancer cell exosomes have not been fully elucidated.

Previous studies have revealed that HR-HPV oncoproteins induce the overexpression and activation of signal transducer and activator of transcription 3 (STAT3) and epidermal growth factor receptor (EGFR) within cervical

cancer cells. This process is strongly associated with a worse prognosis among cervical cancer patients, underscoring the significant roles of STAT3 and EGFR throughout the course of the disease [12]. The present study hypothesized that the EGFR/STAT3 signaling axis may be involved in regulating CD47 expression in cervical cancer cells and its enrichment in exosomes. Prior research has confirmed that there was an extremely important interaction between STAT3 and nuclear factor kappa-B (NF-κB) [13, 14]. The p65 and p50 subunits of NF-κB bind to STAT3 and recruit it to NF-κB and vice versa [15]. In cervical cancer cells, EGFR has the function of STAT3 phosphorylation. HR-HPV oncoproteins may act on NF-κB via the EGFR/STAT3 signaling axis, thereby transcriptionally regulating CD47 expression in cervical cancer cells [13, 16]. The present study used *in vitro* experiments to elucidate whether HR-HPV enhanced p65 phosphorylation and CD47 expression by activating the EGFR-STAT3 signaling pathway. Additionally, the study explored whether the EGFR-STAT3 pathway, when activated by HR-HPV, regulated the expression of RAB31 and promoted CD47 expression in exosomes, consequently fostering cervical cancer progression.

Materials and methods

Experimental reagents. Enzyme linked immunosorbent assay (ELISA) kit (Abcam, #ab314832), p-p65 (Abcam, #ab176647), p-STAT3 (Abcam, #ab126458), LamB1 (Abcam, #ab252351), β-actin (Baililaibio, #BLL-R4404E), Simple-ChIP® Enzymatic Chromatin IP kit (Magnetic Beads, CST, #9003), and Ribo™ Exosome Isolation Reagent (Ribobio, #RN: R11066.5).

***In vitro* culture of human cervical epithelial cells.** HcerEpic human cervical epithelial cells (ATCC® PCS-480-011™) were inoculated into a culture medium containing 10% fetal bovine serum (FBS; Gibco, USA) and 90% high glucose Dulbecco's Modified Eagle medium (DMEM; Gibco, USA) and cultured in an incubator with 5% CO_2 and saturated humidity at 37 °C. The cells were digested with trypsin (Beyotime, China) and passaged when cell confluence reached 80%.

***In vitro* culture of human cervical cancer cells.** HPV-negative cervical cancer cells C33A (ATCC® HTB-31™), HPV18-positive cervical cancer cells HeLa (ATCC® CCL-2™), and HPV16-positive cervical cancer cells SiHa (ATCC® HTB-35™) were used in this study. C33A, HeLa, and SiHa cells were cultured in DMEM containing 10% FBS. All media were supplemented with 1% penicillin-streptomycin (Gibco, USA). Cells were cultured in an incubator at 37 °C, 5% CO_2, and saturated humidity.

Cell transfection. Lipofectamine™ RNAiMAX Transfection Reagent (#13778030) was used for cell transfection of siRNAs. The cells were seeded into a 96-well plate at a concentration of 4×10^4 cells/well, incubated, and cultured *in vitro* until reaching the logarithmic growth phase and 30–50% confluence. Transfection was conducted according to the instructions in the Lipofectamine™ RNAiMAX manual as follows: 50 nmol/l siRNA and 1 μl of Lipofectamine™ RNAiMAX were diluted with 50 μl of Opti-MEM, incubated at room temperature for 5 min, and then mixed. After a 20 min incubation, the siRNA-Lipofectamine™ RNAiMAX mixture was added to the wells containing the cells and Opti-MEM in the culture plate. After 6 h of culture, the medium containing the siRNA-Lipofectamine™ RNAiMAX mixture in the wells was removed and replaced with complete DMEM+10% FBS. Cell transfection efficiency was determined after 1–3 days of culture at 37 °C. The siRNA sequences were as follows: E5 siRNA: sense: 5'-UGGUAUUACUAUUGUGGAUAA-3', antisense: 5'-UUAUCCACAAUAGUAAUACCA-3'; E6 siRNA: sense: 5'-AAAGAGAACUGCAAUGUUU-3', antisense: 5'-UUUCUCUUGACGUUACAAA-3', E7 siRNA: sense: 5'-CACCUACAUUGCAUGAAUA-3, antisense: 5'-GUGGAUGUAACGUACUUAU-3'.

Western blot. Total cellular protein samples were extracted using RIPA lysis buffer (Beyotime, China) and quantified by the bicinchoninic acid assay (BCA) method. Protein samples were first subjected to polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane (Millipore, USA) using the wet transfer method. Next, 5% skimmed milk powder (Beyotime, China) was used for blocking. The corresponding primary antibodies (as listed before) were added and incubated overnight on a shaker at 4 °C. The membrane was then washed three times with Tris-buffered saline and tween 20 (TBST) buffer (Beyotime, China) for 5 min each time. Horseradish peroxidase (HRP) secondary antibodies (Beyotime, China) were added next and incubated at room temperature for 1 h. The membrane was washed three times with TBST buffer (Beyotime, China) for 5 min each time. Subsequently, an ultra-sensitive enhanced chemiluminescence (ECL) luminescence test kit (Beyotime, China) was used for exposure and development. All experiments were technically repeated at least three times, and representative images were shown.

Luciferase reporter assay. The cultured cells were seeded into a 24-well plate at a density of 1×10^5 cells per well 12 h before transfection. The medium was replaced with serum-free DMEM when the cells reached 80% confluence. Then, the cells were transfected using Lipofectamine 2000. The experimental group was transfected with a recombinant luciferase reporter vector containing the enhancer of the wild-type *RAB31* encoding gene and the *STAT3* reporter vector. The control group was transfected with a recombinant luciferase reporter vector containing the enhancer of the mutant *RAB31* encoding gene and the *STAT3* reporter vector. The blank control group was transfected with the corresponding blank vector. After transfection, the cells were cultured in serum-free DMEM for 6 h and then in DMEM containing 10% FBS for 24 h. The luciferase activity was then detected to determine the transcriptional activity of *STAT3* on the *RAB31* encoding gene.

Chromatin immunoprecipitation sequencing (ChIP-seq). SimpleChIP® Enzymatic Chromatin IP kit was used for ChIP-seq. Formaldehyde was added to achieve a final concentration of 1% once the cells reached 80–90% confluence during *in vitro* culture. The culture vessel was gently shaken and incubated at room temperature for 10 min. Subsequently, 10 ml of glycine was added to halt the fixation process. Following gentle shaking, the mixture was incubated at room temperature for 5 min, after which the supernatant was discarded. The cells were washed twice with ice-cold phosphate-buffered saline (PBS) buffer. Then, 1 ml of pre-cooled cell scraping solution (5 µl of protease inhibitor cocktail II and 1 mL of pre-cooled PBS) was added. Next, the solution was transferred to a 1.5 ml centrifuge tube and centrifuged at 800×g for 3 min at 4 °C. The supernatant was then discarded. The cells were resuspended by adding 1 ml of ice-cold cell lysis buffer (5 µl of protease inhibitor cocktail II and 1 ml of pre-cooled SDS lysis buffer). The chromatins were broken into fragments of ~200–1,000 bp using an ultrasonic disruptor (5 watts, four times, 10 s/time, 40 s interval between every two sonications, and operating on ice). After centrifuging at 4 °C and 100,000×g for 10 min, the supernatant was transferred to a new centrifuge tube, and an ultrasonic shearing effect was detected. STAT3 antibodies or the corresponding control antibodies were added and incubated overnight on a shaking table at 4 °C. Then, 60 µl of protein G Sepharose beads were added to the antibody/chromatin complex, shaken at 4 °C (150×g for 1 h), and centrifuged at 6,500×g for 1 min, after which the supernatant was discarded. Low-salt, high-salt, lithium chloride, and Tris-ethylenediamine tetraacetic acid (TE) washing buffers were added in sequence and centrifuged at 6,500×g for 1 min. The supernatant was then discarded. The protein/DNA complex was eluted and the DNA was subsequently de-crosslinked and purified. Finally, 10 µl of PCR products were used for sequencing. DNA sequences bound to STAT3 were also analyzed.

Exosome isolation and ELISA. Ribo™ Exosome Isolation Reagent (Ribobio, #RN: R11066.5) was used to isolate cell exosomes according to the manufacturer's instructions. The cells were harvested and subjected to centrifugation at 2,000×g for 30 min at room temperature to eliminate residual cells and debris. Subsequently, the supernatant was transferred to a fresh centrifuge tube and supplemented with one-third of the Ribo™ Exosome Isolation Reagent volume. The sample was mixed thoroughly and stored in a refrigerator at 4 °C overnight. Then, the mixture was centrifuged at 1,500×g for 30 min at 4 °C the supernatant was discarded, and a small portion of exosomes was retrieved. These steps were repeated using the same centrifuge tube until all exosomes were successfully collected. Extracellular vesicles were quantified using nanoparticle tracking analysis (NanoSight NS300) to determine particle concentration (particles/ml) prior to ELISA, ensuring equivalent exosome numbers per sample. Size distribution was analyzed via dynamic light scattering (DLS), confirming a mode diameter of 90–130 nm consistent with exosome standards. Enzyme linked immunosorbent assay kit was used for ELISA. Microplates were coated with capture antibody (overnight, 4 °C), blocked with 5% BSA (2 h, 37 °C), and incubated with samples. After washing, biotinylated detection antibody and streptavidin-HRP were added sequentially. TMB substrate (15 min) was stopped with H_2SO_4, and absorbance (450 nm) was measured. Antigen levels were quantified via standard curve (4PL regression). Triplicates and controls were included.

Ethical statement. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethics Committee of Yangpu Hospital (No. YZX2021100103B) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis. Statistical analysis was conducted using SPSS 19.0. Each experiment was repeated independently three times, and the mean was taken as the result. The data were expressed as the mean ± standard error of the mean. One-way ANOVA was used to analyze and compare data from more than three groups, while two-tailed Student's t-test was used for single comparisons between two groups. Statistical significance was considered at $p<0.05$.

Results

HPV inhibited EGFR and RAB31 phosphorylation in cervical cancer cells. In order to observe the impact of HPV on EGFR and RAB31 phosphorylation in cervical cancer cells, and investigate the impact of E5, E6, or E7 protein on exosomal CD47 expression, the E5, E6, or E7 HPV fragment was knocked down (Figure 1A). E5, E6, or E7 knockdown significantly weakened the effect of HPV on EGFR and RAB31 phosphorylation in HPV-positive cervical cancer cells (HeLa and SiHa cells; Figure 1B). In addition, ELISA results revealed that E5, E6, or E7 knockdown inhibited exosomal CD47 transport and significantly reduced exosomal CD47 expression (Figure 1C).

EGFR downregulation inhibited STAT3 and p65 phosphorylation and RAB31 signaling pathway in cervical cancer cells. Recent studies have extensively documented the positive regulatory role of EGFR on STAT3. The present study hypothesized that EGFR influenced the RAB31 signaling pathway and CD47 expression by regulating STAT3 and p65, thus regulating CD47 expression in exosomes in cervical cancer cells. Here, we used human cervical epithelial cells HcerEpic as a control and detected these proteins and pathways in HPV-negative C33A cells and HPV-positive HeLa and SiHa cells, which are widely used cell lines for studying cervical cancer. First, EGFR levels were detected in both cervical epithelial and cancer cells. A higher level of EGFR phosphorylation was found in cervical cancer cells, particularly in HPV-positive cells, compared with

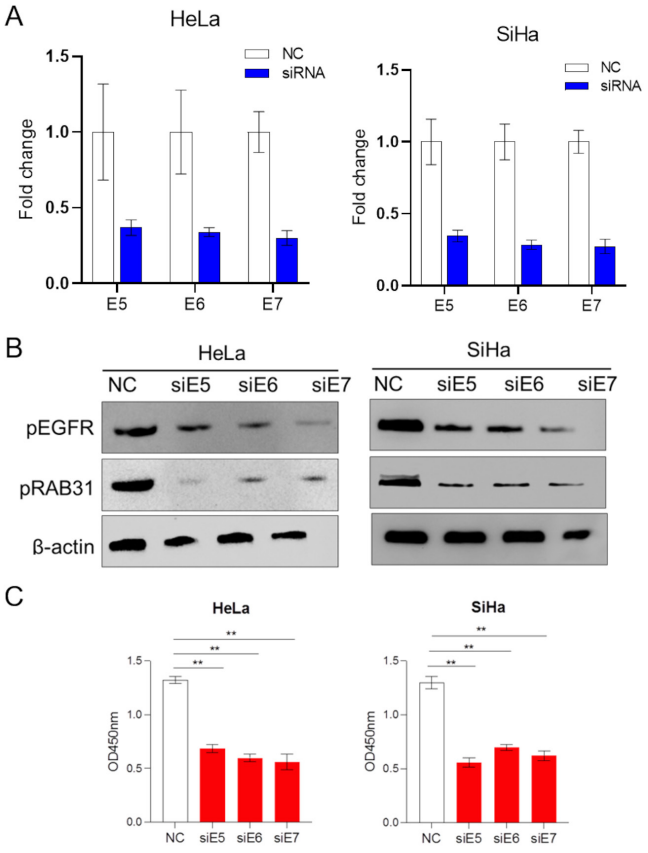

Figure 1. HPV E5, E6, or E7 fragment knockdown inhibits EGFR and RAB31 phosphorylation and exosomal CD47 expression. A) E5/E6/E7 knockdown validated by qPCR assays. B) Effect of E5/E6/E7 knockdown on EGFR and RAB31 phosphorylation levels. C) ELISA results show that E5, E6, and E7 protein knockdown reduced exosomal CD47 transport. **p<0.01

cervical epithelial cells, which showed a positive association with the expression of the RAB31 signaling pathway proteins (Figure 2A). These results suggested that EGFR activity might affect exosomal CD47 level by modulating the expression of RAB31 signaling pathway-related proteins. Furthermore, inhibition of proteins in the RAB31 signaling pathway, which plays important roles in exosomal transport (Figure 2B), along with a notable reduction in exosomal CD47 expression, was observed upon EGFR knockdown (Figures 2C–2F). Beyond assessing the influence of EGFR on CD47 expression in exosomes, its effect on the basal expression levels of STAT3, p65, and CD47 was investigated in cervical cancer cells. A significant reduction in phosphorylation levels of STAT3 and p65, as well as a significant decrease in CD47 expression upon EGFR knockdown, was observed in HeLa and SiHa cells (Figure 2G). These findings indicated that EGFR might influence CD47 transport by modulating the expression of proteins involved in the RAB31 signaling pathway. Additionally, EGFR might affect CD47 expression in cervical cancer cells by regulating STAT3 and p65 phosphorylation levels.

STAT3 downregulation inhibited RAB31 expression and p65 phosphorylation in cervical cancer cells. Our prior results indicated that EGFR might influence the RAB31 signaling pathway to control exosomal CD47 expression. Moreover, numerous studies have demonstrated a direct regulatory effect of EGFR on STAT3. Therefore, we hypothesized that EGFR might modulate the expression of RAB31-associated proteins via STAT3. Indeed, RAB31 expression was significantly decreased after STAT3 knockdown in HeLa and SiHa cells (Figure 3A), suggesting a positive regulatory function of STAT3 in RAB31. Furthermore, the effect of STAT3 knockdown on p65 signaling was observed in HeLa and SiHa cells. These results showed that STAT3 inhibition reduced the phosphorylation of p65 (Figures 3B–3E). Next, we performed ChIP-qPCR assays to investigate whether p-p65 is bound to the promoter region of CD47. The analysis regions of CD47 were defined as P1 to P10 (corresponding primers were listed in Table 1) and shown in Figure 4A. ChIP-qPCR assays were performed in both HeLa and SiHa cells, and results demonstrated that p-p65 bound to the P3 and P7 promoter region of CD47, thereby regulating the expression of CD47 (Figures 4B, 4C). Thus, these results confirmed that EGFR modulated STAT3 phosphorylation, concurrently influencing p65 phosphorylation and exosome transporter RAB31 expression. This dual regulation consequently impacted the expression and secretion of CD47.

Discussion

Over the past few years, immunotherapy has emerged as a highly effective clinical treatment option. CD47 is a transmembrane protein that belongs to the immunoglobulin superfamily and plays a pivotal role as a signaling molecule in the innate immune system. It is highly expressed in almost all human malignant solid tumors, such as breast, ovarian, pancreatic, and prostate cancer, and hematological tumors, and is associated with tumor progression, making it a significant therapeutic target for human cancer. CD47-targeted therapies have shown great potential in solid tumors in recent years [17–19]. The regulation of CD47 is complex, involving multiple levels including transcriptional, epigenetic, and non-coding RNA regulation. PI3K/AKT, NF-κB andHIF-1α pathways are often activated in tumor progression and can enhance CD47 transcription through various mechanisms [20, 21]. In this study, we revealed that CD47 was regulated by the EGFR/STAT3 pathway in cervical cancer.

Studies have validated the significant role of exosomes released by cervical cancer cells and various cell types within their microenvironment in tumor initiation and progression. Active molecules specifically encapsulated within these exosomes hold potential as diagnostic and therapeutic targets for tumors [22, 23]. Cellular exosome quantity and content can be influenced by viral infection [24, 25]. Previous studies

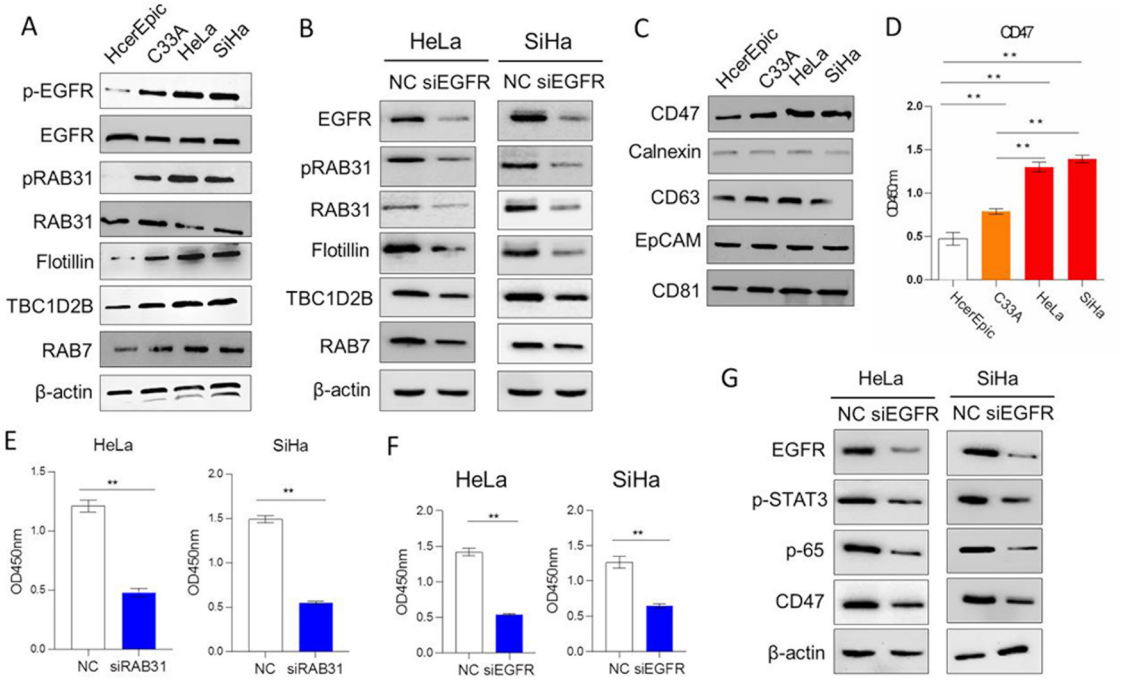

Figure 2. EGFR affects CD47 expression and transport by regulating STAT3, p65, and RAB31 signaling pathway. A) Comparison of the expression of EGFR signaling pathway and exosome vesicle transport-related proteins in cervical cancer and epithelial cells. B) RAB31 signaling pathway-related proteins were extensively inhibited after *EGFR* knockdown, indicating the function of EGFR in regulating the RAB31 signaling pathway. C) Expression of exosome markers (calnexin, CD63, EpCAM, and CD81) confirmed successful exosome extraction. D) Relative CD47 content in exosomes detected by ELISA. E) CD47 expression levels in exosomes derived from HeLa and SiHa cells after *RAB31* knockdown detected by ELISA. F) Effect of EGFR knockdown on exosomal CD47 expression. G) EGFR knockdown effect on the expression of the STAT3/p65/CD47 signaling axis. **p<0.01

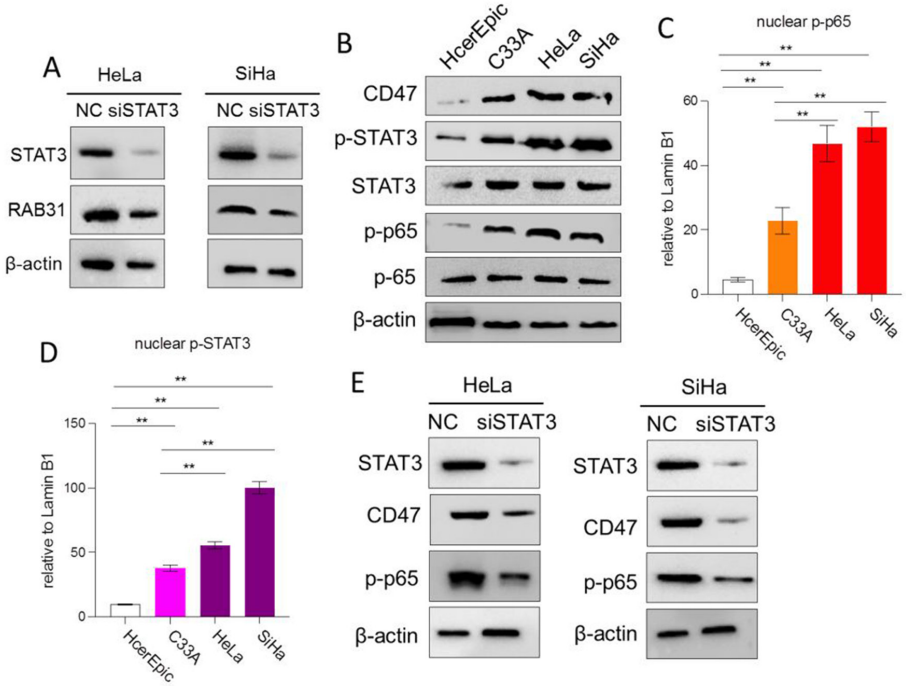

Figure 3. Impact of STAT3 on p65, RAB31, and CD47 expression. A) Influence of *STAT3* knockdown on RAB31 expression. B) Expression comparison of CD47, STAT3 signaling, and NF-κB signaling pathway proteins in cervical epithelial and cancer cells. C) Expression of p-p65 in cervical epithelial and cancer cell nuclei. D) Expression of p-STAT3 in cervical epithelial and cancer cell nuclei. E) Effect of inhibiting STAT3 expression on CD47 and NFκB signaling activities in cervical cancer cells. **p<0.01

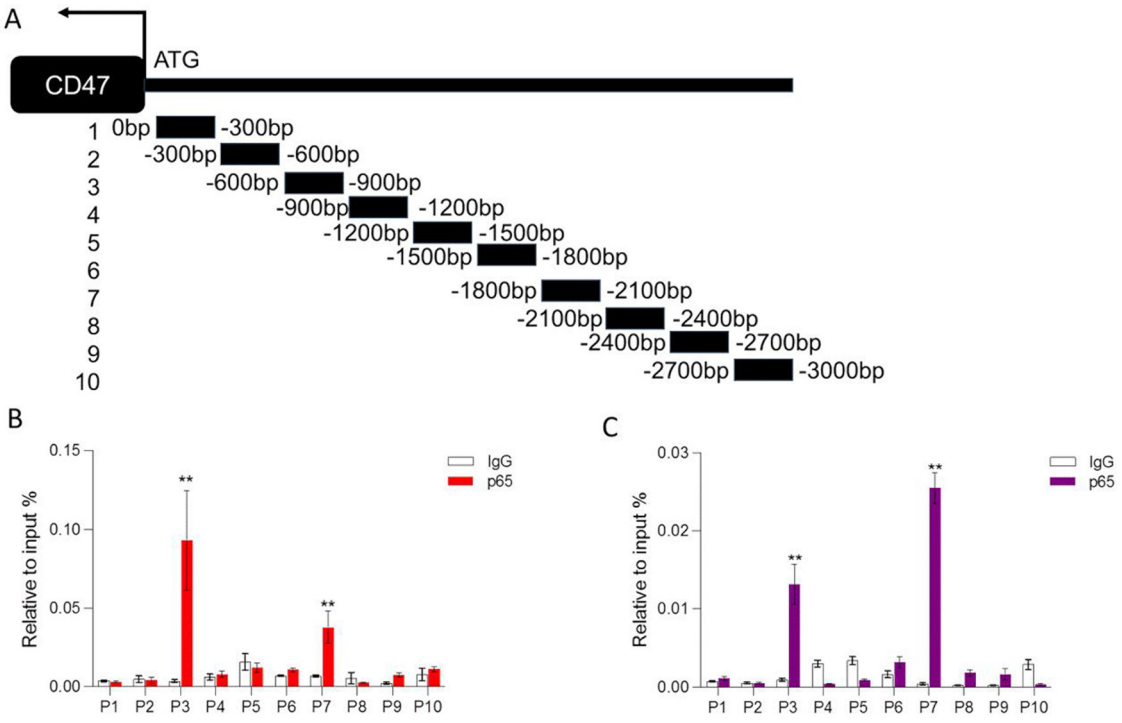

Figure 4. Phosphorylated p65 bound to P3 and P7 CD47 promoter regions to regulate CD47 expression. A) Overview of ChIP-qPCR analysis regions. B, C) ChIP-qPCR reveals p65 enrichment in CD47 promoter region in HeLa and SiHa cells. **p<0.01

Table 1. ChIP-qPCR primers.

Gene	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
CD47-P1	CCGGCCTCCGTGGAGC	CTCCGCGCCCGCCGC
CD47-P2	GGACGTCGGGTCCAGGGAT	GCCTGCTCCAGGGCGG
CD47-P3	ACAATGTTTACCACCGTGAATGGAAC	GGCTGCGCGCGTGGA
CD47-P4	AGAGTACATCAGTTAGGATAACG	AGAGTACATCAGTTAGGATAACG
CD47-P5	AAGAATTGATAATGCTGTGGGG	CTTATCAAAAAGTCAGGCTCTG
CD47-P6	TGGATGATTCTTTTGTATCCACATAAG	TTAAATCCGAGAGCATTTTAAGTAT
CD47-P7	GATATGCCTGCTTTTGCC	CCAGACTTTGAGCATTAGG
CD47-P8	CTCATCTCAAAAAGGGAGATCATAAC	CCAGGAGAGACAGTCCAAAG
CD47-P9	ATGCATTGAGAGAGGAAG	GGAAACAAAGGGAAAGAG
CD47-P10	GAAAGATGCATCTGGCC	AAAAAATTTCCCTTGCCCTC

have found that exosomes secreted by the HPV-18-infected cervical adenocarcinoma cell line HeLa are rich in survivin, cellular inhibitor of apoptosis protein, X-linked inhibitor of apoptosis protein 1/2, and other anti-apoptotic molecules [26–28]. The HR-HPV oncoprotein E6/E7 may alter both the quantity and composition of exosomes released by the HPV-infected cancer cells [29]. E6/E7 can regulate the sorting of exosome miRNAs. The miRNAs found within exosomes are not associated with tumor types or specific HPV strains. Instead, they are more closely linked to the expression of E6/E7 [30]. Based on the combined evidence from existing studies, the present study speculated that HR-HPV oncoproteins may enrich CD47 by regulating exosome production via EGFR/STAT3 signaling. HPV E5 protein, the smallest of the early expressed proteins, primarily promotes cell proliferation by modulating the function of host cell growth factor receptors. E5 promotes the accumulation of EGFR on the cell surface by inhibiting its degradation, thus enhancing the activity of EGFR signaling pathways. Although the direct link is less apparent than with E5, E6, and E7 may indirectly enhance the demand and activity of the EGFR signaling pathway by promoting rapid cell cycle progression.

The present results demonstrated that HPV knockdown of E5, E6, or E7 fragment significantly weakened the effect of HPV on the phosphorylation of EGFR and RAB31 in cervical cancer cells and reduced the exosomal CD47 level. Inter-

fering with *EGFR* in cervical cancer cells significantly inhibited the phosphorylation of STAT3 and p65 and decreased the expression of CD47. In addition, EGFR promoted the expression of CD47 by enhancing the expression of the exosome transporter RAB31. Moreover, inhibiting STAT3 affected the phosphorylation of p65, which can bind to the P3 and P7 promoter regions of CD47, thereby regulating its expression. We demonstrated a crucial dual role of EGFR/STAT3 signaling on CD47 expression and secretion involving transcriptional factor p65 and exosome transporter RAB31. However, further study should utilize *in vivo* models, such as transplanted tumor model and orthotopic tumor model, or patient-derived samples to broaden the applicability of these findings. Moreover, further exploration involving how STAT3 regulates RAB31 and RAB31 functional assays such as exosome secretion quantification will strengthen our conclusion. The present study suggests that CD47 can serve as a therapeutic target for treating cervical cancer, offering a theoretical framework for immunotherapy. Targeting exosomal CD47 (vs. membrane-bound CD47) faces clinical challenges due to overlapping targeting mechanisms of current therapies (e.g., antibodies, fusion proteins), which lack specificity for exosome-specific epitopes. The high heterogeneity of exosomal surface proteins necessitates novel delivery systems, such as engineered exosomes or bispecific antibodies, to enhance precision. While preclinical data suggest potential synergy between exosomal CD47 blockade and PD-1/PD-L1 inhibitors, robust validation in physiologically relevant models is required. Feasibility hinges on resolving technical gaps in exosome-specific targeting and optimizing combination strategies to minimize off-tumor toxicity.

In conclusion, HPV activates EGFR in cervical cancer cells, and EGFR upregulates CD47 expression by promoting p65 phosphorylation and RAB31 expression.

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