Wang *et al.*
European Journal of Medical Research (2025) 30:411
https://doi.org/10.1186/s40001-025-02662-9

European Journal of Medical Research

RESEARCH

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SENP5 promotes endometrial cancer cell growth by regulating β-catenin deSUMOylation to enhance GPX4-resistance to ferroptosis

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Abstract

Background Endometrial cancer (EC) is a significant and serious gynecological cancer, constituting a considerable risk to women's health. The desumoylation of SUMO specific peptidase 5 (SENP5) is intricately linked with various cancers. Nonetheless, the function of SENP5 in EC and its regulation of EC progression through the related mechanism of desumoylation modification remain elusive.

Methods Five samples of EC tumor tissues, along with para-cancerous tissues, were obtained. The expression of SENP5 in EC was assessed using reverse transcription–polymerase chain reaction (RT–qPCR), immunohistochemistry (IHC), and Western blot. HEC-1-B cells were treated with liposomes to interfere SENP5 and/or β-catenin expression. The methodologies employed to assess the impact of SENP5 on the proliferation of EC cells via β-catenin included RT–qPCR, Western blot, CCK8, EDU, and C11-BODIPY methods. Western blot, co-immunoprecipitation (CO-IP), and SUMOylation analysis were conducted to investigate the desumoylation modification of β-catenin by SENP5.

Results SENP5 exhibited elevated expression levels in EC cancer tissues and was correlated with a negative prognosis for patients diagnosed with EC. The suppression of SENP5 inhibited the expression of β-catenin and GPX4, activated ferroptosis, and inhibited HEC-1-B -cell proliferation. Knockdown of β-catenin counteracted the impact of SENP5 overexpression on ferroptosis and HEC-1-B proliferation. In addition, SENP5 stabilized β-catenin level in HEC-1-B cells through desumoylation modification of the β-catenin protein.

Conclusions SENP5 promotes GPX4-mediated ferroptosis resistance, thereby enhancing the proliferation of EC cells by regulating β-catenin desumoylation, this finding indicates that SENP5 may serve as a promising target for therapeutic interventions in the treatment of EC.

Keywords Endometrial cancer, Ferroptosis, DeSUMOylation, SENP5, β-Catenin, GPX4

Background

Endometrial carcinoma (EC) is a prevalent gynecological cancer globally and ranks among the three primary malignancies affecting female reproductive tract. Its incidence tends to rise with advancing age [1]. According to statistics, the 5-year survival rate for patients with early stage EC exceeds 90%, while it is only 20% for advanced stage patients [2]. Currently, with the increasing stress in people's lives, the incidence of EC is becoming younger. However, the treatment options for EC, such as surgical resection, hormone therapy, chemotherapy, and radiation therapy, are mainly suitable for early stage patients and have poor efficacy for advanced-stage patients [3]. Therefore, elucidating the molecular mechanisms of EC

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progression and finding new potential biomarkers are critical for enhancing clinical diagnosis, treatment, and prognosis of EC.

Ferroptosis is a type of non-apoptotic cell death characterized by oxidative damage to cell membrane phospholipids, which is driven by iron. Several studies have highlighted that inducing ferroptosis may become a new cancer treatment method. Much evidences show that the advancement of malignant tumors, including endometrial cancer, prostate cancer, breast cancer, and others, can be effectively hindered by promoting ferroptosis [4–6]. Glutathione peroxidase 4 (GPX4), a member of the glutathione peroxidase family, is essential for reducing lipid peroxides, thereby safeguarding cells against oxidative damage [7]. It is crucial in controlling the process of ferroptosis. Research has indicated that GPX4 can inhibit ferroptosis and promote the metastasis of prostate cancer [8] and gastric cancer [9]. Interestingly, a recent study showed that GPX4 is significantly expressed in EC tissues and inhibits ferroptosis and promotes the aggressive development of EC through the transcriptional activation of ELK1 [4]. However, the upstream molecular mechanisms regulating GPX4 in EC remain shrouded in mystery.

β-Catenin is a protein that has been conserved throughout evolution and is encoded by the CTNNB1 gene, which is dysregulated in the early stages of EC [10]. Dysregulated β-catenin translocates to the nucleus, regulating cancer cell death and proliferation, further inducing earlier and more aggressive endometrial carcinoma [11]. β-Catenin was found to have an inhibitory effect on ferroptosis in cancer in recent years [12]. Dysregulated β-catenin can trigger the expression of genes that regulate ferroptosis, including GPX4 [13], COX2 [14], SCD1 [15], which can prevent ferroptosis in cancer and accelerating the progression of cancer. However, the regulatory mechanism of β-catenin on ferroptosis in EC is remains uncertain.

SUMOylation is crucial in the development of several diseases, including tumors [16]. SUMOylation modification can regulate the stability, degradation, or localization of proteins, thereby exerting regulatory effects in multiple organs and systems of the body, such as the activation of transcription factors and protein–protein interactions [17]. Numerous proteins that play a crucial role in maintaining normal cellular functions are influenced by SUMOylation. Interestingly, relevant research reports have indicated that β-catenin transcriptional activity is affected by SUMOylation [18, 19]. SUMO-specific proteases (SENP) play a crucial role in reversing SUMOylation, helping to regulate the balance between SUMOylated and unmodified proteins within cells [20]. SUMO specific peptidase 5 (SENP5) is a functional SUMO-specific protease [21], and research indicates that SENP5 is instrumental in controlling the function of oncogenes in various cancers, including hepatocellular carcinoma, breast cancer, osteosarcoma, and oral squamous cell carcinoma [22]. Nonetheless, the function of SENP5 in EC progression through the regulation of β-catenin protein deSUMOylation remains unclear.

Therefore, in this study, we collected EC cancer tissues and para-cancerous tissues samples to detect the expression of SENP5. Furthermore, we further explored the specific role of SENP5 in EC in vitro and uncovered a novel mechanism by which SENP5 suppresses ferroptosis by deSUMOylating β-catenin in EC, suggesting that SENP5 could be a potential target for clinical treatment of EC.

Methods

Clinical specimens

Five paired EC cancer tissues and para-cancerous tissues were collected from EC patients who underwent the surgery to remove endometrial cancer at Yinchuan First People's Hospital during February 2023 to May 2024. All patients were pathologically diagnosed with cancer by three pathologists. Inclusion criteria: (1) all patients had no history of other cancer-related treatment; (2) all patients did not receive immunotherapy; and (3) not all patients underwent chemotherapy or radiation treatment. The Ethics Committee of Yinchuan First People's Hospital granted approval for the collection of specimens, and all participants provided their informed consent by signing the necessary forms.

Cells culture

The EC cell line HEC-1-B was purchased from Pricella Biotechnology Co., Ltd. (Wuhan, China). HEC-1-B cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 1×10^5 U/L penicillin, and 100 mg/L streptomycin at a 37 °C, 5% CO_2 incubator.

Cells transfection

When HEC-1-B cells reached 85% confluency, they were transfected using Lipofectamine 2000 (11668-030, Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The transfected cells were divided into three subgroups.

Subgroups one: For the si-SENP5 group and si-NC group, HEC-1-B cells were transfected with si-SENP5 or si-NC utilizing the Lipofectamine transfection reagent.

Subgroups two: For the si-SENP5 + Fer-1 group, si-SENP5 + Lip-1 group, si-SENP5 + Nec-1 group, and si-SENP5 + Z-VAD–FMK group, Liproxstatin-1 (Lip-1) at a concentration of 0.2 μmol/L, Ferrostatin-1 (Fer-1) at a concentration of 1 μmol/L, Necrostatin-1 (Nec-1) at a

concentration of 10 μmol/L, and Z-VAD–FMK at a concentration of 10 μmol/L were separately added to HEC-1-B cells with SENP5 knockdown.

Subgroups three: For the NC + si-NC group, SENP5 + si-NC group, NC + si-β-catenin group, and SENP5 + si-β-catenin group, OE–SENP5 or OE–NC was first transfected into HEC-1-B cells using Lipofectamine, followingly, si-β-catenin or si-NC were transfected into HEC-1-B cells previously treated with OE–SENP5 or OE–NC.

Cells counting kit-8 (CCK-8) assay

HEC-1-B cells from each experimental group were carefully placed into 96-well plates and cultured for 48 h and then the old medium was discarded for fresh medium, and a splash of 10 μL of CCK 8 solution was introduced to each well, setting the stage for the next phase of experimentation. Following a 2-h incubation period, the absorbance was recorded at a wavelength of 450 nm with the help of a microplate reader (Varioskan LUX, Thermo Fisher Scientific, USA).

5-Ethynyl-2′-deoxyuridine (EdU) assay

The HEC-1-B cells in each group were seeded in 96-well plates. 10 μM EdU (C0071L, Beyotime) was introduced and the cells were incubated for 2 h at 25 °C. Following this incubation, the cells were stained with DAPI for 10 min and subsequently examined using a fluorescence microscopy (bx53, Olympus, Japan).

Immunofluorescence staining

HEC-1-B in each experimental group were fixed with 4% paraformaldehyde for 30 min at 25 °C, and another 0.5% immunostained permeable solution (Triton X-100) was added for 15 min. Subsequently, the antibody supplemented with SENP5 (1:200, PA5-104468, Thermo Fisher Scientific, USA) or β-catenin (1:200, MA5-34961, Thermo Fisher Scientific, USA) was incubated for 4℃ overnight. Cells were incubated with fluorescently labeled secondary antibody (1:100, P0186, Beyotime, Shanghai, China) for 1 h before staining nuclei with DAPI in following day. Following, the diluent of the DAPI was added to incubate for 10 min. Finally, fluorescence images were taken under a fluorescence microscope (bx53, Olympus, Japan).

Co-immunoprecipitation (CO-IP) assay

Co-IP assay was conducted in accordance with established protocols as referenced in the literature [23]. In summary, cells were collected using IP lysis buffer, followed by a 40-min incubation on ice. Subsequently, the samples were centrifuged at 12,000×*g* for 10 min at a temperature of 4 °C. Cell lysates were treated overnight with a gentle swirl, using antibodies that target SENP5 (19529-1-AP, Proteintech, Wuhan, China) and β-catenin (17565-1-AP, Proteintech, Wuhan, China), or a negative control IgG, along with protein A/G agarose beads. Once the samples were separated using SDS–PAGE, the resulting immunoprecipitates were then put through the rigorous process of Western blot analysis.

SUMOylation assay

Following previous report [24], in brief, we lysed the cells with RIPA buffer and subsequently sonicated the lysates. The cell lysates were subsequently centrifuged at 14,000 rpm for 10 min at a temperature of 4 ℃, after which the clear supernatant was carefully gathered. The supernatant was subsequently incubated with the primary antibody: β-catenin (1:1000, ab32572, Abcam, Shanghai, China) or SUMO2 (1:1000, 11251-1-AP, proteintech, Wuhan, China) gently shaken for 4 h at 4 ℃, after which 50 μL of magnetic protein A/G was introduced, and the beads were treated for 120 min at 4 ℃ and assessed by protein blot analysis. The fusion protein vector HA-β-catenin, as well as SUMO1, SUMO2 and SUMO3 labeled by Myc for Co-IP cell transfection and Flag–SENP5, were developed and produced by LMAI Bio (Shanghai, China).

Lipid reactive oxygen species (ROS) level assay

Lipid ROS was identified using C11-BODIPY® 581/591 (D3861, Thermo Fisher Scientific). HEC-1-B cells were seeded in six-well plates and stimulated with Liperfluo (1 mM) for 30 min at 37 ℃. The samples were then meticulously examined using FlowJo V10 software (FACS Calibur, BD Biosciences, USA).

Glutathione (GSH) and Fe^{2+} assay

Cells were collected and RIPA buffer was used to lyse the cells. Following the manufacturer's guidelines, we evaluated the levels of GSH and Fe^{2+} within the cells using the GSH ratio assay kit (ab138881, Abcam, Shanghai, China) and the Fe^{2+} assay kit (ab83366, Abcam, Shanghai, China).

Reverse transcription–polymerase chain reaction (RT–qPCR)

Cells were treated with Trizol reagent (T9108, TaKaRa, Japan) to extract their total RNA. cDNA synthesis was performed following the instructions of the reverse transcription kit (RR036 A, Takara, Japan). The reaction parameters included denaturation at 95 °C for 10 min, annealing at 95 °C for 15 s, extension at 60 °C for 1 min, and 40 cycles. The results were calculated by $2^{-\Delta\Delta Ct}$. Primer sequences are shown in Table 1.

Table 1 Primer sequences used for RT–qPCR

Genes	Forward (5′–3′)	Reverse (5′–3′)
SENP5	ATGGCAGTTTGGTTCCACTC	CGTCCATATCCAGCATGTGT
β-Catenin	CGTTTCGCCTTC ATGGACTA	GCCGCTGGGTCCTGATGTCCTGAT
GPX4	AGAGATCAAAGAGTTCGCCGC	TCTTCATCCACTTCCACAGCG
β-Actin	CCCAAGGCCAACCGCGAGAAGATG	GTCCCGGCCAGCCAGGTCCAGA

Western blot

The total protein concentration was measured using the BCA protein quantification kit (ab102536, Abcam, Shanghai, China). A sample of twenty micrograms of protein was put through the exciting process of 10% SDS–polyacrylamide gel electrophoresis, and then it made its way onto a PVDF membrane for further analysis. The membrane was treated with 5% BSA in PBST at 25 °C for 2 h to block it, and afterward, it was incubated overnight at 4 °C with antibodies targeting SENP5 (ab58420, Abcam, Shanghai, China), β-catenin (ab32572, Abcam, Shanghai, China), GPX4 (ab41787, Abcam, Shanghai, China), β-actin (ab8227, Abcam, Shanghai, China), and GAPDH (ab9485, Abcam, Shanghai, China). On the second day, the membrane was incubated with HRP-conjugated secondary antibodies (ab288151, Abcam, Shanghai, China) at 25 ℃ for 1.5 h. The chemiluminescence reagent was applied dropwise onto the membrane. The protein bands were subsequently visualized using a developing solution.

Statistical analysis

We conducted our statistical analysis with the help of SPSS 21.0 and GraphPad Prism 7.0 software. The data were expressed as mean ± standard deviation (SD). To compare two groups, we employed the independent sample *t* test, while for multiple groups, we turned to one-way analysis of variance. A *P* value of less than 0.05 was deemed to hold statistical significance.

Results

High expression of SENP5 in EC tissues is associated with poor prognosis in EC patients

First, we collected 5 pairs of EC cancer tissues and paracancerous tissues to assess SENP5 level in EC. The IHC results showed abnormal expression of SENP5 in EC tissues, with a significant increase in the positive expression levels of SENP5 observed in cancerous tissues (Fig. 1A). Furthermore, RT–qPCR and Western blot results also indicated a notably higher expression of SENP5 in EC cancer tissues when compared to para-cancerous tissues ($P < 0.001$) (Fig. 1B, C). Importantly, through GEPIA bioinformatics analysis, our research indicates that elevated levels of SENP5 expression are strongly associated with unfavorable outcomes in patients with EC, as it notably reduces their overall survival rates (Fig. 1D).

SENP5 promotes proliferation of EC cells by inhibiting ferroptosis

Ferroptosis is an important mechanism in the progression of EC [25]. To explore the role of SENP5 in the progression of EC through the regulation of ferroptosis, we first transfected si-NC or si-SENP5 into HEC-1-B cells and evaluated the transfection efficiency by RT–qPCR and Western blot ($P < 0.001$) (Fig. 2A, B), confirming successful knockdown of SENP5 expression in HEC-1-B cells. Subsequently, we treated HEC-1-B cells with ferroptosis inhibitor Fer-1 and Lip-1, necrosis inhibitor Nec-1, and apoptosis inhibitor Z-VAD–FMK, respectively. CCK-8 results showed that silencing SENP5 obviously reduced the viability of HEC-1-B cells ($P < 0.001$), and treatment with Fer-1 or Lip-1 reversed the effect of si-SENP5 on cell viability, to some extent enhancing the viability of HEC-1-B cells ($P < 0.001$). Treatment with Nec-1 or Z-VAD–FMK also partly reversed the inhibition of cell viability by si-SENP5, but this reversal effect was not as pronounced as that seen after Fer-1 or Lip-1 treatment ($P < 0.05$, Fig. 2C). Similarly, the findings from the EdU assays revealed that silencing SENP5 resulted in a decrease in the proliferation of EC cells ($P < 0.001$). Inhibition of ferroptosis alleviated the growth inhibition caused by SENP5 silencing ($P < 0.001$), while inhibition of necrosis and apoptosis had a limited effect on the inhibition of cell growth caused by SENP5 silencing ($P < 0.05$, Fig. 2D, E). The findings indicate that SENP5 plays a role in inhibiting ferroptosis, and thus facilitates the proliferation of EC.

Silencing SENP5 inhibits the expression of β-catenin and GPX4 to activate ferroptosis in EC cells

We then investigated the molecular mechanisms through which SENP5 influences ferroptosis in EC. As we known, β-catenin and GPX4 are crucial elements that prevent ferroptosis [26, 27], we, therefore, evaluated the effects of SENP5 knockdown on β-catenin and GPX4 expression. The RT-qPCR results showed that silencing SENP5 significantly decreased GPX4 mRNA levels ($P < 0.001$), while only slightly decreased β-catenin mRNA levels

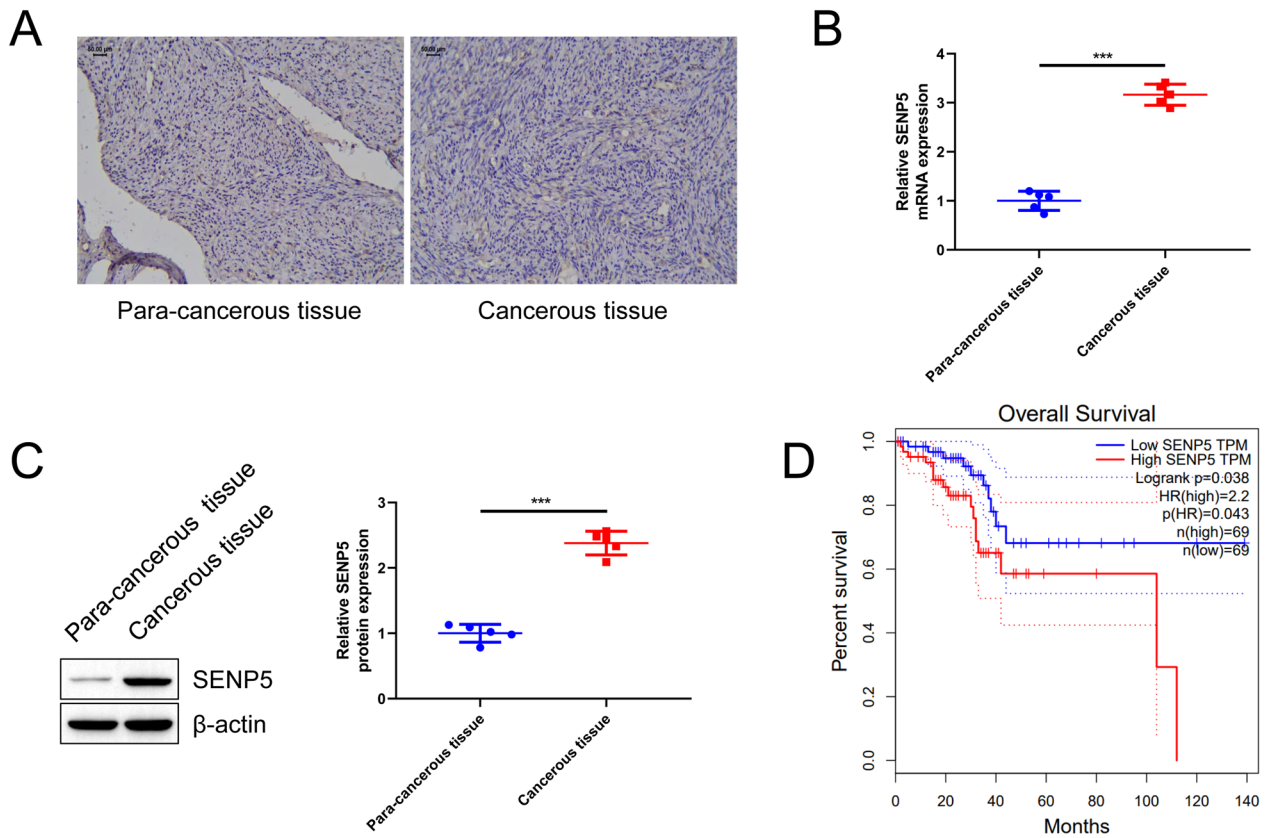

Fig. 1 High expression of SENP5 in EC tissues is associated with poor prognosis in EC patients. Five pairs of EC cancer tissues and para-cancerous tissues were collected. **A** SENP 5 expression in EC cancer tissues and para-cancerous tissues was detected by IHC staining. **B, C** RT–qPCR and Western blot in the evaluation of SENP5 mRNA and protein expression in EC cancer tissues and para-cancerous tissues. **D** GEPIA analysis revealed the correlation between high SENP 5 expression and prognosis in EC patients (***$P < 0.001$ vs. para-cancerous tissue group)

in HEC-1-B cells ($P < 0.05$) (Fig. 3A). The findings from the Western blot analysis revealed silencing SENP5 evidently downregulated β-catenin ($P < 0.001$) and GPX4 ($P < 0.001$) protein levels in HEC-1-B cells (Fig. 3B). Moreover, we found that silencing SENP5 significantly elevated the concentration of Fe^{2+} ($P < 0.001$), promoted ROS accumulation ($P < 0.001$), and decreased the anti-lipid oxidation factor GSH/GSSG level in HEC-1-B cells ($P < 0.001$, Fig. 3C–E). The findings indicate that knockdown of SENP5 activates ferroptosis by inhibiting β-catenin and GPX4 level in EC cells.

SENP5 promotes the expression of GPX4 in EC cells through β-catenin

It is known that GPX4 can act as a cancer-promoting factor by inhibiting ferroptosis to promote cancer progression, including EC [28]. Research has unveiled that β-catenin has the remarkable ability to enhance GPX4 level and inhibit ferroptosis [29]. Based on this, we investigated whether SENP5 affects ferroptosis in EC cells through β-catenin/GPX4. We first transfected OE–NC, OE–SENP5, si-NC, and si-β-catenin into HEC-1-B cells, and the findings from RT–qPCR and Western blot analyses disclosed a successful and significant increase in the levels of SENP5 expression ($P < 0.001$) and a knockdown of β-catenin ($P < 0.001$) in HEC-1-B cells (Fig. 4A–D). The findings revealed that when SENP5 levels were heightened, there was a notable boost in GPX4 expression within HEC-1-B cells ($P < 0.001$), while silencing β-catenin blocked the enhancing influence of SENP5 overexpression on GPX4 expression in HEC-1-B cells, obviously downregulating GPX4 expression ($P < 0.001$, Fig. 4E, F). In all, SENP5 upregulates GPX4 level in EC cells via β-catenin.

SENP5 overexpression inhibits ferroptosis and promotes proliferation of EC cells through β-catenin

We further investigated whether SENP5 regulates ferroptosis and proliferation of EC cells through β-catenin. CCK-8 and EdU results indicated that boosting SENP5 levels enhanced the viability ($P < 0.001$) and proliferation ($P < 0.001$) of HEC-1-B cells; however, this effect

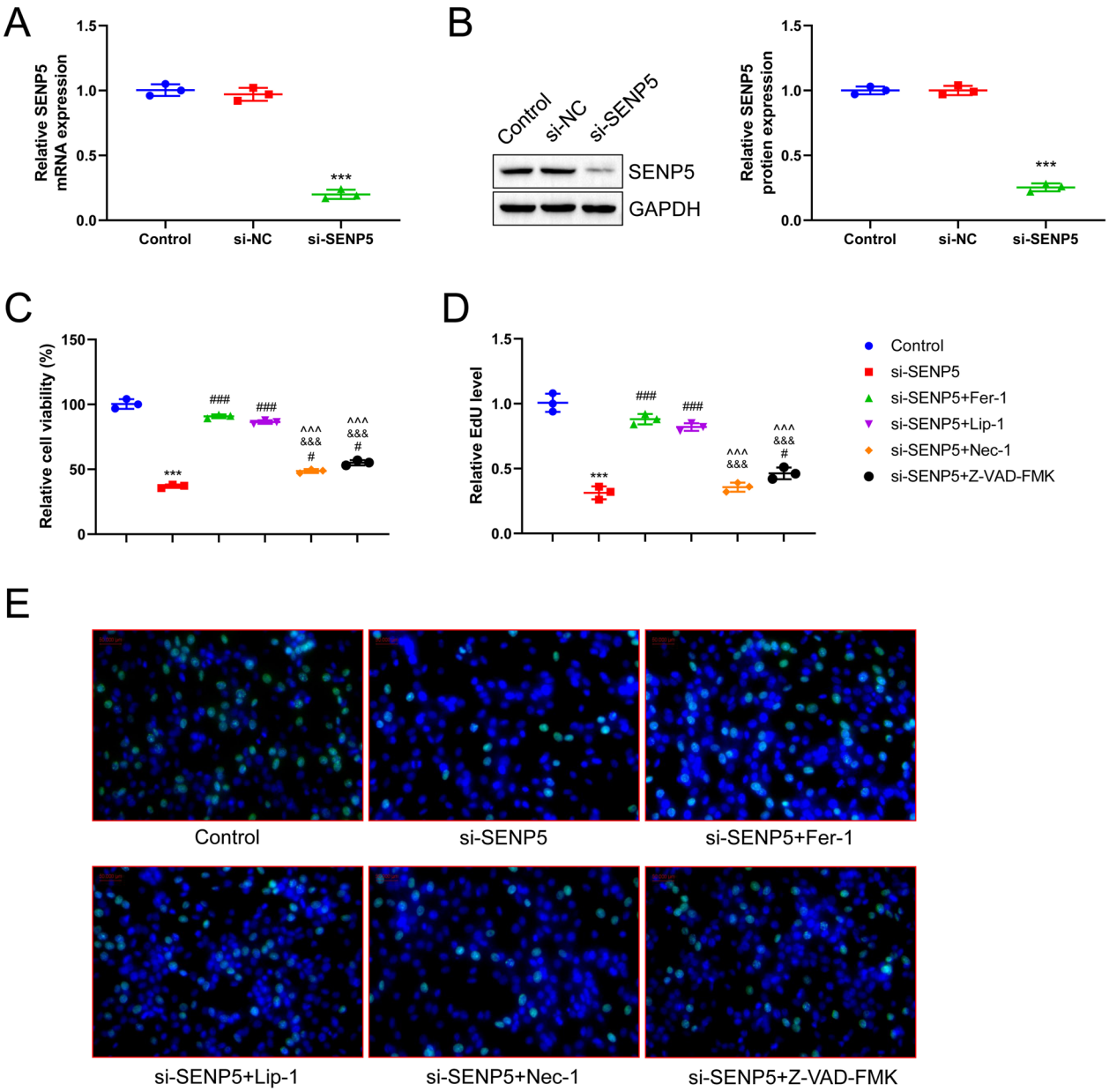

Fig. 2 SENP5 promotes proliferation of EC cells by inhibiting ferroptosis. SENP5 was knocked down in HEC-1-B cells and HEC-1-B cells were subsequently treated with the ferroptosis inhibitors Fer-1 and Lip-1, the necroptosis inhibitor Nec-1, and the apoptosis inhibitor Z-VAD–FMK. **A, B** RT–qPCR and Western blot to assess the transfection efficiency of SENP 5 in HEC-1-B. **C** HEC-1-B-cell viability was measured by CCK-8. **D, E** EdU staining was used to assay HEC-1-B-cell proliferation (***$P < 0.001$ vs. control group; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. si-SENP5 group; $^{\&\&\&}P < 0.001$ vs. si-SENP5 + Fer-1 group; ^^^$P < 0.001$ vs. si-SENP5 + Lip-1 group)

was somewhat diminished in the si-β-catenin group (**P**<0.001, Fig. 5A–C). In addition, overexpression of SENP5 significantly reduced the levels of Fe^{2+} and ROS ($P < 0.001$), but promoted GSH/GSSG level ($P < 0.001$). Consistently, silencing β-catenin reversed these effects, leading to increased accumulation of Fe^{2+} and ROS ($P < 0.001$) and decreased GSH/GSSG levels ($P < 0.001$) after overexpression of SENP5 (Fig. 5D–F). The above results suggest that SENP5 inhibits ferroptosis and promotes proliferation of EC cells through β-catenin.

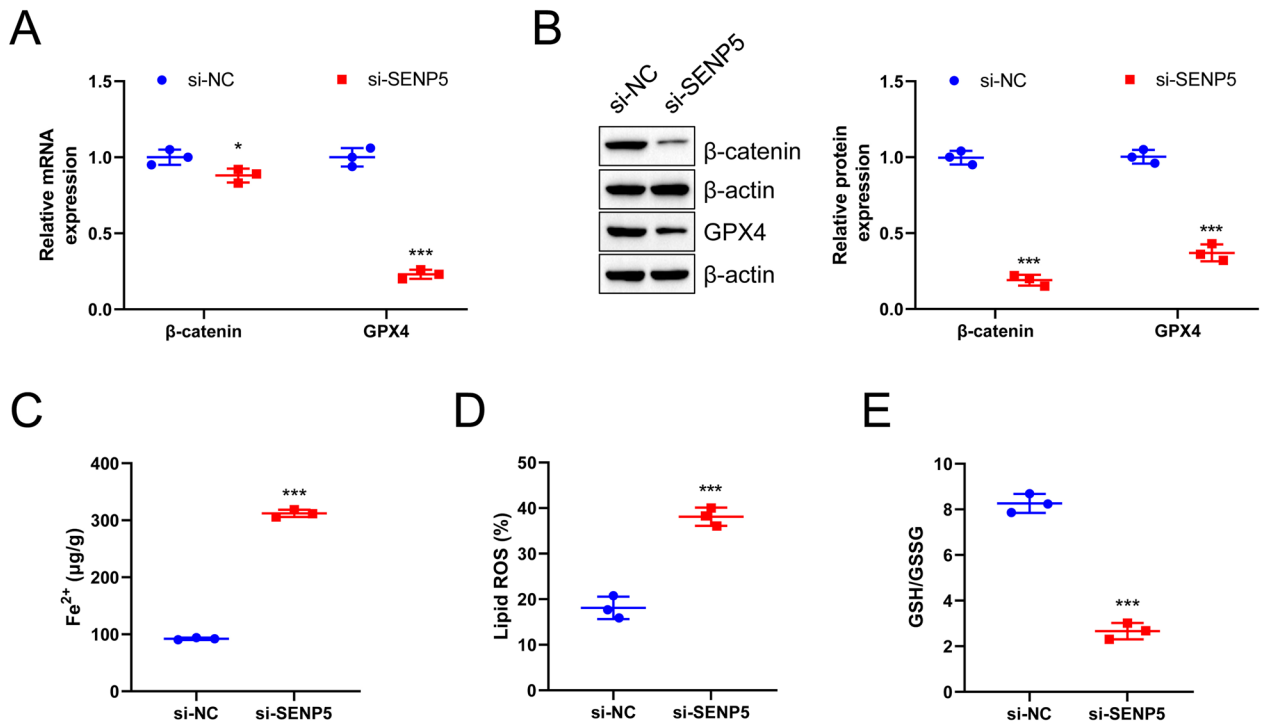

Fig. 3 Silencing SENP5 inhibits the expression of β-catenin and GPX4 to activate ferroptosis in EC cells. Si-NC or si-SENP5 were transfected into HEC-1-B cells. **A, B** RT–qPCR and Western blot were used to detect β-catenin and GPX4 expression in HEC-1-B cells. **C** Fe^{2+} content of HEC-1-B cells was measured by Fe^{2+} assay kit. **D** ROS levels in HEC-1-B cells were determined by C11-BODIPY assay. **E** Level of GSH/GSSG in HEC-1-B cells was detected by ELISA (*$P < 0.05$, ***$P < 0.001$ vs. si-NC group)

SENP5 promotes the β-catenin deSUMOylation to stabilize its expression

Research has indicated that β-catenin is controlled by SUMOylation [30], and SENP5 belongs to the SENP family, which is capable of controlling the deSUMOylation of target proteins [31]. Therefore, we next investigated whether SENP5 regulates the deSUMOylation of β-catenin. First, immunofluorescence staining revealed co-expression of SENP5 and β-catenin in HEC-1-B cells (Fig. 6A). Co-IP analysis also indicated an interaction between SENP5 and β-catenin (Fig. 6B). Next, based on the SUMOplot™ scoring system (https://www.abcepta.com/sumoplot), we predicted potential SUMOylation sites on β-catenin, and K233 was found to have a high probability of being a SUMOylation site, indicating that SUMOylation could be crucial for the stability and expression of β-catenin (Fig. 6C). To confirm that SENP5 regulates the deSUMOylation of β-catenin in EC cells, HEC-1-B cells were simultaneously transfected with HA-tagged β-catenin and Myc-tagged SUMO1, SUMO2, or SUMO3, and then analyzed through immunoblotting using an HA antibody. The results showed that when β-catenin was co-expressed with SUMO2, an HA antibody identified a band with a greater molecular weight than β-catenin, indicating that β-catenin can bind to SUMO2 (Fig. 6D). Furthermore, the addition of a SUMOylation inhibitor (ML-792) reversed the inhibitory effect of SENP5 silencing on β-catenin and promoted β-catenin level ($P < 0.001$, Fig. 6E). In all, SENP5 stabilizes the expression of β-catenin through regulating its deSUMOylation.

Discussion

EC is a type of epithelial malignant tumor that originates from the endometrial gland, accounting for 20–30% of malignant tumors that afflict the female reproductive system, with a high incidence and mortality rate [32]. Therefore, identifying new molecules and related molecular mechanisms that promote the progression of EC will aid in the formulation of more effective EC treatment strategies. SENP5 is a member of the SENP-specific proteinase family and plays a significant role in a range of biological processes, including cell cycle, autophagy, and apoptosis by mediating protein de-SUMOylation [21, 31]. Research has indicated that an imbalance in the expression of SENP5 may result in cellular dysfunction and induce various cancers. Several studies based on clinical tumor samples have reported high expression of SENP5 in various malignancies, including liver cancer, colorectal cancer, and breast cancer, and patients with high SENP5

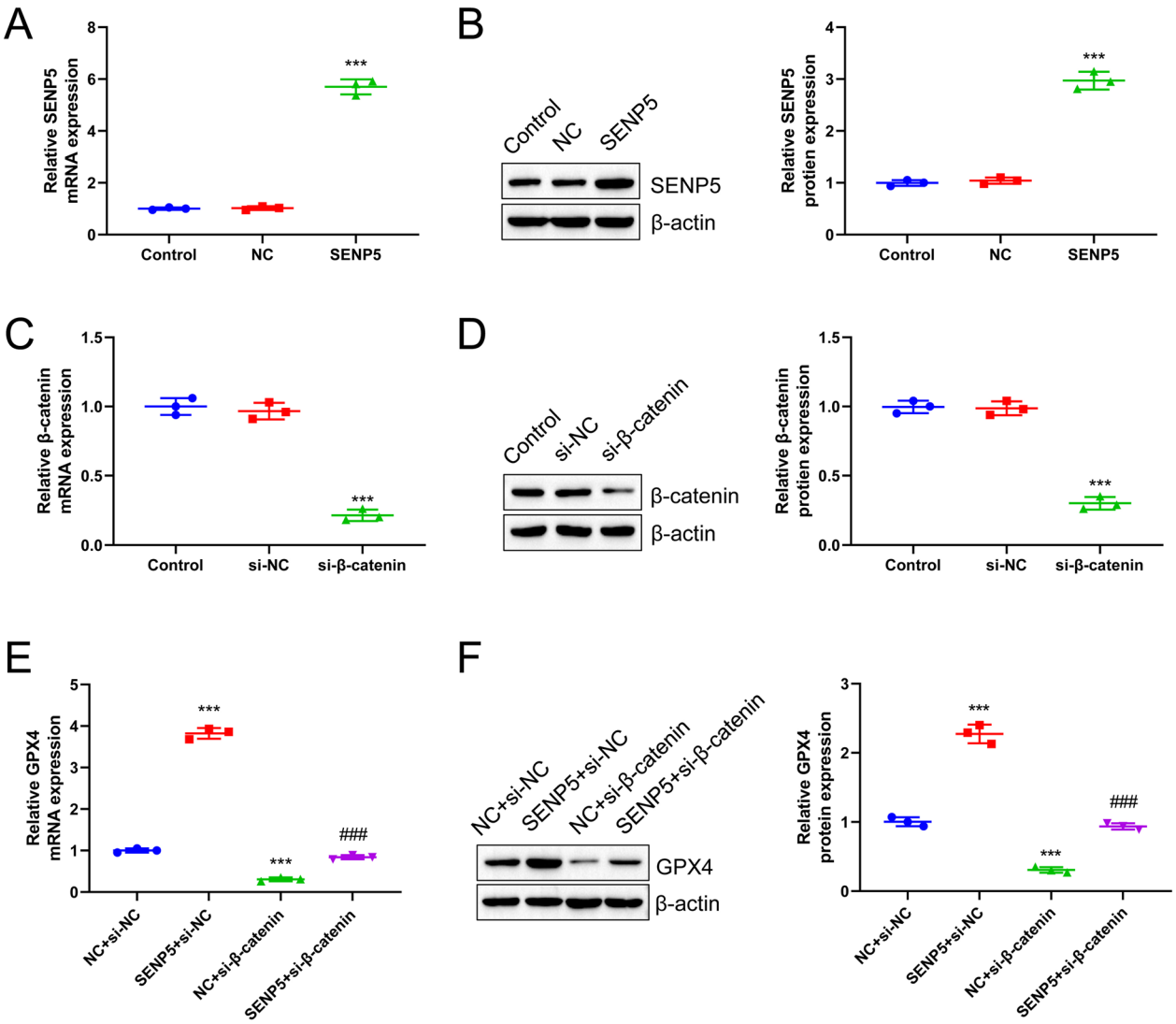

Fig. 4 SENP5 promotes the expression of GPX4 in EC cells through β-catenin. OE–NC, OE–SENP5, si-NC, and si-β-catenin were transfected into HEC-1-B cells, and then transfected si-NC and si-β-catenin into OE–NC or OE–SENP5-treated HEC-1-B cells. **A, B** RT–qPCR and Western blot was used to assess the transfection efficiency of SENP5 in HEC-1-B cells. **C, D** RT–qPCR and Western blot assessed the transfection efficiency of β-catenin in HEC-1-B cells. **E, F** RT–qPCR and Western blot assessed the effects of SENP5 overexpression and β-catenin silencing on GPX4 expression in HEC-1-B cells (***$P < 0.001$ vs. control group and NC + si-NC group; ###$P < 0.001$ vs. SENP5 + si-NC group)

expression have poor prognosis [33–35]. However, the function of SENP5 in EC progression remains ambiguous. In this research, we observed that SENP5 exhibited elevated expression levels in EC cancer tissues and was associated with unfavorable prognostic outcomes in patients diagnosed with EC, which suggests that SENP5 has the potential to function as an oncogene in EC. However, the role and related mechanisms of SENP5 in EC still need further exploration.

Ferroptosis is an emerging form of non-apoptotic regulated cell death pathway, which is marked by the buildup of lipid peroxides that depend on iron and has shown great potential in cancer treatment [36]. Many studies have validated the important function of ferroptosis in eliminating cancer cells and preventing the progression of cancer [37]. Yin et al. [38] found that the NRF1/RAN/DLD protein complex regulated the transcription of CLTRN, thus activating the ferroptosis pathway and increasing the radiation sensitivity of hepatocellular carcinoma cells. Ovarian cancer cells are susceptible to ferroptosis. Greenshields et al. [39] demonstrated that artemisinin has the remarkable ability to trigger ferroptosis in ovarian cancer cells in an ROS-dependent manner, showing strong anti-proliferative

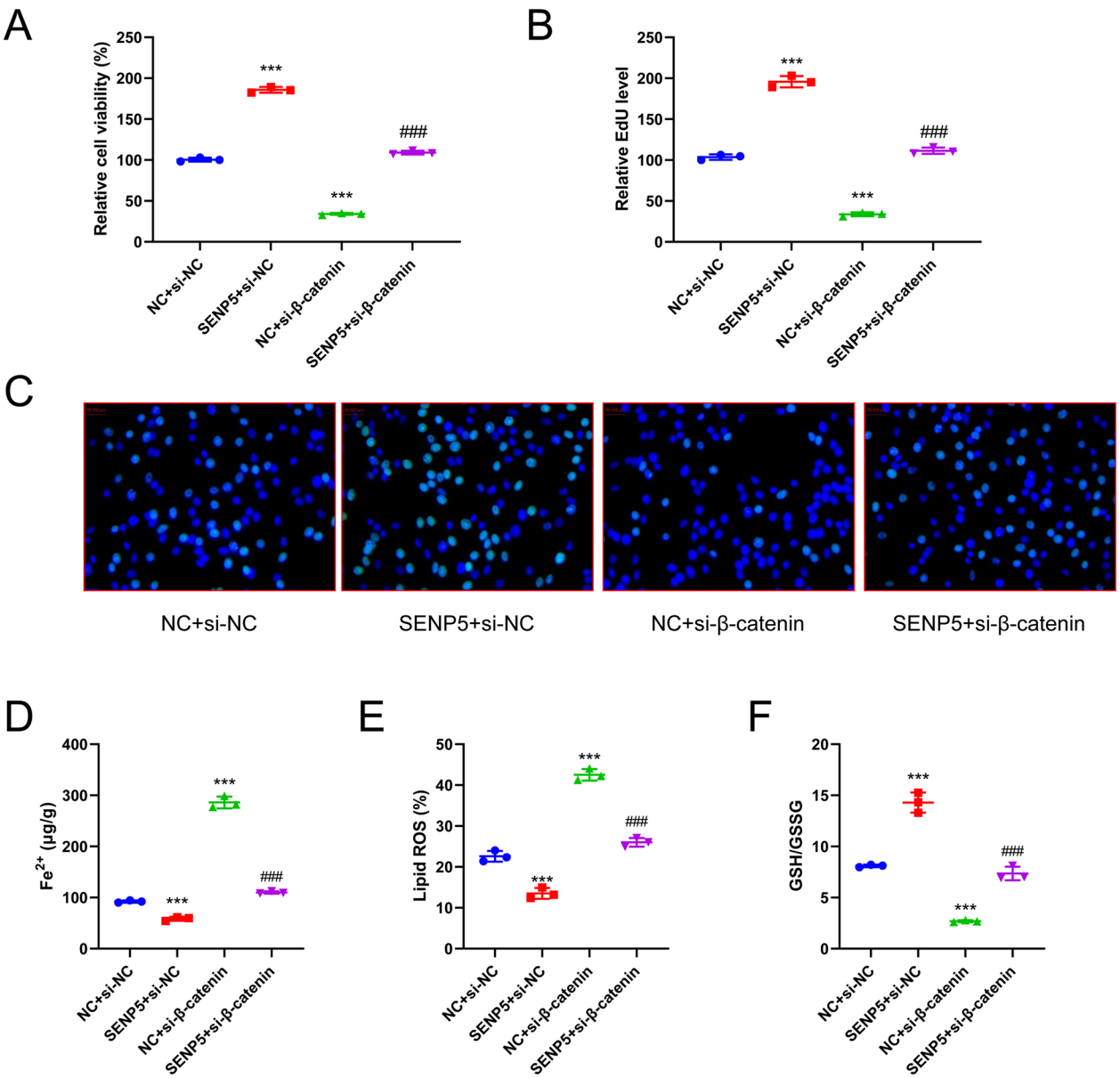

Fig. 5 SENP5 overexpression inhibits ferroptosis and promotes proliferation of EC cells through β-catenin. OE–NC and OE–SENP5 were transfected into HEC-1-B cells, and then transfected si-NC or si-β-catenin into OE–NC or OE–SENP5-treated HEC-1-B cells. **A** CCK8 assay was used to evaluate the viability of HEC-1-B cells. **B, C**. EdU staining was performed to assess the proliferation of HEC-1-B cells. **D** Fe^{2+} content of HEC-1-B cells was measured by Fe^{2+} assay kit. **E** C11-BODIPY staining was used to detect the level of ROS in HEC-1-B cells. **F** Level of GSH/GSSG in HEC-1-B cells was detected by ELISA (***$P < 0.001$ vs. NC + si-NC group; ###$P < 0.001$ vs. SENP5 + si-NC group)

and cytotoxic effects. In addition, researchers have found that in EC, the process of ferroptosis is abnormally regulated, and the application of ferroptosis activators can lead to apoptosis [4]. Interestingly, recent studies have confirmed that SENP5, a homologous protein of SENP1, can inhibit ferroptosis and participate in cancer progression, such as squamous cell carcinoma and lung cancer [40, 41]. However, the role of SENP5 in the progression of EC through the regulation of ferroptosis remains ambiguous. In the present study, we observed that silencing SENP5 significantly decreased cell viability and proliferation in EC cells. Importantly, our results demonstrated that ferroptosis inhibitors, specifically Fer-1 and Lip-1, effectively reversed the effects of SENP5 knockdown on EC cell proliferation and viability. Specifically, treatment of HEC-1-B

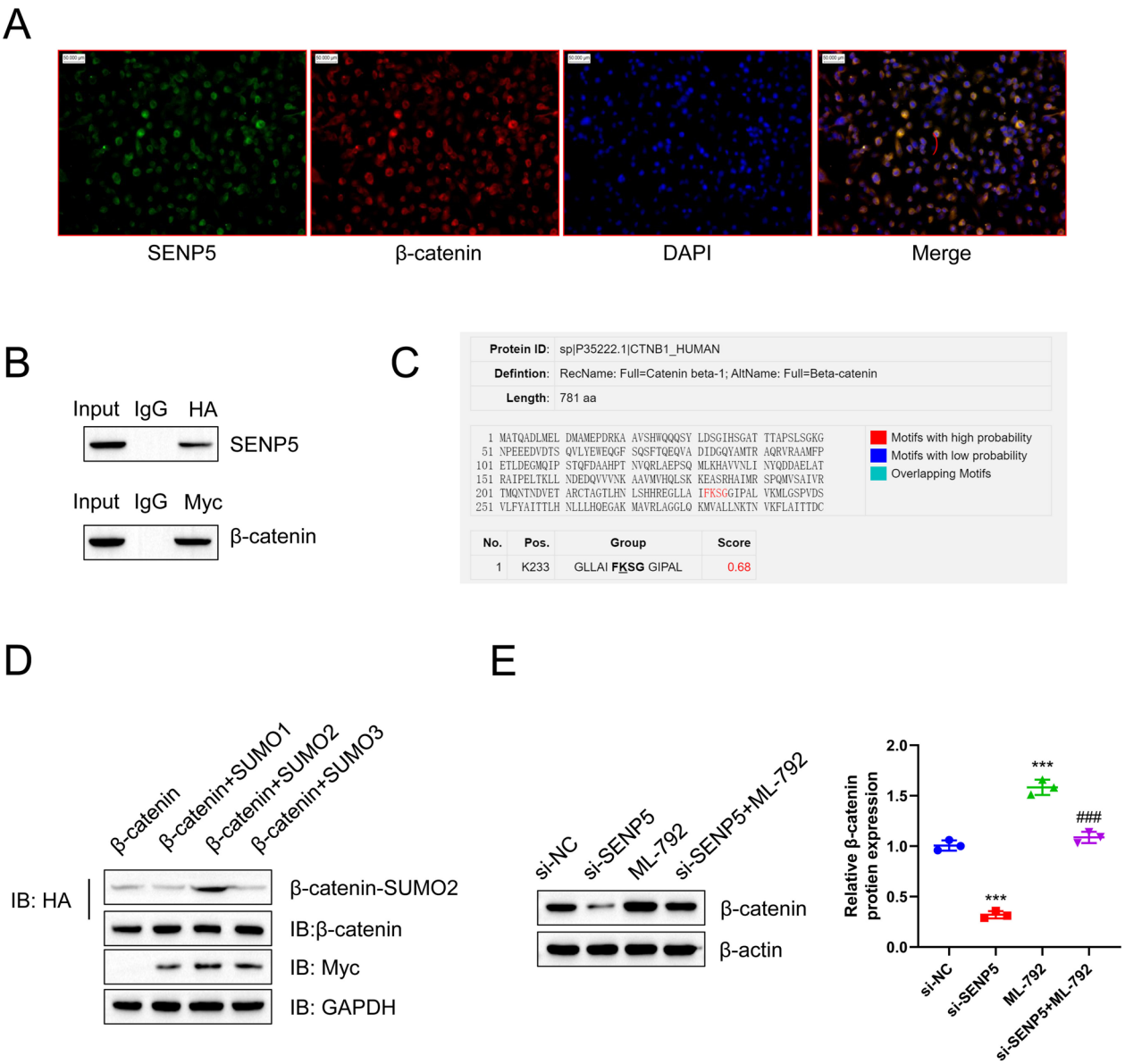

Fig. 6 SENP5 promotes the β-catenin deSUMOylation to stabilize its expression. **A** Immunofluorescence co-localization analysis of SENP5 and β-catenin proteins in HEC-1-B cells. **B** Co-IP analysis of the interaction between SENP5 and β-catenin. **C** SUMOplot™ score system (https://www.abcepta.com/sumoplot) predicted the SUMOylation modification sites of β-catenin protein. **D** HEC-1-B cells were co-transfected with HA-tagged β-catenin and Myc-tagged SUMO1, SUMO2, or SUMO3, and then treated with the SUMOylation inhibitor (ML-792) in SENP5 knockdown HEC-1-B cells. **E** SUMOylation inhibitor (ML-792) was added to SENP5 knockdown HEC-1-B cells, and western blot analysis assessed β-catenin expression (***$P < 0.001$ vs. si-NC group; $^{###}P < 0.001$ vs. si-SENP5 group)

cells with either Fer-1 or Lip-1 significantly alleviated the inhibitory effects of SENP5 knockdown on cell growth, indicating that ferroptosis plays a central role in mediating the biological functions of SENP5 in EC. In contrast, inhibitors of necrosis (Nec-1) and apoptosis (Z-VAD–FMK) had limited effects on reversing the proliferation inhibition caused by SENP5 silencing, further highlighting the specificity of ferroptosis in this context. In addition, we found that silencing SENP5 promoted the accumulation of Fe^{2+} and ROS in EC cells while reducing GSH content, corroborating the activation of ferroptosis-specific metabolic signatures. These findings underscore the importance of targeting ferroptosis pathways in EC therapy and provide mechanistic insights into how SENP5 promotes EC progression by suppressing ferroptosis. Our findings provide the first

evidence that SENP5 promotes EC cell proliferation through inhibition of ferroptosis.

GPX4 serves as a crucial regulatory element in ferroptosis, which employs reduced glutathione to transform lipid hydroperoxides into alcohols, thereby reducing lipid peroxidation and inhibiting ferroptotic cell death [42]. The elevated expression of GPX4 in tumors is strongly correlated with tumor occurrence and metastasis. Multiple studies have confirmed that dysregulated GPX4 facilitates the malignant progression of EC by suppressing ferroptosis [4, 43]. Our research revealed that silencing SENP5 markedly inhibited GPX4 expression in EC cells, suggesting that SENP5 may facilitate the advancement of EC by modulating GPX4 to inhibit ferroptosis.

β-Catenin is a protein that has been conserved throughout evolution and is essential for cell adhesion as well as the transduction of Wnt signaling pathways. In addition, it is significantly involved in the process of tumorigenesis [44]. Specifically, changes in β-catenin or components of the destruction complex have been confirmed as early events in EC [11], but the exact molecular mechanisms regulating β-catenin in EC are largely unknown. Our data suggest that silencing SENP5 significantly inhibits β-catenin expression in EC cells, and knockdown of β-catenin reverses the proliferative influence of SENP5 on EC cells, indicating that SENP5 may participate in the progression of EC by modulating β-catenin activity. Interestingly, several studies have emphasized the inhibitory function of β-catenin in ferroptosis in cancer. Wang et al. [42] demonstrated that the β-catenin/TCF4 transcriptional complex directly interacts with the promoter region of GPX4, resulting in an increase in it and conferring resistance to ferroptotic cell death in gastric cancer. Tang et al. [45] demonstrated that USP8 plays a crucial role in stabilizing the β-catenin protein by preventing K48-specific polyubiquitination, upregulating GPX4 protein levels, and inhibiting ferroptosis, promoting the development of hepatocellular carcinoma. Similarly, our research findings indicate that silencing β-catenin can reverse the effects of SENP5 overexpression on multiple cellular parameters. Specifically, silencing β-catenin counteracted the SENP5-induced increases in GPX4 levels and decreased in intracellular Fe^{2+} levels while also mitigating the reduction in lipid ROS accumulation and the increase in GSH/GSSG ratios. These findings suggest that β-catenin plays a crucial role in mediating the antiferroptotic and proliferative effects of SENP5 through the regulation of GPX4 expression. However, the regulatory mechanism of SENP5 on β-catenin still needs further elucidation.

The activity of β-catenin is a critical factor in the regulation of tumor development. Research has demonstrated that the protein stability of the β-catenin can be modulated via post-translational modification (SUMOylation) [30]. Research has demonstrated that SUMO-specific proteinases (SENPs) regulate the SUMOylation status of β-catenin protein. SENP7S modulates the deSUMOylation process of β-catenin, thereby suppressing β-catenin signaling and the transformation of mammary epithelial cells [20]. SENP2 is instrumental in modulating the proliferation of hepatocellular carcinoma (HCC) cells by regulating the stability of β-catenin through SUMOylation [46]. SENP5 stands out as a key player in the SENP family, but the mechanism of its regulation of β-catenin SUMOylation is not clear. SENP5 exhibits an interaction with β-catenin in EC cells, and silencing SENP5 inhibits β-catenin expression, while treatment with a SUMOylation inhibitor (ML-792) significantly upregulates β-catenin protein expression in EC cells, as found in this study. SENP5 stabilizes the expression of β-catenin in EC cells through deSUMOylation modification, as first demonstrated in this experiment.

Conclusions

Our results indicate that SENP5 is significantly elevated in EC cancer tissues and correlates with a less favorable prognosis for patients with EC. Silencing SENP5 inhibits β-catenin and GPX4 levels, activates ferroptosis, and inhibits HEC-1-B-cell proliferation. Mechanistically, SENP5 stabilizes β-catenin expression in HEC-1-B cells through deSUMOylation modification of β-catenin protein, promoting resistance to GPX4-mediated ferroptosis and further accelerating the progression of EC. Our findings indicate that inhibiting SENP5 could be a key approach in treating EC and offer novel perspectives on the role of post-translational modifications in EC.

Acknowledgements

Not applicable.

Author contributions

Ziyuan Wang, Yongmei Zhang: Methodology, Investigation, Data curation, original draft. Yongfang Zhang,Qiuhong Deng,Yandong Xi, Wanxia He, Xiaolong Ma: Methodology, Investigation, Data curation, original draft. Ziyuan Wang, Yongmei Zhang: Writing, review and editing.

Funding

In 2023, Ningxia Natural Science Foundation, "SENP5 promotes SUMO modification of β-catenin protein, regulates GPX4-mediated iron death Resistance and Promotes endometrial cancer Progression", No. 2023AAC03870.

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request. All the data obtained in the current study were available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate

The Ethics Committee of Yinchuan First People's Hospital granted approval for the collection of specimens, and all participants provided their informed consent by signing the necessary forms.

Consent for publication
All the participants agreed to the publication of the manuscript.

Competing interests
The authors declare no competing interests.

Received: 30 December 2024 Accepted: 4 May 2025
Published online: 24 May 2025

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30.
3. Moxley KM, McMeekin DS. Endometrial carcinoma: a review of chemotherapy, drug resistance, and the search for new agents. Oncologist. 2010;15(10):1026–33.
4. Wei S, Yu Z, Shi R, et al. GPX4 suppresses ferroptosis to promote malignant progression of endometrial carcinoma via transcriptional activation by ELK1. BMC Cancer. 2022;22(1):881.
5. Ma S, Dielschneider RF, Henson ES, et al. Ferroptosis and autophagy induced cell death occur independently after siramesine and lapatinib treatment in breast cancer cells. PLoS ONE. 2017;12(8): e0182921.
6. Viswanathan VS, Ryan MJ, Dhruv HD, et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature. 2017;547(7664):453–7.
7. Xue Q, Yan D, Chen X, et al. Copper-dependent autophagic degradation of GPX4 drives ferroptosis. Autophagy. 2023;19(7):1982–96.
8. Cheng L, He Q, Liu B, et al. SGK2 promotes prostate cancer metastasis by inhibiting ferroptosis via upregulating GPX4. Cell Death Dis. 2023;14(1):74.
9. Li D, Wang Y, Dong C, et al. CST1 inhibits ferroptosis and promotes gastric cancer metastasis by regulating GPX4 protein stability via OTUB1. Oncogene. 2023;42(2):83–98.
10. Dou Y, Katsnelson L, Gritsenko MA, et al. Proteogenomic insights suggest druggable pathways in endometrial carcinoma. Cancer Cell. 2023;41(9):1586-605.e15.
11. Parrish ML, Broaddus RR, Gladden AB. Mechanisms of mutant β-catenin in endometrial cancer progression. Front Oncol. 2022;12:1009345.
12. Li J, Xian L, Zhu Z, et al. Role of CELF2 in ferroptosis: potential targets for cancer therapy (Review). Int J Mol Med. 2023;52(4):88.
13. Wang Y, Zhao G, Condello S, et al. Frizzled-7 identifies platinum-tolerant ovarian cancer cells susceptible to ferroptosis. Can Res. 2021;81(2):384–99.
14. Chen QF, Shi F, Huang T, et al. ASTN1 is associated with immune infiltrates in hepatocellular carcinoma, and inhibits the migratory and invasive capacity of liver cancer via the Wnt/β-catenin signaling pathway. Oncol Rep. 2020;44(4):1425–40.
15. Luo Y, Huang S, Wei J, et al. Long noncoding RNA LINC01606 protects colon cancer cells from ferroptotic cell death and promotes stemness by SCD1-Wnt/β-catenin-TFE3 feedback loop signalling. Clin Transl Med. 2022;12(4): e752.
16. Seeler JS, Dejean A. SUMO and the robustness of cancer. Nat Rev Cancer. 2017;17(3):184–97.
17. Lara-Urea N, Jafari V, Garca-Domnguez M. Cancer-associated dysregulation of sumo regulators: proteases and ligases. Int J Mol Sci. 2022;23(14):8012.
18. Buehler E, Khan AA, Marine S, et al. siRNA off-target effects in genome-wide screens identify signaling pathway members. Sci Rep. 2012;2:428.
19. Shen LN, Geoffroy MC, Jaffray EG, et al. Characterization of SENP7, a SUMO-2/3-specific isopeptidase. Biochem J. 2009;421(2):223–30.
20. Karami S, Lin FM, Kumar S, et al. Novel SUMO-protease SENP7S regulates β-catenin signaling and mammary epithelial cell transformation. Sci Rep. 2017;7:46477.
21. Kim EY, Zhang Y, Beketaev I, et al. SENP5, a SUMO isopeptidase, induces apoptosis and cardiomyopathy. J Mol Cell Cardiol. 2015;78:154–64.
22. Jin ZL, Pei H, Xu YH, et al. The SUMO-specific protease SENP5 controls DNA damage response and promotes tumorigenesis in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci. 2016;20(17):3566–73.
23. Liang M, Wang L, Sun Z, et al. E3 ligase TRIM15 facilitates non-small cell lung cancer progression through mediating Keap1-Nrf2 signaling pathway. Cell Commun Signal. 2022;20(1):62.
24. Ding Y, Chen F, Yang W, et al. SENP5 deteriorates traumatic brain injury via SUMO2-dependent suppression of E2F1 SUMOylation. Acta Biochim Biophys Sin. 2023;55(8):1193–203.
25. Wu J, Zhang L, Wu S, et al. Ferroptosis: opportunities and challenges in treating endometrial cancer. Front Mol Biosci. 2022;9: 929832.
26. Peterson JM, Dyck S. Systems theory facilitates student practice in self-directed learning courses. Nurse Educ. 1986;11(5):12–5.
27. Xie J, Wang H, Xie W, et al. Gallic acid promotes ferroptosis in hepatocellular carcinoma via inactivating Wnt/β-catenin signaling pathway. Naunyn Schmiedebergs Arch Pharmacol. 2024;397(4):2437–45.
28. Žalytė E. Ferroptosis, metabolic rewiring, and endometrial cancer. Int J Mol Sci. 2023;25(1):75.
29. Li B, Wei Z, Wang Z, et al. Fusobacterium nucleatum induces oxaliplatin resistance by inhibiting ferroptosis through E-cadherin/β-catenin/GPX4 axis in colorectal cancer. Free Rad Biol Med. 2024;220:125–38.
30. Huang HJ, Zhou LL, Fu WJ, et al. β-catenin SUMOylation is involved in the dysregulated proliferation of myeloma cells. Am J Cancer Res. 2015;5(1):309–20.
31. Di Bacco A, Gill G. SUMO-specific proteases and the cell cycle. An essential role for SENP5 in cell proliferation. Cell Cycle Georgetown Tex. 2006;5(20):2310–3.
32. Luo J, Chlebowski RT, Hendryx M, et al. Intentional weight loss and endometrial cancer risk. J Clin Oncol. 2017;35(11):1189–93.
33. Zhang CY, Jiang ZM, Ma XF, et al. Saikosaponin-d inhibits the hepatoma cells and enhances chemosensitivity through SENP5-dependent inhibition of gli1 SUMOylation under hypoxia. Front Pharmacol. 2019;10:1039.
34. Liu T, Wang H, Chen Y, et al. SENP5 promotes homologous recombination-mediated DNA damage repair in colorectal cancer cells through H2AZ deSUMOylation. J Exp Clin Cancer Res CR. 2023;42(1):234.
35. Cashman R, Cohen H, Ben-Hamo R, et al. SENP5 mediates breast cancer invasion via a TGFβRI SUMOylation cascade. Oncotarget. 2014;5(4):1071–82.
36. Zhang C, Liu X, Jin S, et al. Ferroptosis in cancer therapy: a novel approach to reversing drug resistance. Mol Cancer. 2022;21(1):47.
37. Tsoi J, Robert L, Paraiso K, et al. Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. Cancer Cell. 2018;33(5):890-904.e5.
38. Yuan Y, Cao W, Zhou H, et al. CLTRN, regulated by NRF1/RAN/DLD protein complex, enhances radiation sensitivity of hepatocellular carcinoma cells through ferroptosis pathway. Int J Radiat Oncol Biol Phys. 2021;110(3):859–71.
39. Greenshields AL, Shepherd TG, Hoskin DW. Contribution of reactive oxygen species to ovarian cancer cell growth arrest and killing by the anti-malarial drug artesunate. Mol Carcinog. 2017;56(1):75–93.
40. Xu X, Mao Y, Feng Z, et al. SENP1 inhibits ferroptosis and promotes head and neck squamous cell carcinoma by regulating ACSL4 protein stability via SUMO1. Oncol Rep. 2024;51(2):34.
41. Gao C, Xiao F, Zhang L, et al. SENP1 inhibition suppresses the growth of lung cancer cells through activation of A20-mediated ferroptosis. Ann Transl Med. 2022;10(4):224.
42. Wang Y, Zheng L, Shang W, et al. Wnt/beta-catenin signaling confers ferroptosis resistance by targeting GPX4 in gastric cancer. Cell Death Differ. 2022;29(11):2190–202.
43. Wang H, Peng S, Cai J, et al. Silencing of PTPN18 induced ferroptosis in endometrial cancer cells through p-P38-mediated GPX4/xCT down-regulation. Cancer Manage Res. 2021;13:1757–65.
44. Liu J, Xiao Q, Xiao J, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022;7(1):3.

45. Tang J, Long G, Xiao L, et al. USP8 positively regulates hepatocellular carcinoma tumorigenesis and confers ferroptosis resistance through β-catenin stabilization. Cell Death Dis. 2023;14(6):360.
46. Jiang QF, Tian YW, Shen Q, et al. SENP2 regulated the stability of β-catenin through WWOX in hepatocellular carcinoma cell. Tumour Biol. 2014;35(10):9677–82.

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