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ABRACL upregulated by transcription factor CBX4 promotes proliferation and migration and inhibits the apoptosis of gastric cancer cells

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Summary. Background. Gastric cancer (GC) is a predominant health concern in many countries. Actin-binding Rho activating C-terminal-like (ABRACL) belongs to a new family of low molecular weight proteins and has been implicated in cancers. This study was implemented to elucidate the role and mechanism of ABRACL in GC.

Methods. The mRNA and protein expression of ABRACL and CBX4 in human gastric epithelium cell line GES-1 and GC cell lines was assessed with RT-qPCR and western blot. The transfection efficacy of sh-ABRACL, oe-CBX4, and sh-CBX4 was examined with RT-qPCR and western blot. AGS cell proliferation, migration, and invasion were evaluated using CCK-8, colony formation assay, wound healing, and Transwell assays, respectively. With western blot analysis, flow cytometry, and caspase-3 assay kits, the expressions of MMP2 and MMP9, cell apoptosis, and caspase-3 activity were estimated. Western blot was adopted to estimate the contents of apoptosis-related proteins. Luciferase reporter and chromatin immunoprecipitation (ChIP) were applied to verify the interaction between ABRACL and CBX4.

Results. The expression of ABRACL and CBX4 was increased in GC tissues and cells. After interfering with ABRACL, the proliferation, migration, and invasion of GC cells were inhibited while apoptosis was promoted. We also discovered that CBX4 could bind to ABRACL and transcriptionally regulate ABRACL expression in AGS cells. Rescue experiments revealed that CBX4 overexpression partially reversed the regulatory effects of ABRACL silencing on the proliferation, migration, invasion, and apoptosis of GC cells.

Conclusion. Collectively, ABRACL transcriptionally upregulated by CBX4 promoted the malignant progression of GC.

Key words: Gastric cancer, ABRACL, CBX4, Proliferation, Migration, Apoptosis

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Introduction

Gastric cancer (GC), the third predominant contributor to cancer-related deaths worldwide, is a common malignancy originating from the gastric mucosa (Wei et al., 2020). It has been reported that over 1 million people were diagnosed with GC in 2020, and more than 768,793 patients died from it (Sung et al., 2021). For the time being, surgical resection is a common treatment strategy for GC patients at an early stage (Sun et al., 2020). However, because of unobvious specific symptoms in the early stage, most GC patients were diagnosed at advanced disease stages with poor prognosis (Torre et al., 2015). In recent years, neoadjuvant chemotherapy and immunotherapy have greatly prolonged the overall survival and improved the life quality of GC patients to a certain extent, yet further improvement is still necessary (Zhang et al., 2023). Hence, it is imperative to develop promising therapeutic strategies.

As a regulator of the actin cytoskeleton and cell motility, actin-binding Rho activating C-terminal-like (ABRACL) belongs to a new family of low molecular weight proteins and only exists in eukaryotes (Lin et al., 2011). It has been reported that ABRACL is closely associated with the invasion and metastasis of a variety of cancers. Take breast cancer as an example, ABRACL expression was increased in MCF-7 cells and the knockdown of ABRACL could inhibit the proliferation, migration, and invasion of MCF-7 cells (Li and Chen, 2022). Interestingly, ABRACL expression was upregulated in GC tissues, and ABRACL upregulation indicated a poor prognosis (Wang et al., 2019). Nevertheless, the role and the reaction mechanism of

ABRACL in GC cells remain obscure.

Chromobox 4 (CBX4), which is a member of the Polycomb group (PcG) family of epigenetic regulatory factors, is involved in the development and occurrence of tumors (Hu et al., 2020). For instance, Wang et al. clarified that CBX4 expression was greatly elevated in human breast cancer cells and the overexpression of CBX4 promoted cell proliferation, migration, and invasion (Wen et al., 2021). It has also been evidenced that CBX4 expression was increased in GC and CBX4 silencing suppressed the proliferation of GC cells (Yang et al., 2021). Of note, the HumanTFDB database (http://bioinfo.life.hust.edu.cn/HumanTFDB#!/) revealed that CBX4 could bind to ABRACL and transcriptionally regulate its expression. However, the mechanism of ABRACL associated with CBX4 in GC remains elusive.

To summarize, this study explored the role of ABRACL in the proliferation, migration, invasion, and apoptosis of GC cells and investigated its hidden reaction mechanism associated with CBX4, which may disclose the potential of ABRACL in treating GC.

Materials and methods

Cell culture and treatment

Human gastric epithelium cell line GES-1 and GC cell lines AGS, HGC-27, and MKN-45 were obtained from the BeNa Culture Collection (Henan, China). All cells were incubated in Dulbecco's modified Eagle's medium (DMEM, Biosharp Life Sciences, China) containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific Inc., USA) and 1% penicillin-streptomycin (Sigma-Aldrich, USA) in a humid incubator at 37°C with 5% CO_2.

Cell transfection

AGS cells were harvested in the logarithmic growth phase and then injected into six-well plates at a density of 1×10^5 cells/mL. Plasmids carrying CBX4 (oe-CBX4), pcDNA3.1 empty vector (oe-NC), short hairpin RNAs (sh-RNAs) targeting ABRACL (sh-ABRACL-1/2) and CBX4 (sh-CBX4-1/2) and the corresponding scrambled sequence as a negative control (sh-NC) were synthesized by Geneseed Biotech Co., Ltd. (Guangzhou, China). 100 nM recombinants were transfected into AGS cells at 37°C for 48h with the employment of Lipofectamine 2000 reagent (Thermo Fisher Scientific Inc.). After 48h, AGS cells were harvested for follow-up experiments.

Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was extracted from sample AGS cells using TRIzol reagent (Biosharp) per the standard protocol. Complementary DNA (cDNA) was obtained using PrimeScript RT Reagent Kit with genomic DNA (gDNA) Eraser (Takara Bio Inc., Japan). Afterward, the templates were amplified with TB Green Premix Ex Taq II on the ABI PRISM 7900 Sequence Detection System (Applied Biosystems) according to the manufacturer's instructions. The primer sequences are listed as follows: ABRACL forward (F), 5'-AAGCCGGCAGAAATGG AACG-3' and reverse (R), 5'-TGTGTAGCACAGGAT CTGCC-3', CBX4 F, 5'-ATAACACGTGGGAACCGG AG-3' and reverse R, 5'-TCAGGACATTGGAACGACG G-3' or GAPDH F, 5'TGTGGGCATCAATGGATTTGG-3' and reverse R, 5'-ACACCATGTATTCCGGGTCAAT-3'. The relative gene expression was determined with $2^{-\Delta\Delta CT}$(Livak and Schmittgen, 2001).

Western blot

AGS cells were harvested in the logarithmic growth phase and then injected into six-well plates at a density of 1×10^5 cells/mL. Total protein, extracted from sample AGS cells with radioimmunoprecipitation assay (RIPA) lysis buffer (Biosharp), was quantified using a bicinchoninic acid (BCA) protein assay kit (Biosharp) according to the manufacturer's instructions. Separated by 10% SDS-PAGE, equal amounts of protein (30 μg/lane) were transferred onto PVDF membranes (Millipore, Burlington, MA, USA). Then, the membranes were blocked by 5% bovine serum albumin (BSA, BioFroxx) at room temperature for 1h and subsequently incubated overnight at 4°C with specific primary antibodies against ABRACL (#HPA030217, 1:2500; Sigma-Aldrich), MMP2 (ab92536; 1:1,000; Abcam), MMP9 (ab76003; 1:1,000; Abcam), Bcl-2 (ab32124; 1:1,000; Abcam), Bax (ab32503; 1:1,000; Abcam), cleaved-PARP (ab32064; 1:1,000; Abcam), PARP (ab32138; 1:1,000; Abcam), CBX4 (ab242149; 1:1,000; Abcam), or GAPDH (ab9485; 1:2,500; Abcam). On the next day, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (ab6721; 1:2,000; Abcam) at 37°C for 1h. Finally, protein bands were visualized with an enhanced chemiluminescence (ECL) detection reagent (Shanghai Yeasen Biotechnology Co., Ltd., China), and ImageJ, version 1.49 (National Institutes of Health, USA) was applied for protein density analysis.

Cell Counting Kit-8 (CCK-8) assay

AGS cells that were harvested in the logarithmic growth phase were injected into 96-well plates at a density of 3×10^4 cells/mL and incubated at room temperature for 24h. After that, 15 μL of CCK-8 solution was added to each well and the cells were incubated for another 3h at 37°C. Finally, the optical density was determined at 450 nm with the help of a microplate reader (Thermo Fisher Scientific Inc.).

Colony formation assay

AGS cells that were harvested in the logarithmic growth phase were injected into six-well plates at a density of $1x10^3$ cells/mL and then incubated for 14 d. Afterward, the colonies formed were subjected to 4% paraformaldehyde fixation for 15 min and 0.1% crystal

violet staining for 5 min at room temperature. Finally, the number of colonies was manually counted using an inverted microscope (Olympus Corp).

Wound healing

AGS cells that were harvested in the logarithmic growth phase were inoculated into six-well plates at a density of $2x10^5$ cells/mL and then incubated until 70-80% confluence was achieved. Then, wounds were created with a 200-μl pipette tip. Following rinsing with PBS, the medium was then replaced with serum-free medium and AGS cells were further incubated for 24h at 37°C. Wounds were recorded at 0h and 24h and analyzed by an inverted microscope (Olympus Corp).

Transwell

Initially, $5x10^4$ AGS cells that were harvested in the logarithmic growth phase were injected into the serum-free medium (200 μl) in the upper chambers, which were pre-coated with Matrigel at 37°C for 1h. RPMI-1640 medium containing 10% FBS was added to the lower chambers. After incubation for 24h at 37°C, the invaded cells on the lower surface were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 30 min. Finally, the images of invasive cells were captured with an inverted microscope.

Flow cytometry

AGS cells that were harvested in the logarithmic growth phase were inoculated into six-well plates at a density of $1x10^5$ cells/mL and then incubated at room temperature. Following centrifugation at 1,500 r/min for 5 min, the cell precipitate was collected and the supernatant was discarded. Then, the collected precipitate was rinsed with pre-chilled PBS and fixed with 75% ethanol at 4°C overnight. Next, AGS cells were subjected to 100 μL RNase A (Sigma-Aldrich) at 37°C for 30 min, and 400 μL propidium iodide (Sigma-Aldrich) was applied for staining at 4°C in the dark for 30 min. Finally, the cells were analyzed using a flow cytometer (Beckman Coulter, Brea, CA, USA).

Detection of caspase-3 activity

AGS cells that were collected in the logarithmic growth phase were injected into six-well plates at a density of 1×10^5 cells/mL and then incubated at 37°C. The activity of caspase-3 was appraised with a caspase-3 assay kit (Elabscience, Houston, TX, USA) according to the manufacturer's instructions. The optical density was determined at 405 nm with a microplate reader (Thermo Fisher Scientific Inc.).

Luciferase reporter assay

HumanTFDB predicted that CBX4 could bind to the ABRACL promoter. A Luciferase reporter assay was adopted for the verification of their interaction on the Luciferase Reporter System (Promega, USA). ABRAC wild-type (WT) and mutant (MUT) reporter plasmids containing CBX4-mimic or mimic-NC binding sites constructed by GenePharma were transfected into AGS cells with Lipofectamine 2000 reagent (Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. Luciferase activity was normalized to that of Renilla.

Chromatin immunoprecipitation (ChIP)

For the ChIP assay, a commercially available kit (Beyotime) was used. Following the sonication of GC cells, DNA fragments ranging from 200 to 500 bp were generated. After that, the CBX4 antibody or IgG antibody was applied for immunoprecipitation with the lysates. Finally, the purified DNA was subjected to PCR amplification.

Statistical analysis

All experiments were replicated three times. All data, displayed as mean ± standard deviation, were analyzed with GraphPad Prism software (version 8.0). Comparisons among multiple groups were demonstrated with One-way analysis of variance (ANOVA) with Tukey's post hoc test. *P* less than 0.05 meant that all experimental data were of statistical significance.

Bioinformatics tools

The expressions of ABRACL and CBX4 in GC tissues were analyzed with GEPIA (http://gepia.cancer-pku.cn/) and HPA databases (https://www.proteinatlas.org).

Results

ABRACL expression was upregulated in GC tissues and cells

According to the GEPIA database, ABRACL expression was significantly increased in GC tissues (Fig. 1A). Data on the HPA database also revealed that ABRACL expression was elevated in GC tissues (Fig. 1B). Then, RT-qPCR and western blot were applied for the detection of ABRACL and the results showed that the mRNA and protein expressions of ABRACL were markedly elevated in GC cell lines compared with GES-1 cells (Fig. 1C). It was noted that ABRACL had the highest expression in AGS cells, thus AGS cells were chosen for follow-up experiments.

ABRACL silencing inhibited the proliferation and migration of GC cells

To reduce ABRACL expression, sh-ABRACL was

transfected into AGS cells and the transfection efficacy was examined with RT-qPCR and western blot. As seen in Figure 2A, the mRNA and protein expression of ABRACL in AGS cells was conspicuously reduced by sh-ABRACL compared with the sh-NC group. It was evident that ABRACL had a lower expression in the sh-ABRACL-1 group by contrast with the sh-ABRACL-2 group; hence, sh-ABRACL-1 was chosen for subsequent experiments. Abnormal proliferation is a typical characteristic of cancer cells while cell migration and invasion are responsible for cancer metastasis. Considering this, the role of ABRACL in GC cell proliferation, migration, and invasion was explored. Results obtained from CCK-8 and colony formation assays showed that the proliferation and colony-forming ability of AGS cells were clearly inhibited by ABRACL silencing compared with the sh-NC group (Fig. 2B,C). In addition, the migration and invasion of AGS cells were remarkably reduced after depleting ABRACL expression in comparison with the sh-NC group (Fig. 2D,E). Moreover, ABRACL silencing markedly reduced the expression of MMP2 and MMP9 in AGS cells (Fig. 2F).

ABRACL silencing promoted the apoptosis of GC cells

Apoptosis has been implicated as a critical regulator in cancers. The effects of ABRACL silencing on GC cell apoptosis were investigated through the implementation of flow cytometry and the results revealed that the apoptosis of AGS cells was greatly facilitated when compared with the sh-NC group (Fig. 3A). In contrast with the sh-NC group, the activity of caspase-3 in AGS cells was markedly increased by ABRACL silencing (Fig. 3B). Furthermore, it was found that ABRACL deficiency reduced Bcl-2 expression, whereas it increased the expression of Bax and cleaved-PARP relative to the sh-NC group (Fig. 3C).

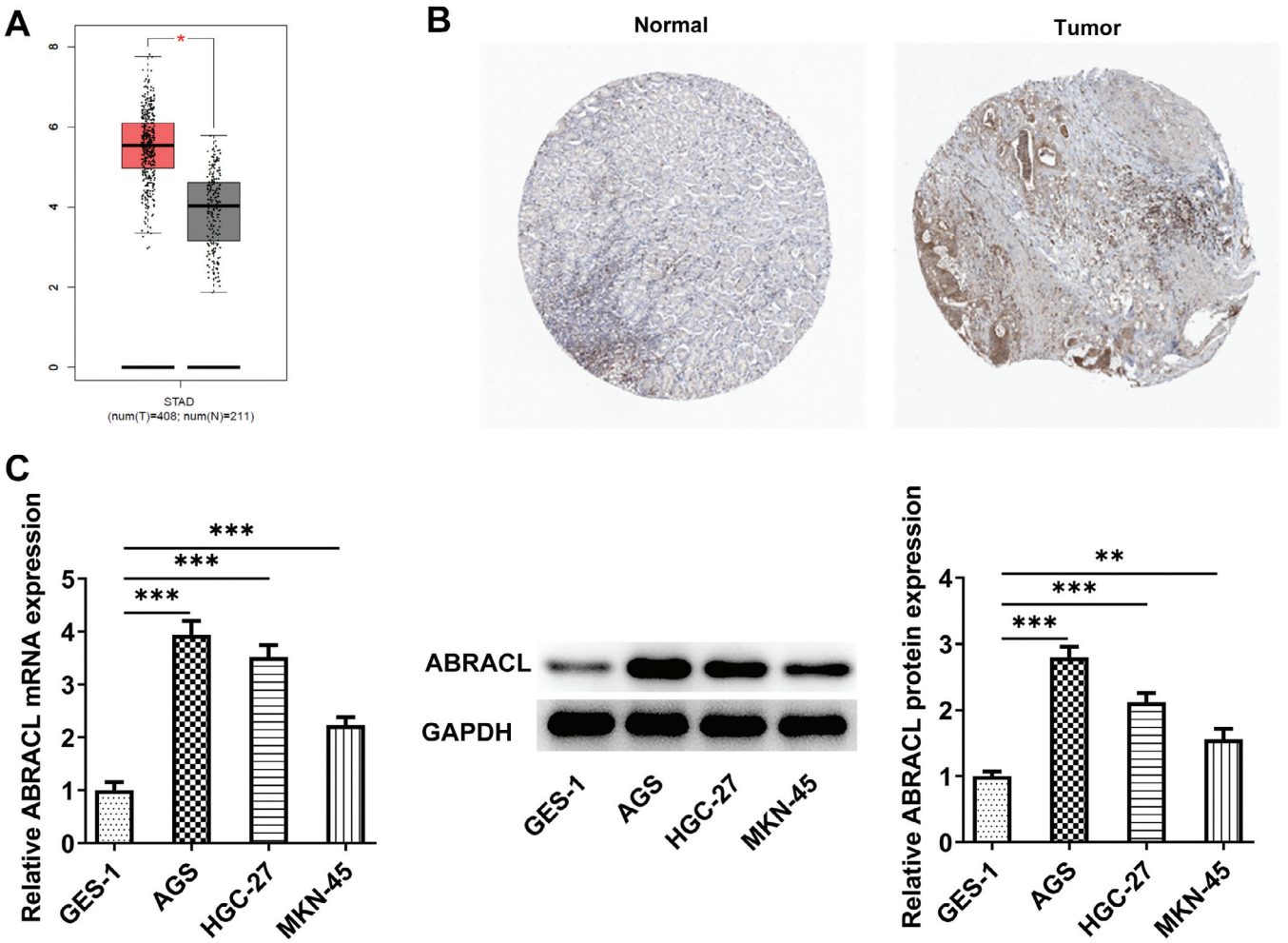

Fig. 1. ABRACL expression was upregulated in GC tissues and cells. The GEPIA **(A)** and HPA databases **(B)** revealed the upregulation of ABRACL in GC tissues. **C.** The mRNA and protein expression of ABRACL was detected using RT-qPCR and western blot. **p*<0.05, ***p*<0.01, ****p*<0.001, n=3.

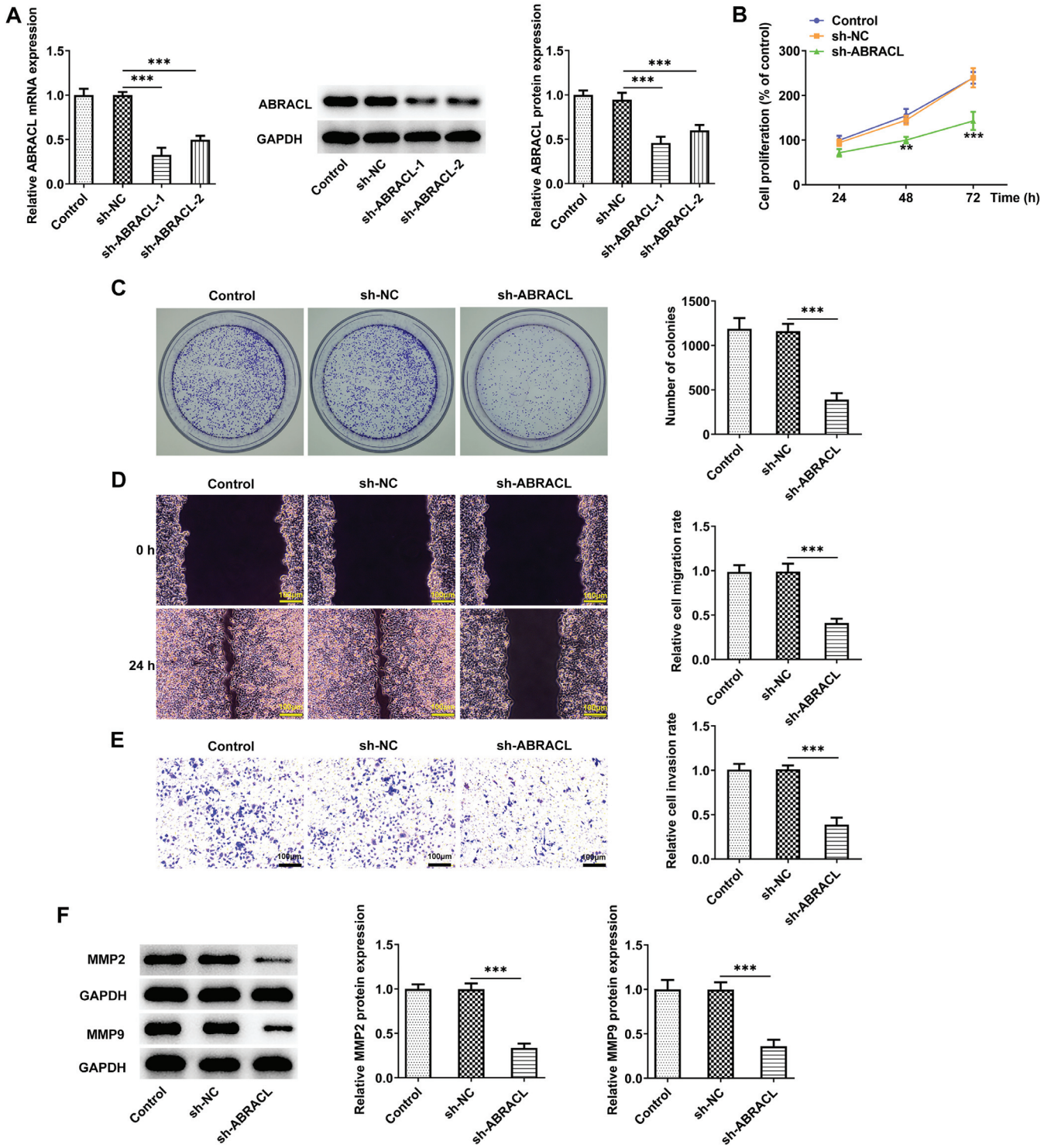

Fig. 2. ABRACL silencing inhibited the proliferation and migration of GC cells. **A.** The transfection efficacy of sh-ABRACL in AGS cells was examined with RT-qPCR and western blot. **B.** The proliferation of ABRACL-silenced AGS cells was assessed with the CCK-8 assay. $^{**}p<0.01$ and $^{***}p<0.001$ vs. sh-NC. **C.** The colony-forming ability of ABRACL-silenced AGS cells was assessed with the colony formation assay. **D.** The migration of ABRACL-silenced AGS cells was assessed with the wound healing assay. **E.** The invasion of ABRACL-silenced AGS cells was assessed with the Transwell assay. **F.** The expression of MMP2 and MMP9 in ABRACL-silenced AGS cells was assessed with western blot. $^{***}p<0.001$, n=3.

CBX4 expression was upregulated in GC tissues and cells and could bind to and transcriptionally regulate ABRACL expression

According to GEPIA and HPA database, CBX4 expression was greatly elevated in GC tissues (Fig. 4A,B). The mRNA and protein expression of CBX4 was estimated with RT-qPCR and western blot and the results demonstrated that CBX4 expression was significantly increased in AGS cells in contrast to that in GES-1 cells (Fig. 4C). The transfection efficacy of oe-CBX4 and sh-CBX4 were examined with RT-qPCR and western blot. As seen in Figure 4D, CBX4 expression was markedly upregulated after AGS cells were transfected with oe-CBX4. Compared with the sh-NC group, the mRNA and protein expression of CBX4 in AGS cells was conspicuously decreased by CBX4 deficiency. It is worthwhile mentioning that CBX4 had a lower expression in the sh-CBX4-1 group, hence, sh-CBX4-1 was chosen for subsequent experiments. After overexpressing or interfering with CBX4, the expression of ABRACL in transfected AGS cells was assessed and the results showed that CBX4 overexpression increased ABRACL expression while CBX4 interference reduced it (Fig. 4E). Besides, compared with ABRACL-WT+oe-NC, CBX4 overexpression conspicuously increased the activity of the ABRACL promoter (Fig. 4F). Additionally, results obtained from ChIP showed that ABRACL expression was enriched in CBX4 antibodies (Fig. 4G).

CBX4 overexpression partially reversed the inhibitory effects of ABRACL silencing on the proliferation and migration of GC cells

As Figure 5A,B depicts, the reduced proliferation and colony-forming ability of AGS cells due to ABRACL silencing was partially revived by CBX4 overexpression. Similarly, the inhibited migration and

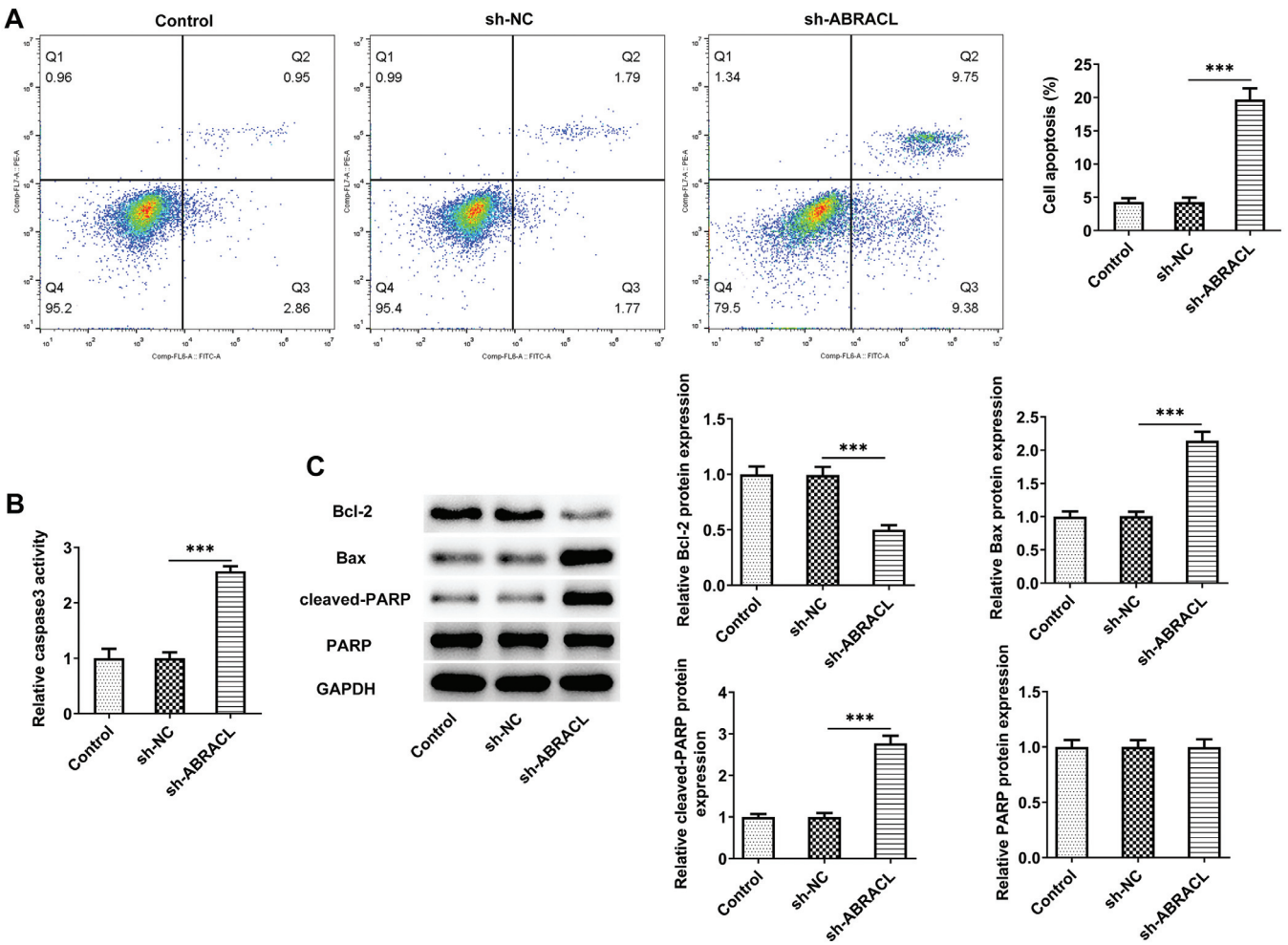

Fig. 3. ABRACL silencing promoted the apoptosis of GC cells. **A.** The apoptosis of ABRACL-silenced AGS cells was assessed with flow cytometry. **B.** The activity of caspase-3 in ABRACL-silenced AGS cells was assessed with a caspase-3 assay kit. **C.** The expression of apoptosis-related proteins in ABRACL-silenced AGS cells was detected using western blot. ***p<0.001, n=3.

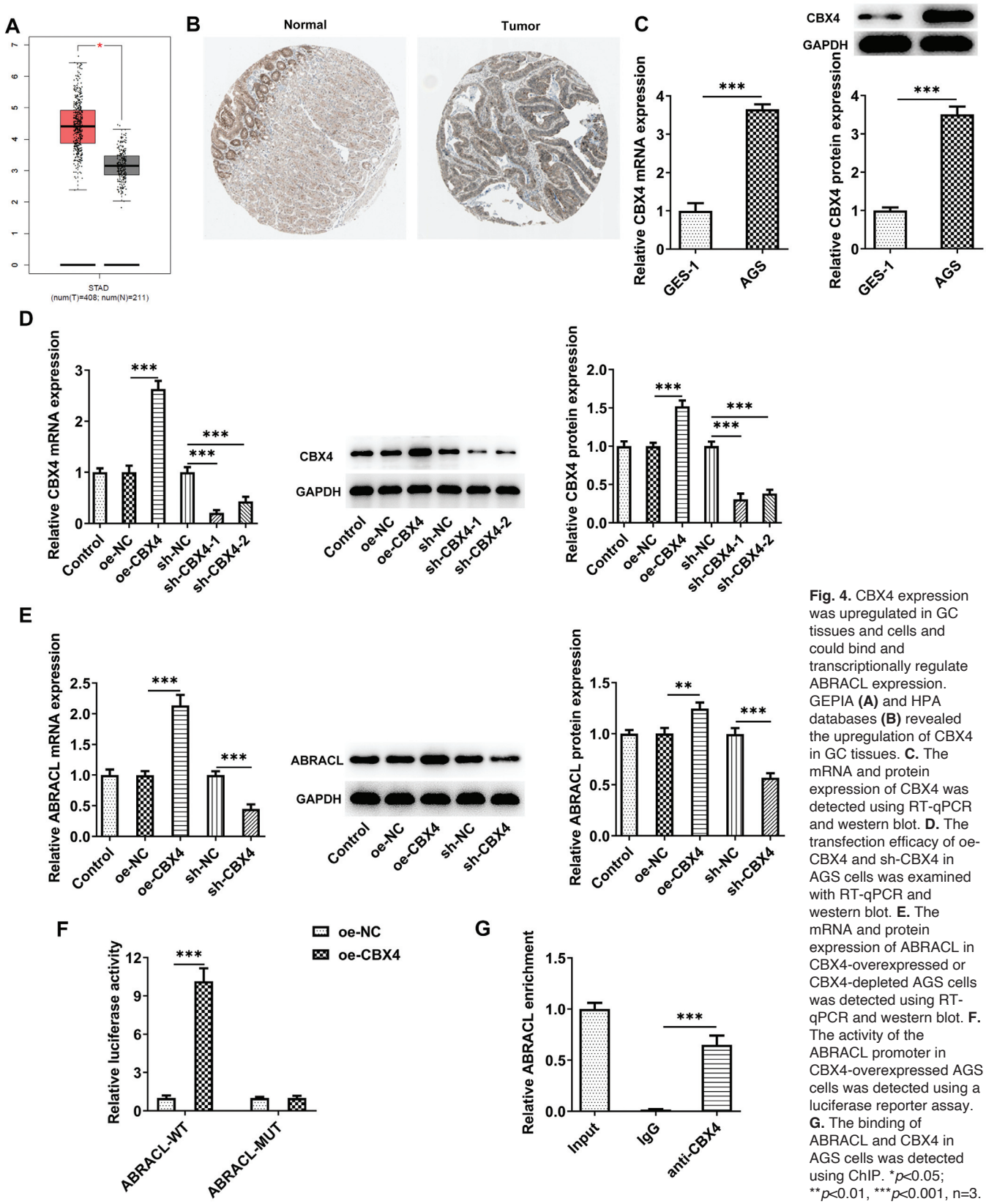

Fig. 4. CBX4 expression was upregulated in GC tissues and cells and could bind and transcriptionally regulate ABRACL expression. GEPIA **(A)** and HPA databases **(B)** revealed the upregulation of CBX4 in GC tissues. **C.** The mRNA and protein expression of CBX4 was detected using RT-qPCR and western blot. **D.** The transfection efficacy of oe-CBX4 and sh-CBX4 in AGS cells was examined with RT-qPCR and western blot. **E.** The mRNA and protein expression of ABRACL in CBX4-overexpressed or CBX4-depleted AGS cells was detected using RT-qPCR and western blot. **F.** The activity of the ABRACL promoter in CBX4-overexpressed AGS cells was detected using a luciferase reporter assay. **G.** The binding of ABRACL and CBX4 in AGS cells was detected using ChIP. *p<0.05; **p<0.01, ***p<0.001, n=3.

invasion of ABRACL-depleted AGS cells were increased after overexpressing CBX4 (Fig. 5C,D). In addition, ABRACL interference reduced the expression of MMP2 and MMP9 in AGS cells compared with the sh-NC group, which were then increased by CBX4 overexpression (Fig. 5E).

CBX4 overexpression partially reversed the promotive effects of ABRACL silencing on the apoptosis of GC cells

Compared with the sh-ABRACL+ oe-NC group, the increased apoptosis in ABRACL-silenced AGS cells was inhibited after transfection with oe-CBX4 (Fig. 6A).

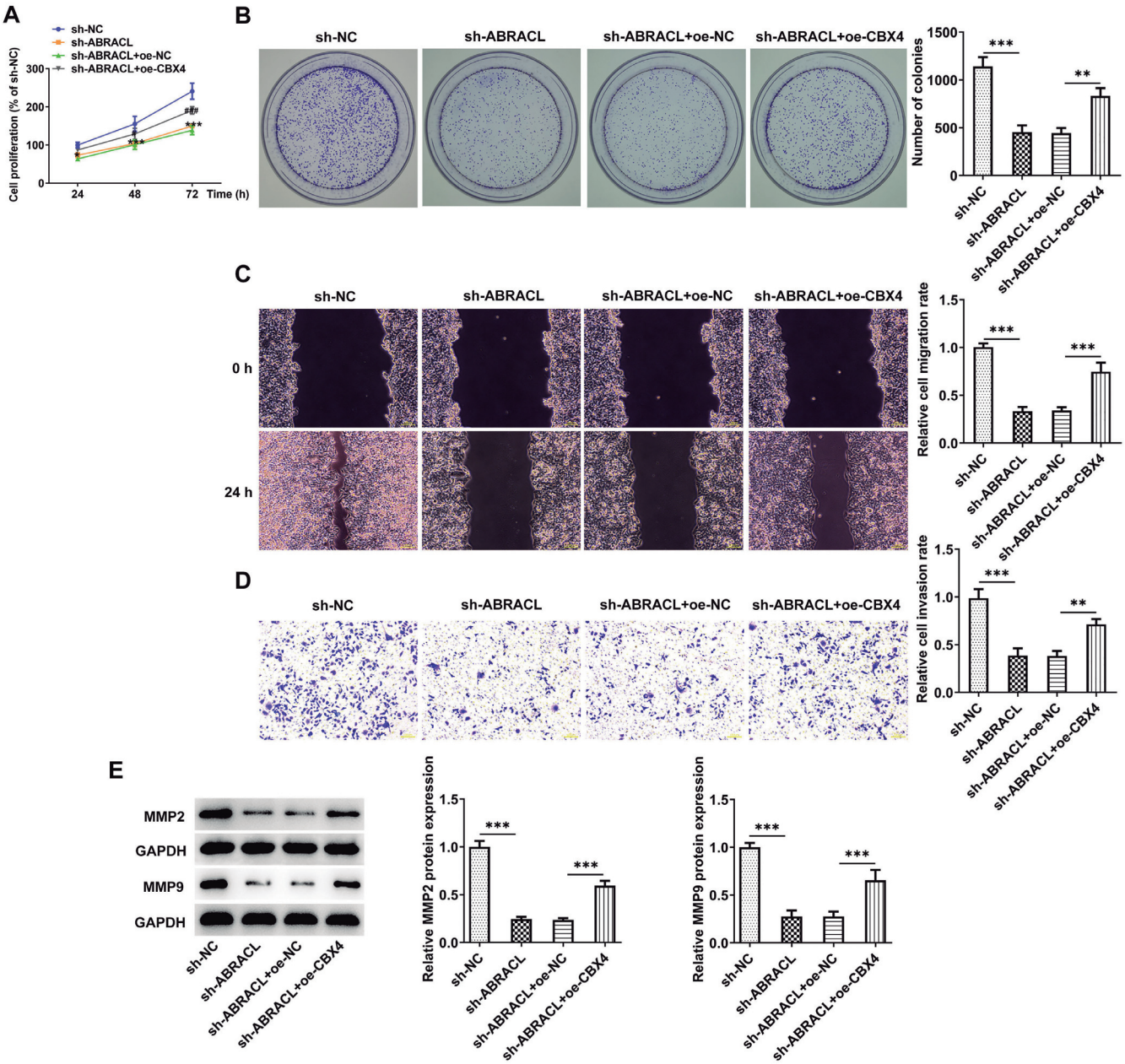

Fig. 5. CBX4 overexpression partially reversed the inhibitory effects of ABRACL silencing on the proliferation and migration of GC cells. **A.** The proliferation of AGS cells co-transfected with sh-ABRACL and oe-CBX4 was detected using the CCK-8 assay. *p<0.05 and ***p<0.001 vs. sh-NC, $^{\#}p$<0.05 and $^{\#\#\#}p$<0.001 vs. sh-ABRACL+oe-NC. **B.** The colony-forming ability of AGS cells co-transfected with sh-ABRACL and oe-CBX4 was detected using the colony formation assay. **C.** The migration of AGS cells co-transfected with sh-ABRACL and oe-CBX4 was detected using a wound healing assay. **D.** The invasion of AGS cells co-transfected with sh-ABRACL and oe-CBX4 was detected using the Transwell assay. **E.** The expression of MMP2 and MMP9 in AGS cells co-transfected with sh-ABRACL and oe-CBX4 was detected using western blot. **p<0.01, ***p<0.001, n=3.

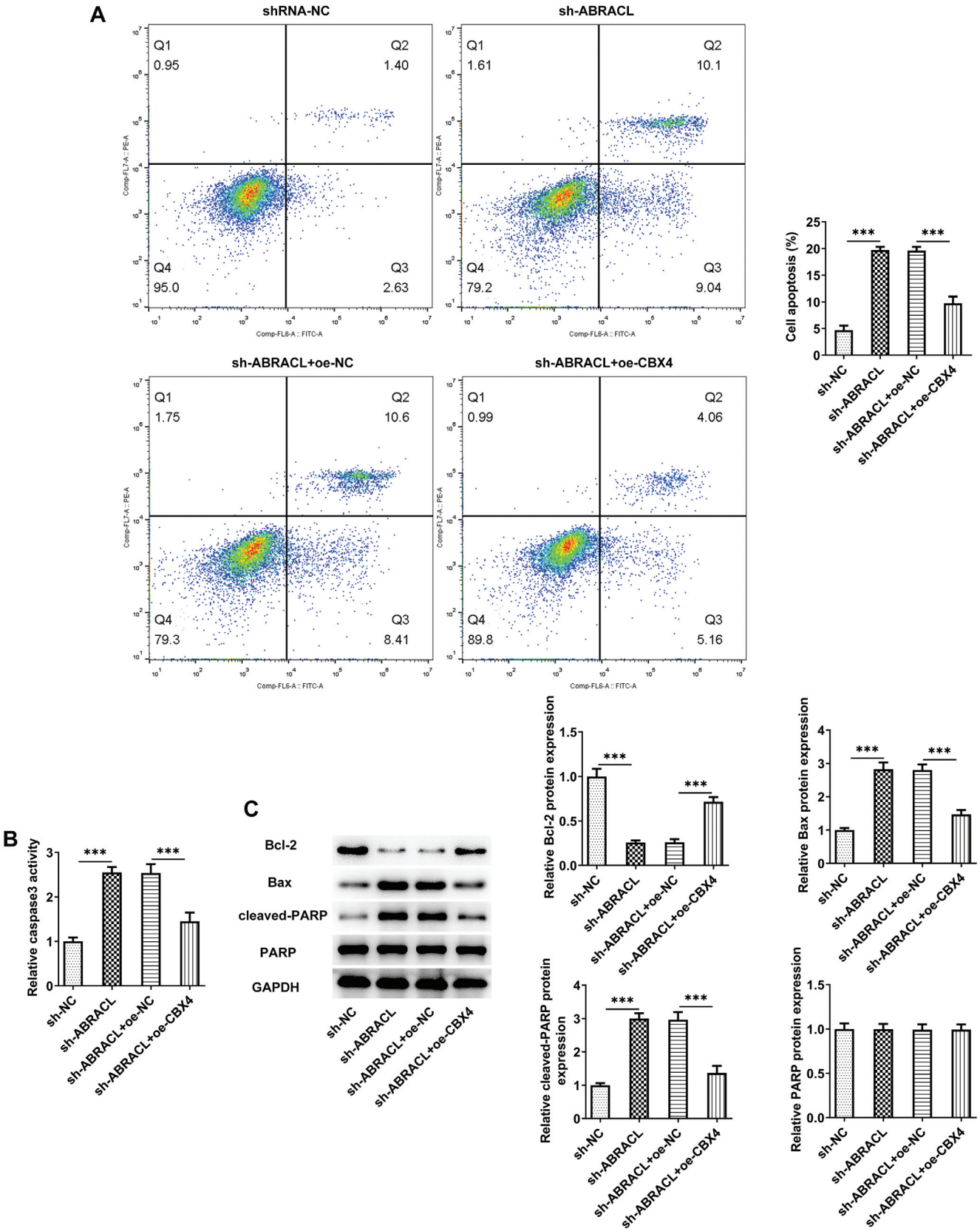

Fig. 6. CBX4 overexpression partially reversed the promotive effects of ABRACL silencing on the apoptosis of GC cells. **A.** The apoptosis of AGS cells co-transfected with sh-ABRACL and oe-CBX4 was assessed with flow cytometry. **B.** The activity of caspase-3 in AGS cells co-transfected with sh-ABRACL and oe-CBX4 was assessed with a caspase-3 assay kit. **C.** The expression of apoptosis-related proteins in AGS cells co-transfected with sh-ABRACL and oe-CBX4 was detected using western blot. ***$p<0.001$, n=3.

Likewise, the increased caspase-3 activity in AGS cells due to ABRACL deficiency was decreased by CBX4 overexpression (Fig. 6B). Relative to the sh-NC group, ABRACL silencing decreased Bcl-2 expression and increased that of Bax and cleaved-PARP in AGS cells, while CBX4 overexpression exhibited opposite effects on these proteins, evidenced by increased Bcl-2 expression and decreased expression of Bax and cleaved-PARP in the sh-ABRACL + oe-CBX4 group (Fig. 6C).

Discussion

The high incidence and mortality rates of malignancies remain major problems that plague human life and health. Although tremendous advances have been made in radical surgery, radiotherapy, chemotherapy, immunological therapy, as well as new hormone therapy, patients still suffer from cancer recurrence and metastasis (Vanneman and Dranoff, 2012). In recent years, targeted therapies have emerged as promising treatment methods for cancer (Lee et al., 2018). In GC, several molecules, such as CXCR4, CD47, and Rac1 have been identified as potential therapeutic targets (Koizumi et al., 2012; Ji et al., 2015; Yoshida et al., 2015). Results of the present study showed that ABRACL transcriptionally upregulated by CBX4 promoted proliferation, migration, and invasion whereas it inhibited the apoptosis of AGS cells, preliminarily indicating that ABRACL might be a potential candidate for the treatment of GC.

A previous study showed that ABRACL expression was upregulated in GC tissues compared with normal tissues and high ABRACL expression was associated with tumorigenesis and affected clinic outcomes in GC (Wang et al., 2019). Similarly, the elevation of ABRACL was also observed in GC cells in this study. As known to all, the imbalance between proliferation and apoptosis is the direct inducement of tumor formation (Qiao et al., 2023). Facilitating apoptosis and suppressing the proliferation of tumor cells are supposed to be effective ways to impede the advancement of cancers (Zhao et al., 2021). A previous study demonstrated that ABRACL expression was increased in esophageal carcinoma cells and ABRACL deficiency could suppress cell proliferation (Fan et al., 2021). Additionally, the interference of ABRACL could inhibit the proliferation and the colony-forming ability of cells in breast cancer (Li and Chen, 2022). Similarly, ABRACL depletion hereby could inhibit the proliferation and colony-forming ability of AGS cells whereas it facilitated cell apoptosis. The Bcl-2 and caspase families are closely related to tumor cell apoptosis. In the Bcl-2 family, the Bcl-2 protein repressed cell apoptosis through the interruption of early linkage to programmed cell death, and the Bax protein facilitated cell apoptosis via the formation of a dimer with the Bcl-2 protein to inactivate Bcl-2 (Ghasemi et al., 2018). Caspase-3, which is an executor of apoptosis, will cleave itself into an active cleaved-caspase-3 state to induce apoptosis when it is inactivated (Zhang et al., 2019). ABRACL deficiency hereby was discovered to reduce Bcl-2 expression and increase the expression of Bax and cleaved-PARP as well as caspase-3 activity in AGS cells.

It was evidenced that cancer cells and tissues are characterized by the expression of voltage-gated sodium channels, which can facilitate the migration of cancer cells (Fraser et al., 2014). In addition, the migration and invasion of cancer cells into the surrounding tissue and vasculature are important steps in cancer metastasis, which is the predominant cause of cancer death (Duff and Long, 2017). As reported, the targeting of ABRACL by miR-145-5p could suppress the migration and invasion of esophageal carcinoma cells (Fan et al., 2021). Moreover, Hsiao et al. attested that ABRACL silencing could repress the migration and invasion of colorectal cancer cells (Hsiao et al., 2021). Evidently, the intervention in the migration and invasion of cancer cells is effective to retard cancer progression. Consistently, this study discovered that ABRACL silencing suppressed the migration and invasion of AGS cells.

CBX4, which is also known as hPC2 or NBP16, has been reported to be abnormally expressed in various malignancies (Ismail et al., 2012); e.g., elevation of CBX4 expression could be observed in lung adenocarcinoma cells (Wang et al., 2021). Additionally, CBX4 expression was increased in osteosarcoma tissues and osteoblast cells (Wang et al., 2020). Of note, CBX4 expression is greatly elevated in GC cells (Li et al., 2022). Per this, this study discovered that CBX4 expression was greatly increased in AGS cells and CBX4 could bind to and transcriptionally regulate the ABRACL promoter. The upregulation of CBX4 by circular RNA hsa_circ_0008039 could promote the proliferation, migration, and invasion of breast cancer cells (Huang et al., 2020). Jiang et al. provided evidence supporting that CBX4 knockdown promoted HDAC inhibitor (HDACi)-induced cell apoptosis in clear cell renal cell carcinoma (Jiang et al., 2020). To explore the mechanism of ABRACL associated with CBX4 in GC, rescue experiments were conducted and the results showed that CBX4 overexpression reversed the effects of ABRACL knockdown on cell proliferation, migration, invasion, and apoptosis in GC.

Conclusion

To sum up, this study discussed the functional role of ABRACL in cell proliferation, migration, invasion, and apoptosis in GC. We identified that the ABRACL promoter could bind to CBX4 and that ABRACL could be positively regulated by CBX4, thus revealing the mechanism by which ABRACL transcriptionally activated by CBX4 promoted the progression of GC. However, the functional experiments we conducted were only limited to AGS cells, which is a limitation of the present study.

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Ethics approval and consent to participate. Not applicable.

Availability of data and materials. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. KG and XG conceived the experiments. KG performed the experiments. KG analyzed the data. KG and XG confirmed the authenticity of all the raw data. Both authors have read and approved the final manuscript.

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