Transcriptional activation of GSTP1 by ANXA3 is associated with cisplatin sensitivity in colorectal cancer cells

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Abstract. Although platinum-based chemotherapeutic agents demonstrate efficacy against colorectal cancer (CRC), the development of resistance to these agents remains a notable clinical challenge, with the underlying mechanisms still incompletely understood. In the present study, public tumor databases revealed that annexin A3 (ANXA3) was significantly upregulated in CRC primary tumors. This upregulation was then validated at the protein level using a tissue microarray, which confirmed elevated ANXA3 expression in CRC tumor tissues. The Kaplan-Meier curve analysis further demonstrated that patients with CRC with high ANXA3 expression exhibited shorter overall survival compared with those with low expression. Notably, CRC cell lines with high ANXA3 expression showed reduced sensitivity to cisplatin. Moreover, overexpression of ANXA3 significantly diminished cisplatin sensitivity in CRC cells, whereas silencing ANXA3 enhanced this sensitivity. These results collectively indicated that ANXA3 modulated cisplatin sensitivity in CRC cells. Subsequent investigations revealed that ANXA3 upregulated glutathione S-transferase P1 (GSTP1) expression in CRC cells in an NF-κB-dependent manner, a pathway mechanistically implicated in clinically observed chemotherapy resistance. In addition, ANXA3-overexpressing exosomes were found to upregulate GSTP1 transcriptional expression and decrease cisplatin sensitivity in CRC cells. Finally, *in silico* molecular docking predicted K201 as a potential ANXA3-interacting compound. Further investigations revealed that combination treatment with K201 and cisplatin significantly enhanced tumor inhibition compared with cisplatin monotherapy in CRC cell models. In conclusion, using *in vitro* experiments, the current study demonstrated that targeting ANXA3 may represent a promising strategy for overcoming cisplatin resistance in CRC. However, further *in vivo* validations, particularly regarding the therapeutic relevance of the ANXA3-GSTP1 axis and K201, are warranted to substantiate its clinical potential.

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Introduction

Colorectal cancer (CRC) is the most prevalent malignant tumor among gastrointestinal malignancies in China, ranking fifth in both incidence and mortality among all tumors (1-4). Notably, over the past 30 years, the incidence and mortality rates of CRC in patients <50 years of age have steadily increased, gradually showing a younger trend (5). Unlike in Western countries where CRC is often diagnosed at earlier stages due to well-established population-based screening programs, universal health insurance coverage for preventive services and higher public awareness of early warning signs, CRC in China is often diagnosed at an advanced stage, with the majority of patients presenting with local progression or distant metastasis. Consequently, the 5-year survival rate for late-stage patients remains at ~14% (6-9). For patients with metastatic or recurrent CRC, chemotherapy remains a main treatment strategy. Among platinum-based drugs, such as oxaliplatin and cisplatin, oxaliplatin is more commonly used in clinical practice for CRC, whereas cisplatin retains clinical relevance, particularly in cases of oxaliplatin resistance or intolerance (10-13). In the present study, cisplatin was used to investigate the chemoresistance mechanisms in CRC, with the

aim of identifying novel strategies to overcome platinum-based chemotherapy resistance.

Annexin A3 (ANXA3), a member of the membrane protein family, is a calcium-dependent phospholipid-binding protein (14). Alterations in the expression level or subcellular localization of membrane-associated proteins can trigger various pathological processes, including tumorigenesis (14). ANXA3 has been reported to serve important roles in numerous biological processes, such as angiogenesis, adipocyte differentiation, tumorigenesis and chemotherapy resistance (15). For example, ANXA3 has been reported to be upregulated in ovarian cancer and to serve as a potential biomarker for chemotherapy resistance (16). It may also enhance platinum resistance in ovarian cancer cells via the regulation of p53 (16). Additionally, ANXA3 has been identified as being upregulated in lung cancer, and positively associated with clinical staging and lymph node metastasis (17). The relationship between ANXA3 and the development of liver cancer has also been reported, yet its specific mechanism remains unclear (18).

Notably, the precise mechanism by which ANXA3 mediates cisplatin resistance in CRC, especially whether it acts by regulating key cellular detoxification pathways, remains elusive. Glutathione S-transferase P1 (GSTP1), an important member of the GST family, has a key role in cellular detoxification and drug resistance (19). Specifically, GSTP1 catalyzes the binding of glutathione with various electrophilic compounds, including platinum-based chemotherapeutic drugs, thereby promoting their metabolism and excretion, and reducing the cytotoxicity of these drugs to cancer cells (20). Previous studies have demonstrated that GSTP1 upregulation is closely associated with chemotherapeutic resistance in multiple tumors, including CRC (21-23). Based on the role of ANXA3 in chemotherapeutic resistance and the key function of GSTP1, it is hypothesized that ANXA3 may mediate cisplatin resistance in CRC through GSTP1. Moreover, exosomes, key mediators of intercellular communication, can transfer drug resistance between cancer cells (24,25). Membrane-associated proteins, such as ANXA2, are routinely detected in exosomes (26,27), which prompts the further hypothesis that ANXA3 may also be transmitted between cells via the exosome pathway and amplify this GSTP1-mediated drug resistance.

The present study aimed to investigate whether ANXA3 modulates cisplatin resistance in CRC through the regulation of GSTP1 expression and to explore the availability of potential inhibitors targeting this pathway. By addressing these issues, the present study aimed to provide novel insights into the molecular mechanisms underlying cisplatin resistance in CRC and to identify a promising therapeutic strategy for overcoming chemotherapy resistance.

Materials and methods

Cells, tissues and chemicals. CRC cell lines HCT116, LoVo, RKO, SW480 and SW620 were obtained from American Type Culture Collection. The 293T cell line was obtained from the Shanghai Branch, Cell Bank, Chinese Academy of Sciences and maintained in our laboratory. All cell lines were cultured in Dulbecco's Modified Eagle Medium (HyClone™; Cytiva) with 10% fetal bovine serum (cat. no. 10099141; Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin (cat. no. C0222; Beyotime Biotechnology) in a humidified atmosphere of 5% CO_2 at 37˚C. Primary CRC tumors (from 91 male patients and 78 female patients) were collected from Shanghai East Hospital (Shanghai, China) between March 2024 and February 2026, with ages ranging from 36 to 82 years and a median age of 62 years. The present study was approved by the Ethics Committee of Shanghai East Hospital (approval no. 2024-220). Cisplatin and the NF-κB inhibitor JSH-23 were purchased from Selleck Chemicals. K201 was purchased from TargetMol Chemicals Inc.

Gene expression analysis. ANXA3 expression in colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) was analyzed using Gene Expression Profiling Interactive Analysis (GEPIA), an online tool that integrates tumor tissue data from The Cancer Genome Atlas (TCGA) and normal tissue data from the Genotype-Tissue Expression (GTEx) project. The COAD cohort comprised 275 tumor samples and 349 normal samples, while the READ cohort included 92 tumor samples and 318 normal samples (http://gepia2.cancer-pku.cn). Pearson correlation analyses among ANXA3 expression and excision repair cross-complementing 1 (ERCC1), GSTP1 or ATP7B in CRC were also performed using the GEPIA database. The online public tumor database UALCAN, which integrates protein expression data from the Clinical Proteomic Tumor Analysis Consortium (CPTAC), was used to analyze the protein expression of ANXA3 in colon cancer (https://ualcan.path.uab.edu/).

To evaluate the mRNA levels, reverse transcription-quantitative PCR (RT-qPCR) was performed. Briefly, total RNA was extracted from cultured cells using RNAiso Plus (Takara Bio, Inc.), and cDNA was reverse-transcribed with the PrimeScript™ RT reagent Kit (Takara Bio, Inc.) according to the manufacturer's protocol. Subsequently, qPCR was carried out using SYBR Green qPCR Master Mix (Clontech; Takara Bio, Inc.) with Roche LightCycler® 480II real-time PCR system (Roche Diagnostics). GAPDH was used as the reference gene, and relative expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method as described previously (28). Thermal cycling conditions included initial denaturation at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. Primer sequences used for qPCR are shown in Table SI.

Immunohistochemistry (IHC) analysis. Tissue arrays containing 169 pairs of CRC tissues and corresponding paracancerous tissues were prepared for IHC, and the patients' information has been described in our previous study (29). Tissues were formalin-fixed at room temperature for 24 h, paraffin-embedded and cut into 4-μm sections. Sections were permeabilized with 0.1% Triton X-100, blocked with 5% BSA at room temperature for 1 h, and incubated with an anti-ANXA3 antibody (cat. no. ab127924; Abcam; 1:250) at 4˚C overnight. HRP-conjugated goat anti-rabbit IgG (cat. no. ab205718; Abcam; 1:500) was applied for 1 h at room temperature, followed by DAB chromogen detection. Staining was visualized using a light microscope. The staining of ANXA3 was scored using a semi-quantitative scale, as follows: 0, negative; 1, weak; 2, moderate; 3, strong. The scoring was performed in a blinded manner. For survival analysis, the ANXA3 score was categorized as follows: Scores of 0-1 indicated

low ANXA3 expression, while scores of 2-3 indicated high ANXA3 expression.

Western blot analysis. Western blot analysis was carried out as described previously (30). Briefly, the total protein from cells or exosomes was extracted using RIPA lysis buffer (Beyotime Biotechnology) supplemented with protease and phosphatase inhibitors. The total protein concentration was detected using the BCA Protein Assay Kit (cat. no. P0009; Beyotime Biotechnology), and 30 μg protein per lane was separated by 10% SDS-PAGE. Subsequently, the proteins were transferred onto polyvinylidene fluoride membranes (MilliporeSigma), and membranes were blocked with 5% (w/v) non-fat milk at room temperature for 2 h. After blocking, the membranes were incubated with the indicated primary antibodies overnight at 4˚C. The anti-ANXA3 antibody (cat. no. ab127924; 1:1,000), anti-CD63 antibody (cat. no. ab68418; 1:500) and anti-CD81 antibody (cat. no. ab79559; 1:500) were obtained from Abcam. The anti-Flag-tag antibody (cat. no. M185-3; 1:2,000) was purchased from Medical & Biological Laboratories Co., Ltd. The anti-GAPDH antibody (cat. no. 97166; 1:1,000), anti-phosphorylated (p)-NF-κB p65 (Ser536) antibody (cat. no. 3033; 1:1,000) and anti-NF-κB p65 antibody (cat. no. 8242; 1:1,000) were purchased from Cell Signaling Technology, Inc. The anti-GSTP1 antibody (cat. no. F0781; 1:500) was obtained from Selleck Chemicals. After washing with TBS-Tween (0.1% v/v), the membranes were incubated with appropriate HRP-conjugated secondary antibodies (cat. no. A0208 or A0216; Beyotime Biotechnology; 1:500) for 1 h at room temperature. Finally, the immunoreactive bands were visualized using an ECL substrate (Beyotime Biotechnology) and captured with a chemiluminescence imaging system (Tanon Science and Technology Co., Ltd.). Semi-quantitative analysis of western blot band intensities was performed using ImageJ software (version 2.9.0, National Institutes of Health).

Measurement of cell viability. Cell viability was detected using the Cell Counting Kit-8 (CCK-8) assay (cat. no. C0042; Beyotime Biotechnology) as described previously (31,32). Briefly, CRC cells (5,000 cells/well) were seeded in 96-well plates and allowed to adhere overnight. For the cisplatin sensitivity assay, various CRC cell lines, lentivirus-infected cells, or siRNA-transfected cells were incubated with increasing concentrations of cisplatin (0, 10, 20, 40, 80 and 160 μM) for 24 h at 37˚C. For combination treatment, the cells were pretreated with 10 μM K201 or vehicle (0.1% DMSO in cell culture medium) for 1 h at 37˚C, followed by co-exposure to increasing concentrations of cisplatin (0, 10, 20, 40, 80 and 160 μM) for 24 h at 37˚C in the continued presence of K201 or vehicle. Subsequently, 10 μl CCK-8 reagent was added to each well and the cells were cultured for a further 1 h at 37˚C. After incubation with CCK-8 reagent, OD values were measured. Wells were assigned randomly and the investigator performing the OD measurement was blinded to the treatment groups. Experiments were repeated independently three times.

Plasmid construction, lentivirus preparation and small interfering RNA (siRNA) transfection. The human full-length ANXA3 cDNA sequence was amplified by PCR and cloned into the lentivirus vector PLVX (Addgene, Inc.) with a Flag tag (PLVX-Flag-ANXA3). An empty PLVX vector (PLVX-NC) was used as the negative control. The ANXA3-overexpressing plasmids and packaging plasmids were co-transfected into 293T cells to produce the lentivirus and the viral particles were prepared as described previously (33). In detail, lentiviral particles were produced by using a second-generation lentiviral packaging system in the present study. When 293T cells reached 70-80% confluence, they were co-transfected with the lentiviral expression plasmid, psPAX2 and pMD2.G at a mass ratio of 4:3:1 by using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instruction. The total amount of plasmid DNA used for each 10-cm dish was 20 μg. The culture supernatant containing lentiviral particles was collected at 48 and 72 h after transfection. LoVo and RKO cells were infected with the indicated lentiviral particles at a multiplicity of infection of 20 and 30, respectively, in the presence of 5 μg/ml polybrene (cat. no. H9268; Sigma-Aldrich; Merck KGaA). Following 48 h infection, stable cells were selected using 2 μg/ml puromycin (cat. no. P9620; Sigma-Aldrich; Merck KGaA) for 7 days and maintained in medium containing 1 μg/ml puromycin.

The siRNA against ANXA3 (siANXA3), the siRNA against GSTP1 (siGSTP1) or the negative control siRNA (siNC) were transfected into CRC cells at a final concentration of 10 nM using Lipofectamine® RNAiMAX (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Transfections were carried out at 37˚C in a humidified incubator with 5% CO_2, and the transfection medium was replaced with complete culture medium after 6 h. These cells were prepared for subsequent experiments 24 or 48 h post-transfection. The sequences were as follows: siANXA3 sense strand, 5'-GGAAUAUCAAGCAGCAUAUdTdT-3'; antisense strand, 5'-AUAUGCUGCUUGAUAUUCCdTdT-3'; siGSTP1 sense strand, 5'-CAUCAAUGGCAACGGGAAAdTdT-3'; antisense strand, 5'-UUUCCCGUUGCCAUUGAUGdTdT-3'; and siNC sense strand, 5'-UUCUCCGAACGUGUCACGUdTdT-3'; antisense strand, 5'-ACGUGACACGUUCGGAGAAdTdT-3' (all synthesized by Shanghai GenePharma Co., Ltd.).

NF-κB inhibitor treatment. For pharmacological inhibition of NF-κB signaling, LoVo cells were seeded in 6-well plates at $5x10^5$ cells/well and allowed to adhere overnight at 37˚C with 5% CO_2. And then, cells were incubated with 5 μM NF-κB inhibitor JSH-23 (Selleck Chemicals) or vehicle control (DMSO) for 48 h at 37˚C with 5% CO_2, followed by RNA and protein extraction for further analysis.

Isolation of exosomes. Exosomes were isolated as described previously (34). Briefly, LoVo cells infected with PLVX-NC or PLVX-Flag-ANXA3-derived lentiviruses were incubated for 72 h at 37˚C. Cell culture supernatants were then collected and subjected to sequential centrifugation: 300 x g for 10 min at 4˚C to remove cells, 2,000 x g for 20 min at 4˚C to remove debris and 10,000 x g for 30 min at 4˚C to remove large vesicles. Exosomes were then isolated from the resulting supernatant using the Hieff® Quick Exosome Isolation Kit Plus (cat. no. 41205ES10; Shanghai Yeasen Biotechnology Co., Ltd.) according to the manufacturer's instructions. The total protein concentration of the isolated exosomes was detected using BCA Protein Assay Kit according to the

manufacturer's protocol. The expression levels of exosomal biomarkers CD63 and CD81 were then detected by western blot analysis as aforementioned. For exosome treatment, LoVo cells were incubated with 50 μg/ml OE-ANXA3-exosomes or NC-exosomes at 37˚C with 5% CO_2 for 24 h.

Luciferase assay. The GSTP1 luciferase reporter gene construct (-336 to +74) was generated as reported previously (35). For the luciferase assay, LoVo cells ($5x10^4$ cells/well) were seeded in 24-well plates and cultured overnight. The cells were then co-transfected with 400 ng GSTP1 luciferase reporter plasmid (constructed by inserting GSTP1 promoter into PGL4 vector, and 40 ng pRL-TK *Renilla* internal control vector (Promega Corporation) using Lipofectamine 3,000 according to the manufacturer's instructions. After 24 h, the cells were infected with the PLVX-Flag-ANXA3-derived lentiviruses for 48 h or incubated with the ANXA3-overexpressing exosomes for 24 h, followed by luciferase assay using the Dual-Luciferase Reporter Assay System (Promega Corporation) according to the manufacturer's instructions. Firefly luciferase activity was normalized to the corresponding *Renilla* luciferase activity for each well. All transfections and treatments were performed in three independent replicates.

Virtual screening. Virtual screening was performed using molecular docking as described previously (36). ANXA3 structure (UniProt ID: P12429; https://www.uniprot.org/uniprotkb/P12429/entry) was obtained from AlphaFold database (version 2.3.2; https://alphafold.ebi.ac.uk/entry/P12429) and the scoring of the molecular docking was completed using the Schrödinger Suite (HTVS/SP/XP modes, Schrödinger LLC), followed by Sybyl clustering conducted in Sybyl-X 2.0 (Certara L.P.). Finally, the candidate compound K201 was screened out based on the scores and PubMed literature search (https://pubmed.ncbi.nlm.nih.gov). The docking score of K201 was -5.378 kcal/mol and Molecular Mechanics/Generalized Born Surface Area of K201 was -54.204 kcal/mol.

Statistical analysis. *In vitro* experiments were independently repeated at least three times unless otherwise indicated. The software GraphPad Prism 5 (Dotmatics) was used to generate figures and perform statistical analysis. Continuous data are presented as the mean ± SD, and were compared between two independent groups using unpaired Student's t-test. When data were paired or matched, paired Student's t-test was used. Categorical data are presented as median with interquartile range (IQR); the Wilcoxon signed-rank test was applied for non-continuous data (such as IHC scores). One-way or two-way ANOVA was used to compare three or more groups, followed by Tukey's post hoc test for multiple comparisons. The Kaplan-Meier method and log-rank test were used for survival analysis. For IHC score distributions (scores 0, 1, 2 and 3), Bowker's test was used to compare CRC and NT groups, and Fisher's exact test was used for comparisons across clinical stages I-IV. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Elevated ANXA3 is a negative index for patients with CRC. In our previous study, ANXA3 was identified as being upregulated in both primary and liver metastatic CRC (37). To further validate this finding, the publicly available cancer data from the GEPIA database were analyzed, which integrates TCGA normal and GTEx datasets, and the results revealed significantly upregulated mRNA expression levels of ANXA3 in COAD and READ tissues (Fig. 1A). Concurrently, data from the UALCAN database (matched with CPTAC samples) revealed that the protein levels of ANXA3 were elevated in the primary tumor tissues of colon cancer (Fig. 1B). To validate these findings, a tissue array was prepared and subsequently evaluated by IHC staining. These results confirmed that ANXA3 was significantly upregulated in CRC tumor tissues (Fig. 1C and 1D; Table SII). Furthermore, Kaplan-Meier survival analysis revealed that high expression of ANXA3 was associated with poor prognosis in patients with CRC (Fig. 1E). Taken together, these preliminary data indicated that ANXA3 was upregulated in CRC tumors and elevated ANXA3 was associated with a poor prognosis for patients with CRC.

ANXA3 mediates cisplatin sensitivity and regulates GSTP1 expression in CRC cells. First, the expression levels of ANXA3 in five CRC cell lines were detected, including HCT116, LoVo, RKO, SW480 and SW620. As shown in Fig. 2A, ANXA3 was markedly downregulated in LoVo and RKO cell lines compared with in the other cell lines. Subsequently, these five CRC cell lines were incubated with cisplatin and CCK-8 assays were performed to assess drug sensitivity. The results showed that CRC cell lines with high ANXA3 expression exhibited reduced sensitivity to cisplatin compared with those with low ANXA3 (Fig. 2B). To further validate this relationship, ANXA3 was overexpressed by lentiviral plasmid transduction in LoVo and RKO cells, which exhibited low endogenous ANXA3 (Fig. 2C), and the results showed that overexpression of ANXA3 significantly reduced the sensitivity of cisplatin in CRC cells (Fig. 2D and E). By contrast, when ANXA3 was silenced in HCT116 and SW480 cells with high endogenous ANXA3 (Fig. 2F and G), these CRC cells became more sensitive to cisplatin (Fig. 2H and I). Taken together, the aforementioned results suggested that ANXA3 mediates cisplatin sensitivity in CRC cells.

Next, the public tumor database GEPIA was analyzed and it was found that ANXA3 expression in CRC was positively correlated with the expression of several important genes associated with chemoresistance, including ERCC1 ($r=0.24$; $P<0.05$), GSTP1 ($r=0.45$; $P<0.05$) and ATP7B ($r=0.27$; $P<0.05$) (Fig. 3A). The expression levels of these genes were also confirmed using RT-qPCR and GSTP1 was revealed to be the most prominently upregulated following ANXA3 overexpression in CRC cells (Fig. 3B and C). Western blot analysis further revealed that ANXA3 overexpression markedly increased GSTP1 protein levels in CRC cells (Fig. 3D). Combined with the mRNA levels, it was hypothesized that ANXA3 may regulate the transcriptional expression of GSTP1. To assess this, a luciferase reporter system driven by the GSTP1 promoter was used, and the luciferase assay showed that ANXA3 significantly increased the activity of the GSTP1 promoter (Fig. 3E). By contrast, silencing ANXA3 decreased GSTP1 expression (Fig. 3F). The aforementioned results indicate that ANXA3 promoted the transcriptional expression of GSTP1 in CRC cells. Given that NF-κB has been reported

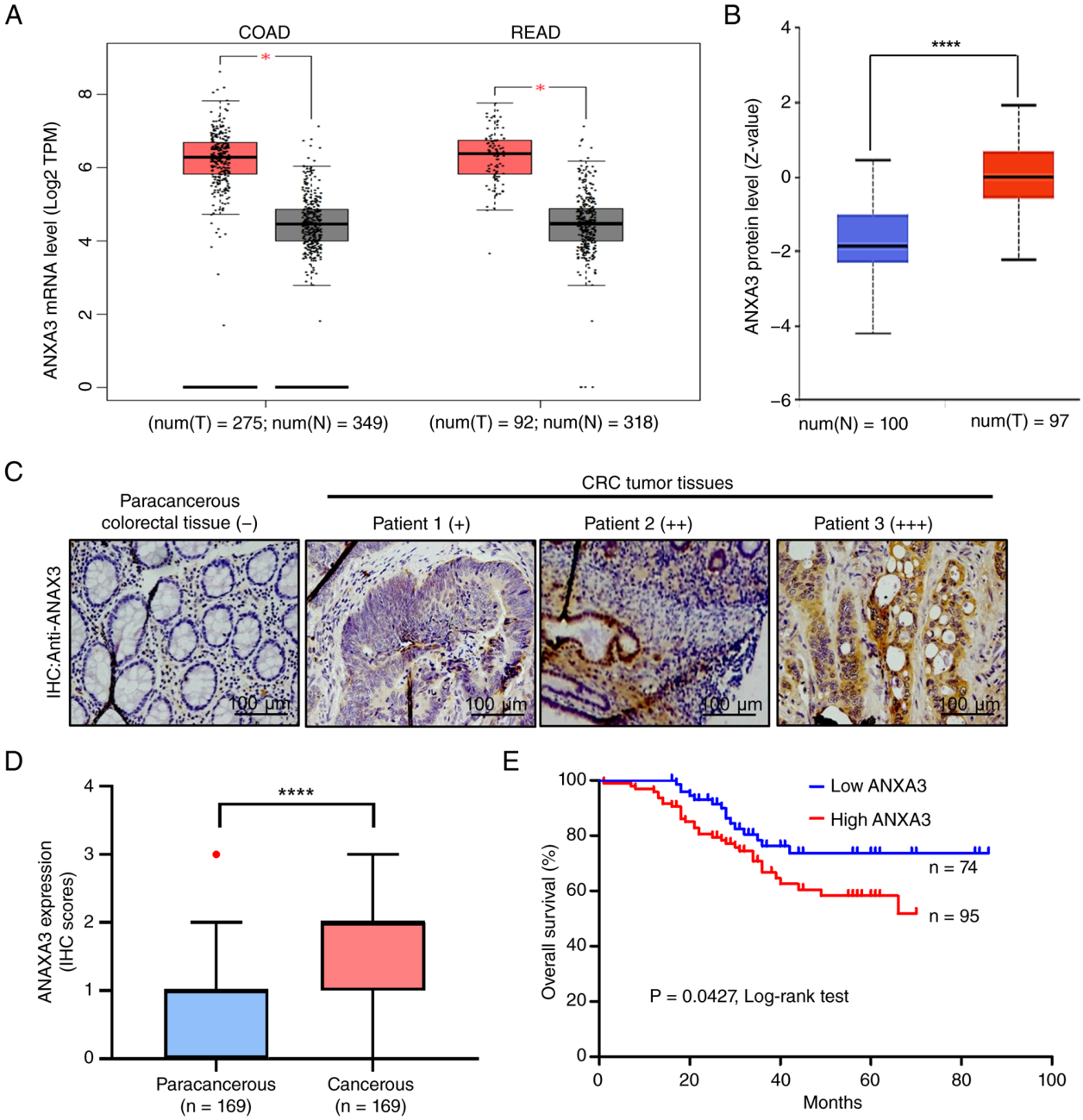

Figure 1. Elevated ANXA3 is a negative index for patients with CRC. (A) Online public tumor database Gene Expression Profiling Interactive Analysis was used to analyze the mRNA expression of ANXA3 in COAD and READ. Data were analyzed using unpaired Student's t-test and are presented as the mean ± SD. (B) Online public tumor database UALCAN was used to analyze the protein expression of ANXA3 in colon cancer. Data were analyzed using unpaired Student's t-test and are presented as the mean ± SD. IHC staining was performed to detect the expression of ANXA3 in a tissue microarray containing 169 pairs of CRC cancerous tissues and corresponding paracancerous tissues, and the (C) representative images and (D) statistical analysis are shown. '-' represents a score of 0, '+' represents a score of 1, '++' represents a score of 2, '+++' represents a score of 3. Data are presented as box plots showing the median and interquartile range, and statistical analysis was performed using the Wilcoxon signed-rank test. (E) Overall survival of patients with CRC with low or high ANXA3 (n=169) was analyzed by Kaplan-Meier curve analysis. *P<0.05, ****P<0.0001. ANXA3, annexin A3; T, tumor tissues; N, normal tissues; COAD, colon adenocarcinoma; CRC, colorectal cancer; IHC, immunohistochemistry; READ, rectum adenocarcinoma.

to be an important transcription factor mediating the expression of GSTP1 (38), whether ANXA3 exerted its function by activating NF-κB was investigated. As shown in Fig. 3G, overexpression of ANXA3 upregulated the expression levels of p-NF-κB p65. Moreover, the regulatory effect of ANXA3 on GSTP1 expression was also blocked by the NF-κB inhibitor JSH-23 (Fig. 3H and I), which indicated that ANXA3 regulated GSTP1 expression in CRC cells in an NF-κB-dependent manner. In addition, as demonstrated in Fig. S1, knockdown of GSTP1 significantly suppressed ANXA3-mediated cisplatin resistance, which further suggested that GSTP1 may serve as a key downstream molecule through which ANXA3 exerts its effect on drug sensitivity.

ANXA3-overexpressing exosomes (OE-ANXA3-exosomes) upregulate GSTP1 expression and reduce cisplatin sensitivity in CRC cells. For the present study, OE-ANXA3-exosomes were also generated. As shown in Fig. 4A and B,

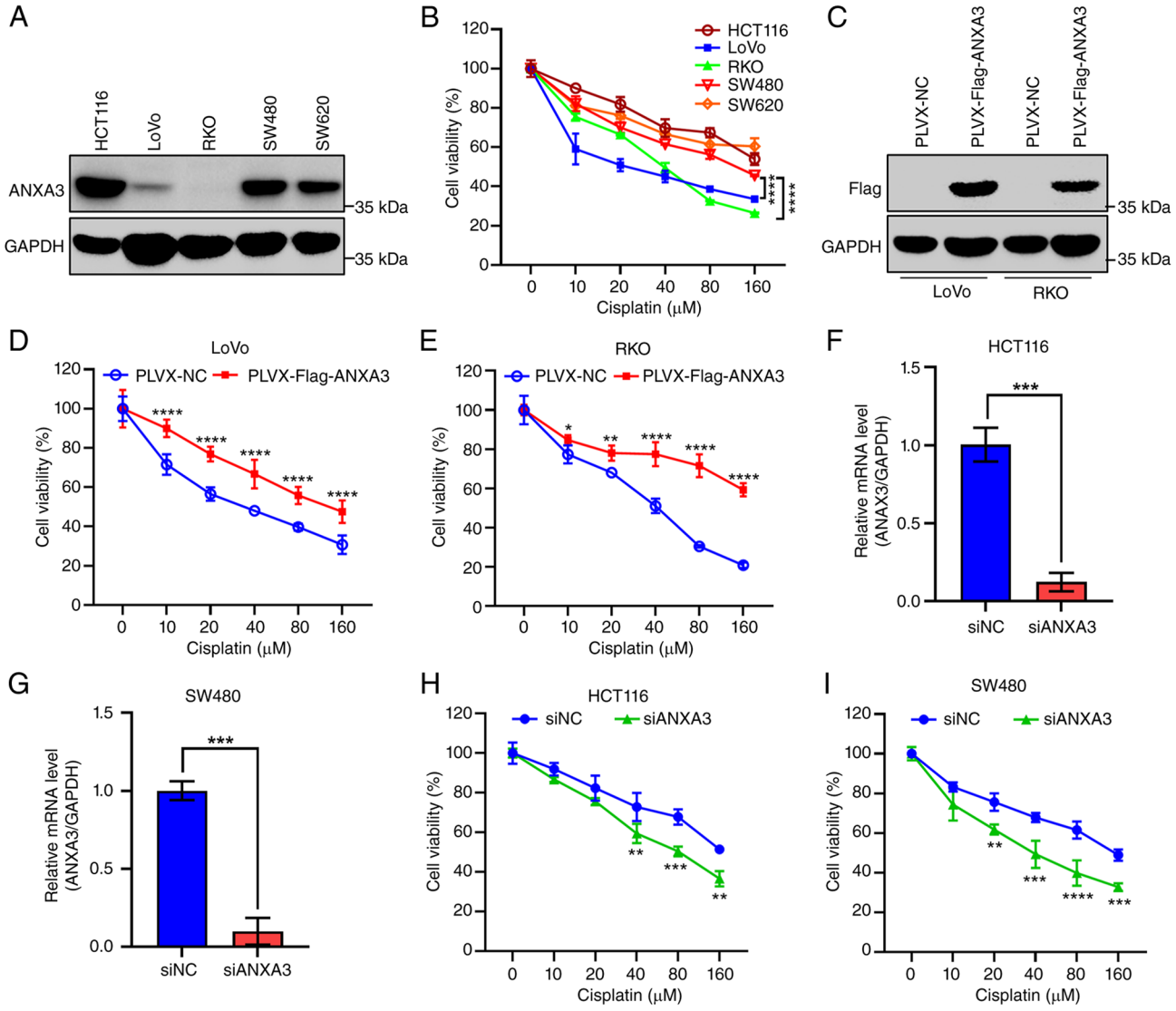

Figure 2. Elevated ANXA3 reduces the sensitivity of cisplatin in CRC cells. (A) CRC cell lines, including HCT116, LoVo, RKO, SW480 and SW620, were lysed for western blot analysis to detect the protein levels of ANXA3. (B) CRC cell lines were incubated with increasing concentrations of cisplatin for 24 h, followed by CCK-8 assay. Data are presented as the mean ± SD and P-values were calculated via two-way ANOVA. ****P<0.0001. (C) Western blotting was used to detect the effectiveness of the PLVX-derived ANXA3-overexpressing lentivirus system in LoVo and RKO cells. (D) LoVo and (E) RKO cells infected with PLVX-NC or PLVX-Flag-ANXA3-derived lentiviruses were incubated with increasing concentrations of cisplatin for 24 h, followed by CCK-8 assay. Data are presented as the mean ± SD and P-values were calculated via two-way ANOVA. *P<0.05, **P<0.01, ****P<0.0001 vs. PLVX-NC group at that concentration. (F) HCT116 and (G) SW480 cells were transfected with siNC or siANXA3 for 24 h, followed by reverse transcription-quantitative PCR analyses to assess the mRNA levels of ANXA3. GAPDH was used as an internal control. Data were analyzed using unpaired Student's t-test and are presented as the mean ± SD. ***P<0.001. (H) HCT116 and (I) SW480 cells transfected with siNC or siANXA3 were incubated with increasing concentrations of cisplatin for 24 h, followed by CCK-8 assay. Data are presented as the mean ± SD and P-values were calculated via two-way ANOVA. **P<0.01, ***P<0.001, ****P<0.0001 vs. siNC at that concentration. All experiments were repeated independently three times. ANXA3, annexin A3; CCK-8, Cell Counting Kit-8; CRC, colorectal cancer; NC, negative control; si, small interfering.

ANXA3-overexpressing CRC cells were successfully established, and exosomes isolated from the culture supernatants were subsequently characterized by western blot analysis, which confirmed the expression of exosomal biomarkers CD63 and CD81. Subsequently, CRC cells were incubated with OE-ANXA3-exosomes or NC-exosomes, and the results indicated that OE-ANXA3-exosomes significantly upregulated the transcriptional expression of GSTP1 (Fig. 4C and D). Additionally, OE-ANXA3-exosomes significantly increased the luciferase activity of the GSTP1 promoter (Fig. 4E). Subsequently, CRC cells were incubated with OE-ANXA3-exosomes or cisplatin, and the results indicated that OE-ANXA3-exosomes significantly decreased the drug sensitivity of cisplatin in CRC cells (Fig. 4F). Moreover, the cisplatin-triggered downregulation of GSTP1 was partially reversed by the treatment with OE-ANXA3-exosomes (Fig. 4G and H). Based on these findings, it is speculated that ANXA3 may be involved in the process of exosome-mediated cisplatin resistance in CRC cells through regulating GSTP1 expression.

Combined K201 and cisplatin treatment significantly inhibits CRC viability. Finally, molecular docking-based virtual screening identified K201 as a potential compound

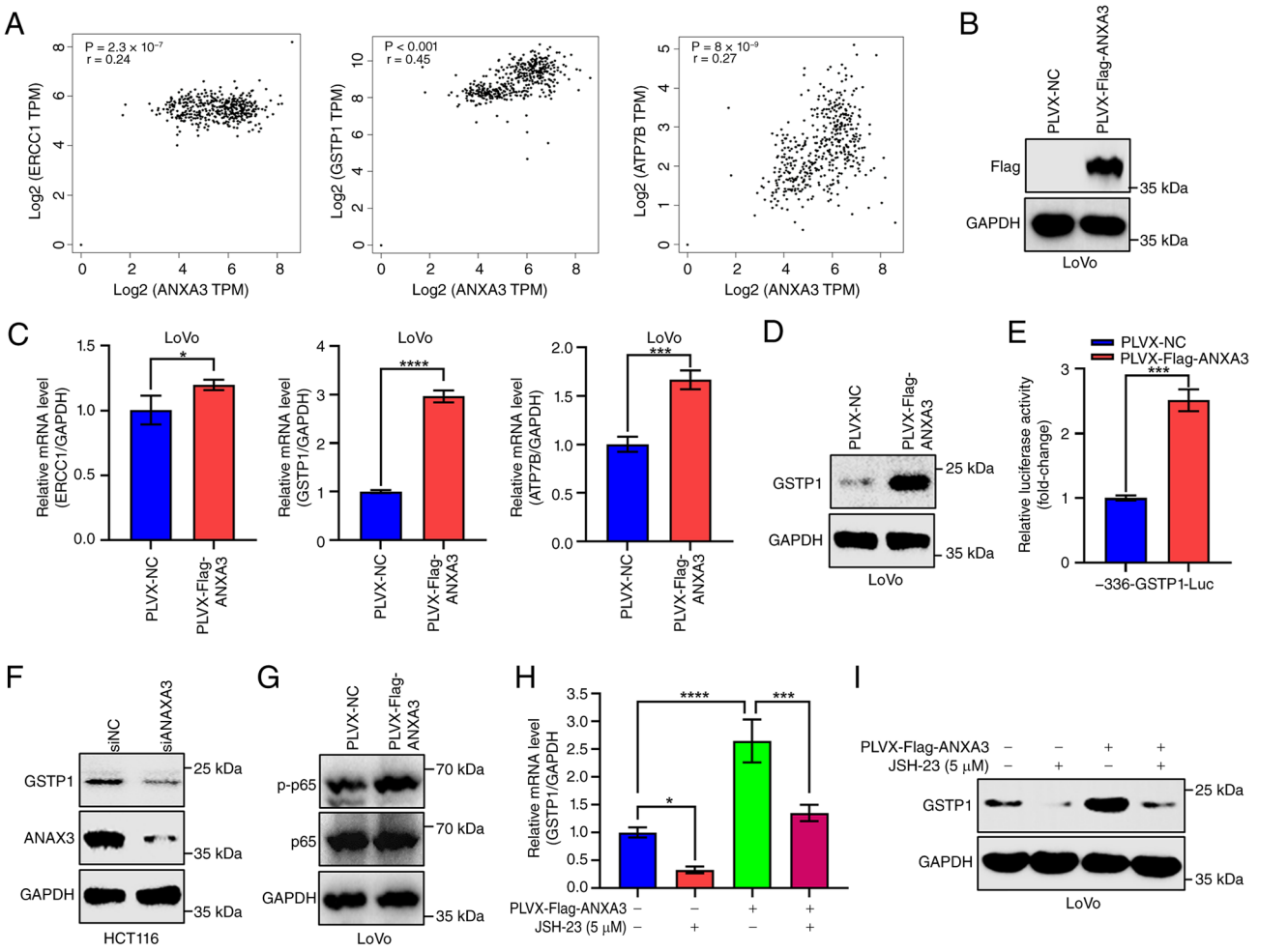

Figure 3. ANXA3 regulates GSTP1 expression in colorectal cancer cells in an NF-κB-dependent manner. (A) ANXA3 gene co-expression analyses were conducted using the publicly available cancer database Gene Expression Profiling Interactive Analysis, including ERCC1, GSTP1 and ATP7B. LoVo cells were infected with PLVX-NC or PLVX-Flag-ANXA3 lentiviruses, and cultured for 48 h. (B) Then, western blotting was performed to assess the expression of Flag-ANXA3. (C) RT-qPCR was performed to assess the mRNA levels of ERCC1, GSTP1 and ATP7B. GAPDH was used as an internal control. (D) Western blotting was performed to assess the expression of GSTP1. (E) Luciferase assay was conducted to assess the effects of ANXA3 on the activity of the GSTP1 promoter (-336 to +74). (F) HCT116 cells were transfected with siNC or siANXA3 for 48 h, followed by western blotting to assess the protein levels of GSTP1 and ANXA3. GAPDH was used as a loading control. (G) LoVo cells were infected with PLVX-NC or PLVX-Flag-ANXA3 for 48 h, followed by western blotting to assess the expression levels of p-NF-κB p65, NF-κB p65 and GAPDH. LoVo cells were infected with PLVX-Flag-ANXA3-derived lentivirus or incubated with 5 μM NF-κB inhibitor JSH-23 for 48 h at 37˚C, followed by (H) RT-qPCR analyses to assess the mRNA levels of GSTP1 or (I) western blotting to assess the protein expression of GSTP1. GAPDH was used as an internal control. Data are presented as the mean ± SD and P-values were calculated by unpaired Student's t-test or one-way ANOVA. All experiments were repeated independently three times. *P<0.05, ***P<0.001, ****P<0.0001. ANXA3, annexin A3; ERCC1, excision repair cross-complementing 1; GSTP1, glutathione S-transferase P1; NC, negative control; p-, phosphorylated; RT-qPCR, reverse transcription-quantitative PCR; si, small interfering.

interacting with ANXA3. As shown in Fig. 5A-C, K201 formed π-π interactions with His8 (HIE8) and Phe206, as well as a hydrogen bond and a salt bridge with Asp283 of ANXA3. Subsequently, CRC cells harboring different expression levels of ANXA3 were incubated with K201, and the results showed that K201 exhibited stronger cytotoxicity in HCT116 cells with high ANXA3 expression than in RKO and LoVo cells with low ANXA3 expression (Fig. S2). CRC cells with high ANXA3 expression (including HCT116 and SW480) were then incubated with cisplatin alone or in combination with K201, and K201 was shown to significantly enhance the cytotoxicity of cisplatin in CRC cells (Fig. 5D and E). Critically, the enhanced cytotoxicity observed in the combination group (K201 + cisplatin) was significantly greater than that of K201 alone, arguing against a simple additive toxic effect and supporting a specific sensitizing mechanism (Fig. 5F). Moreover, ectopic overexpression of ANXA3 partially reversed the K201-mediated cisplatin sensitization in CRC cells (Fig. 5F), which suggested that the level of ectopically expressed ANXA3, while sufficient to significantly attenuate K201 activity, may not fully saturate all available targets in the presence of the drug. These results collectively suggested that targeting ANXA3 activity with K201 may enhance cisplatin efficacy in CRC cells.

Discussion

ANXA3 exhibits high structural homology with other membrane-associated proteins, particularly ANXA5 (39). It has been reported that the mRNA levels of ANXA5 are upregulated in tumor tissues of colorectal adenocarcinoma, and increased ANXA5 expression is associated with higher

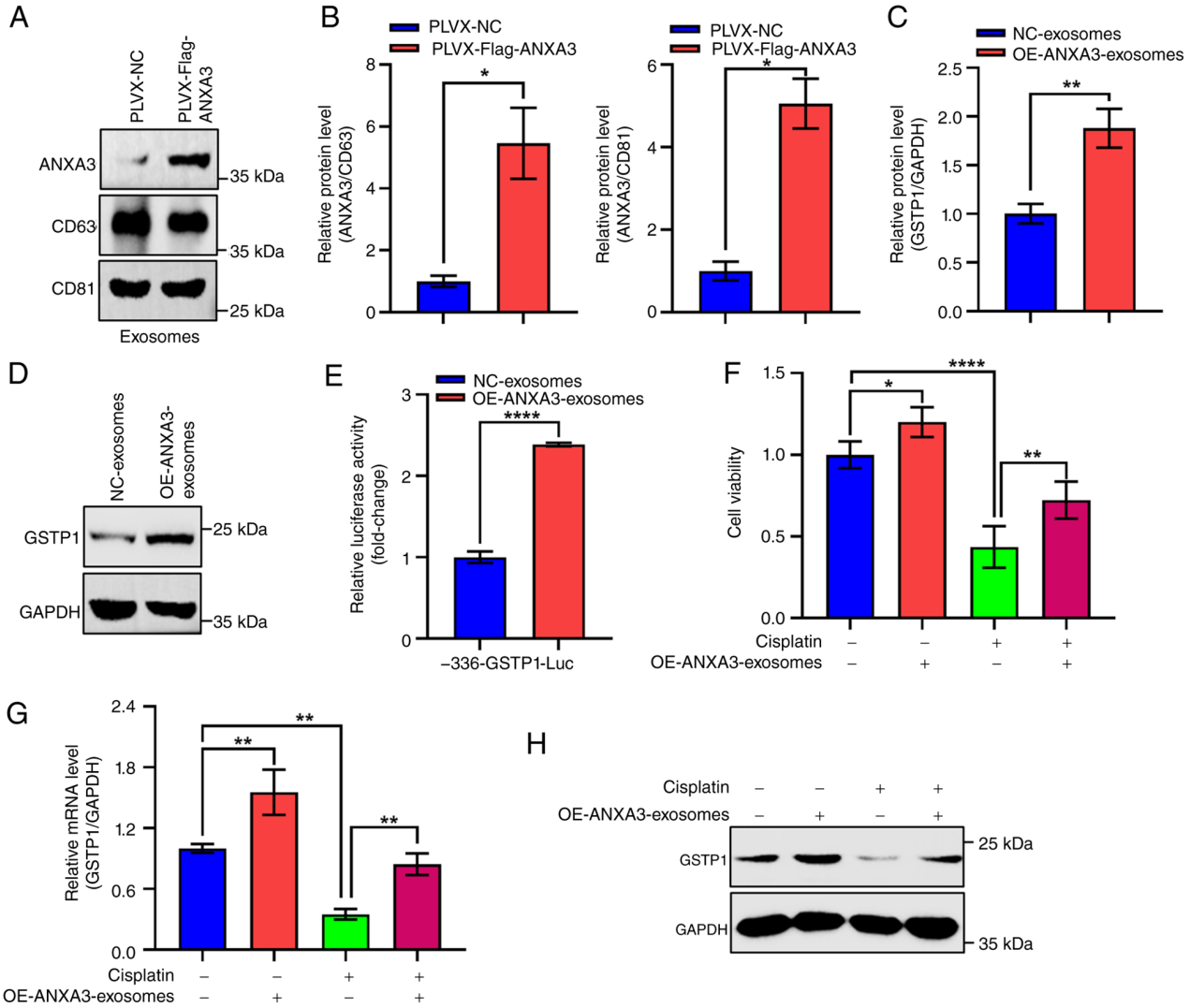

Figure 4. OE-ANXA3-exosomes increase GSTP1 expression and decrease sensitivity to cisplatin in colorectal cancer cells. (A) Exosomes from LoVo cells infected with PLVX-NC or PLVX-Flag-ANXA3-derived lentiviruses (namely NC-exosomes and OE-ANXA3-exosomes) were isolated and identified by western blotting via detection of the expression levels of the indicated biomarkers. (B) Protein expression of ANXA3 was semi-quantified. LoVo cells were incubated with 50 μg/ml OE-ANXA3-exosomes or NC-exosomes for 24 h, followed by (C) RT-qPCR or (D) western blotting to assess the expression of GSTP1. (E) Luciferase assay was conducted to assess the effects of OE-ANXA3-exosomes on the activity of the GSTP1 promoter (-336 to +74). (F) LoVo cells were incubated with 40 μM cisplatin or 50 μg/ml OE-ANXA3-exosomes for 24 h, followed by Cell Counting Kit-8 assay. The aforementioned cells were also prepared for (G) RT-qPCR to assess the mRNA levels of GSTP1 or (H) western blotting to assess the protein expression of GSTP1. GAPDH was used as an internal control. Data are presented as the mean ± SD and P-values were calculated via unpaired Student's t-test or one-way ANOVA. All experiments were repeated independently three times. *P<0.05, **P<0.01, ****P<0.0001. ANXA3, annexin A3; GSTP1, glutathione S-transferase P1; NC, negative control; OE-ANXA3-exosomes, ANXA3-overexpressing exosomes; RT-qPCR, reverse transcription-quantitative PCR.

tumor stage and poor prognosis (40). This aligns with the broader phenomena observed within the annexin family, where some members are dysregulated in tumors. The present study showed that ANXA3 was significantly upregulated in CRC primary tumors, and its high expression was associated with shorter overall survival, which reinforces the existing literature reporting ANXA3 upregulation across various tumor types, such as breast cancer and pancreatic cancer (41).

Beyond its expression patterns, the role of ANXA3 in chemoresistance has emerged as another important function. For example, cervical cancer cells have been shown to become sensitive to cisplatin after ANXA3 is silenced, whereas the opposite effect has been observed when ANXA3 is overexpressed in cervical cancer cells (42). Similarly, in breast cancer, ANXA3 knockdown enhances the sensitivity of cells to doxorubicin (DOX) by promoting drug uptake, and the combination of DOX treatment and ANXA3 downregulation can synergistically suppress tumor growth and metastasis (43). Moreover, ANXA3 has been shown to be upregulated in oxaliplatin-resistant lung cancer cells, indicating that downregulation of ANXA3 may overcome such resistance (44). Consistent with these reports across different cancer types, the present study provided CRC-specific insights: It was demonstrated that ANXA3 expression was associated with cisplatin resistance, and ANXA3 overexpression promoted the expression of GSTP1, a known drug-resistance gene in CRC, through the NF-κB signaling pathway. Collectively, these findings position ANXA3 within the specific pathway of cisplatin response in CRC cells. While ANXA3 can regulate GSTP1 to modulate cisplatin sensitivity, alternative mechanisms may also

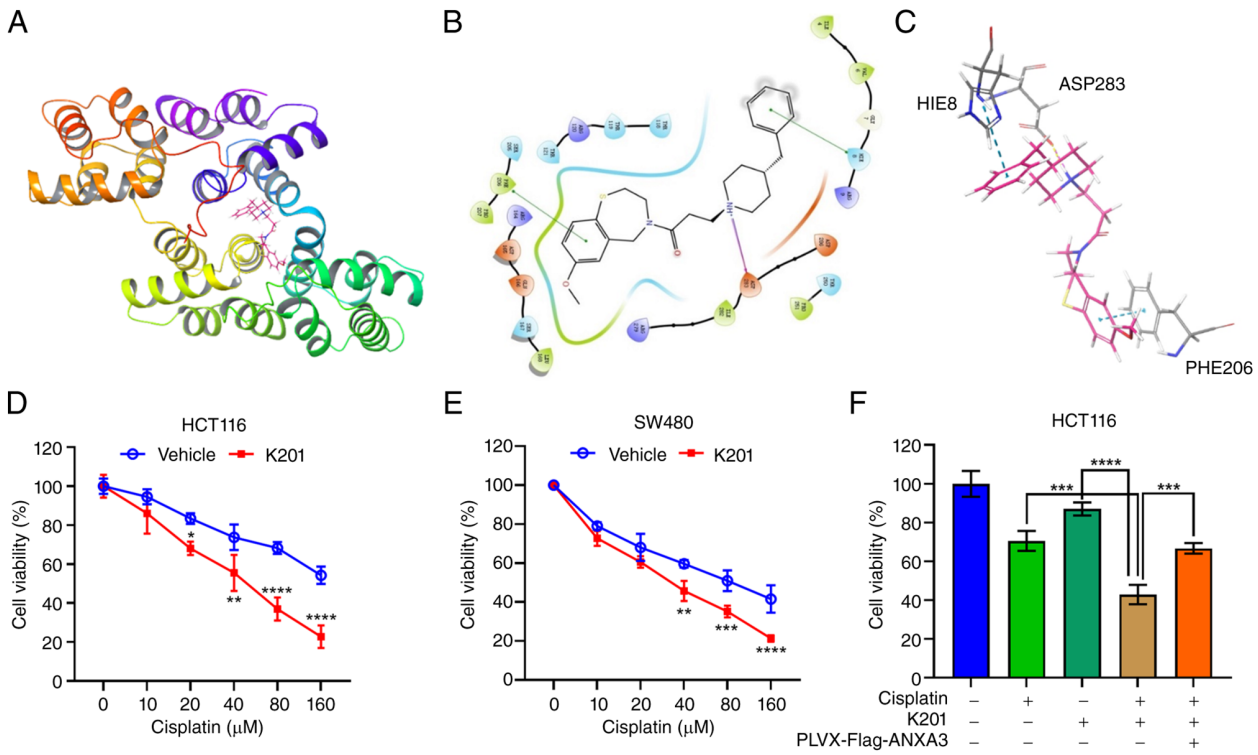

Figure 5. Combined K201 and cisplatin treatment significantly inhibits colorectal cancer cell viability. Molecular modeling of K201/ANXA3 complex: (A) 3D presentation of the molecular docking complex pose, and (B) 2D and (C) 3D presentations of the interactions between K201 and the key residues of ANXA3. (D) HCT116 and (E) SW480 cells with high endogenous ANXA3 were incubated with the indicated concentrations of cisplatin or 10 μM K201 for 24 h, followed by CCK-8 assay. Data are presented as the mean ± SD and P-values were calculated using a two-way ANOVA. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. vehicle group at that concentration. (F) HCT116 cells infected with PLVX-negative control or PLVX-Flag-ANXA3 were incubated with 40 μM cisplatin or 10 μM K201 for 24 h, followed by CCK-8 assay. Data are presented as the mean ± SD and P-values were calculated using a one-way ANOVA. ***P<0.001, ****P<0.0001. All experiments were repeated independently three times. ANXA3, annexin A3; CCK-8, Cell Counting Kit-8.

contribute to cisplatin resistance in CRC cells. For example, ANXA3 might modulate drug influx/efflux transporters, interfere with DNA damage repair pathways, or regulate broader oxidative stress responses beyond GSTP1-mediated conjugation (45-47). Future studies are needed to investigate the potential relationship between these pathways and the GSTP1-dependent mechanism proposed in the present study.

It is well known that exosomes can induce cancer cells to develop resistance to chemotherapeutic drugs, a topic that has attracted extensive attention in recent years. It has been reported that exosomes enriched with ANXA3 can effectively abolish ferroptosis in laryngeal squamous cell carcinoma (LSCC) cells and induce lymphatic metastasis, thereby promoting the progression of LSCC (48). The data in the present study are consistent with and extend this phenomenon, demonstrating that OE-ANXA3-exosomes could markedly decrease the sensitivity of CRC cells to cisplatin by regulating the transcriptional expression of GSTP1. This indicates a potential common mechanism where ANXA3-rich exosomes mediate drug sensitivity across different tumor types, with ANXA3 regulating GSTP1 as an important pathway worthy of further investigation. In addition to the aforementioned mechanism, recent studies have found other mechanisms underlying CRC chemoresistance. For example, GIPC1 has been reported to suppress CRC chemoresistance by regulating the TTC7B/mTOR/NF-κB axis (49). ARL4C has also been reported to promote oxaliplatin resistance in CRC by activating the RAP1/PI3K-Akt-mTOR signaling axis and inhibiting its own ubiquitination to enhance protein stability (50). Whether ANXA3 also affects these newly discovered chemoresistance mechanisms remains to be explored in future work.

Additionally, to overcome the insensitivity of cells to cisplatin caused by ANXA3 overexpression, the compound K201 was identified through virtual screening. The present study revealed that K201 significantly reduced cisplatin resistance in ANXA3-elevated cells, and overexpression of ANXA3 partially reversed the K201-mediated cisplatin sensitization. While the results from the molecular docking and functional assays indicated that K201 interacts with ANXA3, direct binding evidence (for example, cellular thermal shift assay or drug affinity responsive target stability assay) is currently lacking. This will be further investigated in future work.

Despite the findings of the present study, several limitations should be acknowledged. First, the findings are limited to *in vitro* experiments and the therapeutic relevance of the ANXA3-GSTP1 axis and K201 remains unvalidated *in vivo*. Second, elucidating the specific cell death mechanisms regulated by ANXA3 represents another important direction for future research and we plan to address this in subsequent studies. Third, the characterization of exosomes in the current study remains preliminary and a more comprehensive isolation and validation protocol is needed. Finally, the survival analysis is limited by insufficient clinical adjustment, such as patient comorbidities and treatment regimens. Collectively, these limitations underscore the need for further validation in future investigations.

In conclusion, the present study suggested that ANXA3 may mediate cisplatin sensitivity in CRC cells via regulation of GSTP1. These *in vitro* findings indicate that targeting ANXA3 could be a potential strategy for overcoming chemoresistance in CRC. However, these results remain preliminary and require further validation in more complex models and clinical studies to establish their therapeutic relevance.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YY and XJ designed the study and supervised the research process. XC, XX, XY, KH, HW, SZ, CM and RH performed the experiments, and collected and analyzed the data. XC and XX generated the figures. XX and YY wrote the original draft. YY and XJ confirmed the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shanghai East Hospital (approval no. 2024-220). All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Eng C, Yoshino T, Ruiz-Garcia E, Mostafa N, Cann CG, O'Brian B, Benny A, Perez RO and Cremolini C: Colorectal cancer. Lancet 404: 294-310, 2024.
2. Zuo S, Huang Y and Zou J: The role of the gut microbiome in modulating immunotherapy efficacy in colorectal cancer. IUBMB Life 76: 1050-1057, 2024.
3. Yang W, Huang X, Lv W, Jin Y and Zhu Y: LINC00365 promotes miR-221-5p to inhibit pyroptosis via Dicer in colorectal cancer. Acta Biochim Biophys Sin (Shanghai) 57: 529-541, 2024.
4. Zhang Y, Gao T, Wu M, Xu Z and Hu H: Value analysis of ITLN1 in the diagnostic and prognostic assessment of colorectal cancer. Transl Cancer Res 13: 2877-2891, 2024.
5. Dharwadkar P, Zaki TA and Murphy CC: Colorectal cancer in younger adults. Hematol Oncol Clin North Am 36: 449-470, 2022.
6. Siegel RL, Wagle NS, Cercek A, Smith RA and Jemal A: Colorectal cancer statistics, 2023. CA Cancer J Clin 73: 233-254, 2023.
7. Zhao S, Mi Y, Zheng B, Wei P, Gu Y, Zhang Z, Xu Y, Cai S, Li X and Li D: Highly-metastatic colorectal cancer cell released miR-181a-5p-rich extracellular vesicles promote liver metastasis by activating hepatic stellate cells and remodelling the tumour microenvironment. J Extracell Vesicles 11: e12186, 2022.
8. Wang X, Sun T, Fan J, Zuo X and Mao J: Gastrin-related circRNA_0017065 promotes the proliferation and metastasis of colorectal cancer through the miR-3174/RBFOX2 axis. Biol Direct 19: 75, 2024.
9. Wang Z, Jia L, Sun Y, Li C, Zhang L, Wang X and Chen H: CORO1C is associated with poor prognosis and promotes metastasis through PI3K/AKT pathway in colorectal cancer. Front Mol Biosci 8: 682594, 2021.
10. Benson AB, Venook AP, Adam M, Chang G, Chen YJ, Ciombor KK, Cohen SA, Cooper HS, Deming D, Garrido-Laguna I, *et al*: Colon cancer, version 3.2024, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 22: e240029, 2024.
11. Zhang C, Xu C, Gao X and Yao Q: Platinum-based drugs for cancer therapy and anti-tumor strategies. Theranostics 12: 2115-2132, 2022.
12. Zhang Q, Miao Y, Fu Q, Hu H, Chen H, Zeng A, Jin Y, Jiang Y, Qian L, Wu L, *et al*: CircRNACCDC66 regulates cisplatin resistance in gastric cancer via the miR-618/BCL2 axis. Biochem Biophys Res Commun 526: 713-720, 2020.
13. Fei Y, Cao D, Li Y, Wang Z, Dong R, Zhu M, Gao P, Wang X, Cai J and Zuo X: Circ_0008315 promotes tumorigenesis and cisplatin resistance and acts as a nanotherapeutic target in gastric cancer. J Nanobiotechnology 22: 519, 2024.
14. Yang L, Lu P, Yang X, Li K and Qu S: Annexin A3, a calcium-dependent phospholipid-binding protein: Implication in cancer. Front Mol Biosci 8: 716415, 2021.
15. Liu C, Li N, Liu G and Feng X: Annexin A3 and cancer. Oncol Lett 22: 834, 2021.
16. Li DQ, Lin M and Abdelrahman Z: High expression of the ANXA3 gene promotes immune infiltration and improves tumor prognosis in ovarian serous carcinoma using bioinformatics analyses. Ann Transl Med 10: 1055, 2022.
17. Liu YF, Liu QQ, Zhang YH and Qiu JH: Annexin A3 knockdown suppresses lung adenocarcinoma. Anal Cell Pathol (Amst) 2016: 4131403. 2016.
18. Guo C, Li N, Dong C, Wang L, Li Z, Liu Q, Ma Q, Greenaway FT, Tian Y, Hao L, *et al*: 33-kDa ANXA3 isoform contributes to hepatocarcinogenesis via modulating ERK, PI3K/Akt-HIF and intrinsic apoptosis pathways. J Adv Res 30: 85-102, 2021.
19. Wang G, Cao Y, Hu T, Cai Z, Chen C, Geng Q, Luo X, Liu Y, Wang W, Jin J and Sheng W: A mutual interaction between GSTP1 and p53 improves the drug resistance and malignant biology of pancreatic cancer. Cancer Sci 116: 1268-1281, 2025.
20. Shen Q, Yang K, Li Q, Qin T, Yu Y, Hong H, Yao H and Xu X: Nanoparticles (NPs)-mediated silencing of GSTP1 expression to reverse chemoresistance for effective breast cancer therapy. J Colloid Interface Sci 685: 38-48, 2025.
21. Wang F, Xu H, Yan Y, Wu P, Wu J, Zhu X, JiaoYing L, JingBo S, Kun Z, XiaoLi R, *et al*: FBX8 degrades GSTP1 through ubiquitination to suppress colorectal cancer progression. Cell Death Dis 10: 351, 2019.
22. Wang SQ, Chen JJ, Jiang Y, Lei ZN, Ruan YC, Pan Y, Yam JWP, Wong MP and Xiao ZJ: Targeting GSTP1 as therapeutic strategy against lung adenocarcinoma stemness and resistance to tyrosine kinase inhibitors. Adv Sci (Weinh) 10: e2205262, 2023.
23. Simic P, Pljesa I, Nejkovic L, Jerotic D, Coric V, Stulic J, Kokosar N, Popov D, Savic-Radojevic A, Pazin V and Pljesa-Ercegovac M: Glutathione Transferase P1: Potential therapeutic target in ovarian cancer. Medicina (Kaunas) 58: 1660, 2022.
24. Ling H, Li Y, Wang P, Zhang Z and Yang Z: Diffuse large B-cell lymphoma cell-derived exosomal NSUN2 stabilizes PDL1 to promote tumor immune escape and M2 macrophage polarization in a YBX1-dependent manner. Arch Biochem Biophys 766: 110322, 2025.

25. Zhang Y, Li X, Wang D, Jiang X, Zhang M and Lv K: Serum exosome microRNA panel as a noninvasive biomarker for molecular diagnosis of fulminant myocarditis. Mol Ther Methods Clin Dev 20: 142-151, 2021.
26. Liu Y, Li X, Zhang T and Liu G: The roles of exosomes in ovarian cancer chemo-resistance. J Cancer 14: 2128-2144, 2023.
27. Obal F Jr, Jancso G, Hajos M and Obal F: Differences in the mechanisms of the thermoregulatory impairment induced by capsaicin in newborn and adult rats. Acta Physiol Hung 69: 437-445, 1987.
28. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
29. Xu X, Huang A, Cui X, Han K, Hou X, Wang Q, Cui L and Yang Y: Ubiquitin specific peptidase 5 regulates colorectal cancer cell growth by stabilizing Tu translation elongation factor. Theranostics 9: 4208-4220, 2019.
30. Xu X, Wu G, Han K, Cui X, Feng Y, Mei X, Yang P, You W and Yang Y: Inhibition of OTUB2 suppresses colorectal cancer cell growth by regulating beta-Catenin signaling. Am J Cancer Res 13: 5382-5393, 2023.
31. Gu M, Jiang B, Li H, Zhu D, Jiang Y and Xu W: Aldolase A promotes cell proliferation and cisplatin resistance via the EGFR pathway in gastric cancer. Am J Transl Res 14: 6586-6595, 2022.
32. Zhu Y, Li K, Yan L, He Y, Wang L and Sheng L: miR-223-3p promotes cell proliferation and invasion by targeting Arid1a in gastric cancer. Acta Biochim Biophys Sin (Shanghai) 52: 150-159, 2020.
33. Xu X, Han K, Tang X, Zeng Y, Lin X, Zhao Y, Zhang Z, Cao B, Wu D and Mao X: The ring finger protein RNF6 induces leukemia cell proliferation as a direct target of pre-B-cell leukemia homeobox 1. J Biol Chem 291: 9617-9628, 2016.
34. Abbasi S, Movassaghpour A, Soleimani M and Molabashi ZA: The effects of released exosomes from NK-92 cells with IL15 on the apoptosis of HL-60 cell line. Int J Hematol Oncol Stem Cell Res 19: 126-137, 2025.
35. Nishinaka T, Ichijo Y, Ito M, Kimura M, Katsuyama M, Iwata K, Miura T, Terada T and Yabe-Nishimura C: Curcumin activates human glutathione S-transferase P1 expression through antioxidant response element. Toxicol Lett 170: 238-247, 2007.
36. Xu X, Han K, Zhu J, Mao H, Lin X, Zhang Z, Cao B, Zeng Y and Mao X: An inhibitor of cholesterol absorption displays anti-myeloma activity by targeting the JAK2-STAT3 signaling pathway. Oncotarget 7: 75539-75550, 2016.
37. Ku X, Xu Y, Cai C, Yang Y, Cui L and Yan W: In-depth characterization of mass spectrometry-based proteomic profiles revealed novel signature proteins associated with liver metastatic colorectal cancers. Anal Cell Pathol (Amst) 2019: 7653230, 2019.
38. Xia C, Hu J, Ketterer B and Taylor JB: The organization of the human GSTP1-1 gene promoter and its response to retinoic acid and cellular redox status. Biochem J 313: 155-161, 1996.
39. Wu W, Jia G, Chen L, Liu H and Xia S: Analysis of the expression and prognostic value of annexin family proteins in bladder cancer. Front Genet 12: 731625, 2021.
40. Xue G, Hao LQ, Ding FX, Mei Q, Huang JJ, Fu CG, Yan HL and Sun SH: Expression of annexin a5 is associated with higher tumor stage and poor prognosis in colorectal adenocarcinomas. J Clin Gastroenterol 43: 831-837, 2009.
41. Zhou T, Liu S, Yang L, Ju Y and Li C: The expression of ANXA3 and its relationship with the occurrence and development of breast cancer. J BUON 23: 713-719, 2018.
42. Huang J, Wei W, Kang F, Tan S, Li Y, Lu X and Wang N: ANXA3, associated with YAP1 regulation, participates in the proliferation and chemoresistance of cervical cancer cells. Genes Genomics 45: 1575-1586, 2023.
43. Du R, Liu B, Zhou L, Wang D, He X, Xu X, Zhang L, Niu C and Liu S: Downregulation of annexin A3 inhibits tumor metastasis and decreases drug resistance in breast cancer. Cell Death Dis 9: 126, 2018.
44. Jin YF, Huang YT and Chen PF: ANXA3 deletion inhibits the resistance of lung cancer cells to oxaliplatin. Eur Rev Med Pharmacol Sci 24: 3741-3748, 2020.
45. Zhou Z, Mao Y, Li J, Yang J, Zhang Y, Zhao Y and Deng Y: Lactobacillus plantarum ameliorates high-fat-diet-induced dyslipidemia via cholesterol metabolism and gut microbiota modulation. Food Funct 17: 6125-6143, 2026.
46. Yang L, Lu P, Yang X, Li K, Chen X, Zhou Y and Qu S: Downregulation of annexin A3 promotes ionizing radiation-induced EGFR activation and nuclear translocation and confers radioresistance in nasopharyngeal carcinoma. Exp Cell Res 418: 113292, 2022.
47. Su J, Wang L, Guan X and Sun L: Knocking-down annexin A3 suppresses inflammation, oxidative stress, apoptosis, and endoplasmic reticulum stress to attenuate sepsis-induced acute kidney injury in HK2 cells. Cytojournal 21: 75, 2025.
48. Xu L, Li W, Liu D, Cao J, Ge J, Liu X, Wang Y, Teng Y, Liu P, Guo X, *et al*: ANXA3-rich exosomes derived from tumor-associated macrophages regulate ferroptosis and lymphatic metastasis of laryngeal squamous cell carcinoma. Cancer Immuno Res 12: 614-630, 2024.
49. Gan D, Yang C, Shen X, Shi J, Wang R, Zhu L, Li H, Luo J, Zhao T, Li J, *et al*: GIPC1 restrains the progression and chemoresistance of colorectal cancer by regulating TTC7B/mTOR/NF-kappaB axis. Int J Biol Sci 22: 786-801, 2026.
50. Wang Y, Chang Z, Sun Z, Wang L, Li J, Li G, Wang Y, Zhang J, Ai L, Zhang Z, *et al*: Targeting the ARL4C/RAP1/PI3K-Akt-mTOR signaling loop promotes ARL4C ubiquitination and reverses oxaliplatin resistance in colorectal cancer. Theranostics 16: 99-122, 2026.