LncRNA BANCR Attenuates the Killing Capacity of Cisplatin on Gastric Cancer Cell through the ERK1/2 Pathway

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Research

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Abstract

Background

Chemotherapy-based comprehensive treatment is the most important therapeutic method for patients with advanced gastric cancer, but chemoresistance often causes treatment failure. Long non-coding RNA (LncRNA) BRAF-activated non-coding RNA (BANCR) has been shown to participate in many biological behaviors of multiple cancers. However, the biological roles of LncRNA BANCR in chemoresistance of gastric cancer remain unclear. Here, we aimed to evaluate the functions of LncRNA BANCR in the therapy of gastric cancer.

Methods

In this study, LncRNA BANCR expression was detected in GC patient samples and cell lines by quantity polymerase chain reaction (qPCR). Cell proliferation and viability in cisplatin treated cells were measured using clonogenic survival assay and cell counting kit-8. The levels of ERK1/2 pathway molecules were tested with western blot. Ly3214996, an inhibitor of ERK signal pathway, administration was used to assess the effects of BANCR overexpression on GC cell cisplatin-treated resistance. Moreover, the role of BANCR in cisplatin resistance of GC was certified in xenograft mouse models *in vivo*.

Results

our study showed that LncRNA BANCR expression was also significantly increased in GC tissues compared with adjacent normal tissues. Furthermore, we found that BANCR overexpression promoted, while BANCR inhibited, GC cell resistance to cisplatin *in vitro*. Ly3214996 treatment abolished the BANCR overexpression-mediated GC cell cisplatin resistance via regulating the phosphorylation of ERK protein. Knock-down of BANCR delayed significantly tumor growth in xenograft mouse models.

Conclusion

BANCR promoted cisplatin resistance of GC cells by activating ERK1/2 pathway. Inhibition of BANCR was markedly suppressed the growth of gastric cancer cells *in vitro* as well as *in vivo*. This result provided a new strategy for gastric cancer via targeting BANCR

Background

Gastric cancer (GC)is the fourth most common malignant tumors and the second leading cause of cancer-correlated death all over the world (1, 2). Surgery is currently the most effective treatment for non-metastatic GC (3). However, more than three fourths patients with GC were diagnosed at advanced stages and metastasis, whose tumors could not be surgically removed. Therefore, chemotherapy-based

comprehensive treatments are the most important therapeutic method for patients with advanced GC. However, treatments fail in many patients due to chemoresistance, which is the biggest obstacles to overcome for the successful cure of GC, and solving this problem is an urgent concern.

Emerging evidences suggested that long non-coding RNAs (LncRNAs) controls tumorigenesis and development through regulating expressions of target genes (4–6). Importantly, a number of aberrant LncRNAs have been reported to be involved in cancer chemoresistance(7, 8). For example, LncRNA MALAT1 silencing sensitized GC cells to autophagy associated chemotherapeutics via competing endogenous RNA for miR-23b-3p (2). Unregulated LncARSR promoted doxorubicin resistance via regulating PTEN-PI3K/Akt pathway in hepatocellular carcinoma (9). LncRNA BLACAT1 accelerated the oxaliplatin-resistance through upregulating ABCB1 expression by targeting miR-361 in GC (10). These data indicated that LncRNAs play a critical role in the chemoresistance of various cancers.

BRAF-activated non-coding RNA (BANCR), was first identified by Flockhart et al., exerted crucial roles in regulating cell proliferation and migration in malignant melanoma (11). Li et al. reported that the level of BANCR was upregulated in GC tissues and was positively associated with clinical stage, tumor depth, lymph node metastasis, and distant metastasis in GC patients (12). Furthermore, high expression of BANCR was an independent poor prognostic factor for GC patients (12). However, the biological roles of BANCR in chemoresistance of GC remain unclear. In this study, LncRNA BANCR expression level was investigated in GC tissues and adjacent normal tissues. We also explored the mechanism of LncRNA BANCR in cisplatin based chemoresistance in GC patients.

Materials And Methods

Cell lines, clinical samples, animals, and reagents

The GES-1 non-malignant gastric epithelial cell line, AGS, HGC27, SGC7901 and MKN45 gastric cancer cell lines with different differentiation degrees were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China) and were cultured at 37 °C in 5% CO2. AGS: human gastric adenocarcinoma cell; HGC 27, human gastric cancer cell with metastatic lymph node; SGC7901: human gastric cancer cell (lymph node metastasis of patients with gastric adenocarcinoma); MKN45: human gastric cancer cell. The GES-1 and SGC7901 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI)-1640 (Gibco, Rockville, MD, USA), while AGS, HGC27, and MKN45 cells were cultured in Dulbecco's modified Eagle's medium (Gibco). All medium were supplemented with 10% fetal bovine serum (Hyclone, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (100 mg/mL) (Invitrogen, Carlsbad, CA, USA).

35 paired GC tissues and adjacent normal tissues from GC patients who underwent surgical resection were included in this study. In the 35 GC cases, there were 20 males and 15 females with a median age of 64.2 years (range 23–87 years). This study was approved by the ethics committee of the Lianyungang

Municipal Oriental Hospital (Liangyungang, Jiangsu, China). In addition, we obtained informed consents from each patient for experiment.

6–8 weeks female BALB/C nude mice were purchased from the experimental animal center of Shanghai, China and fed in a pathogen-free mice facility, which were cultured at 22–25 °C temperature and 40–60% humidity, respectively. All animal study protocol was approved by our hospital ethical committees.

Cisplatin and Erk inhibitor Ly3214996 were purchased from Sigma (Sigma, USA, Cat#:BP809 for cisplatin and A668057 for Ly3214996) and stored at 10 mmol/L stock in DMSO solution.

Cell transfection and infection

Expression plasmids encoding BANCR and corresponding empty vectors as control were obtained from GenePharma Co. Ltd. (Shanghai, China). Three BANCR siRNAs were also obtained from GenePharma Co. Ltd. We transfected BANCR plasmids or BANCR siRNAs into GC cells using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions.

RNA isolation and qRT-PCR

Total RNA was isolated from GC tissue samples or cell lines by TRIzol® (Invitrogen) following the manufacturer's protocol. The complementary DNAs (cDNA) were synthesized using First Stand cDNA Synthesis kit (Roche, Basel, Switzerland), and then performed quantitative PCR analysis by Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix kit (Thermo Fisher Scientific, Waltham, MA, USA in Applied Biosystems QuantStudio™ 12 K Flex Software (Thermo Fisher Scientific). The primer sequences were as follows: BANCR forward, 5'-ACAGGACTCCATGGCAAACG-3' and BANCR reverse, 5'-ATGAAGAAAGCCTGGTG-3'; GAPDH forward, 5'-ACCACAGTCCATGCCATCAC-3' and 5'-TCCACCACCCTGTTGCTGTA-3'. GAPDH acted as internal standard. The BANCR relative quantitative expression was calculated by the $2^{-\Delta\Delta Ct}$ methods.

Cell viability assays

We performed cell viability analysis with a cell Counting Kit-8 (CCK-8, DoJinDo, Tokyo, Japan) according to the manufacturer's protocol. Briefly, 5000 cells/well were seeded in 96-well plates and treated with cisplatin at different concentration gradient for 24 hours. Finally, we measured the absorbance at 450 nm to determine cell viability.

Colony formation assays

We seeded GC cells (500/well) into 6-well plates and treated with cisplatin for 12 days. We added fresh medium into the plates every 3 days. After 12 days, we fixed colonies with 4% paraformaldehyde and then stained the colonies with 1% crystal violet. Finally, we photographed and counted the cell colonies.

Western blot

The total protein from cultured cells and tumors were obtained with Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) and separated by 10% polyacrylamide gel electrophoresis, followed by transferring

onto polyvinylidene fluoride membranes (Millipore, MA, USA). The protein membrane was incubated with primary antibodies (Anti-pERK1/2, anti-total ERK1/2, Anti-pMEK1/2, Anti-MEK1/2, and Anti-GAPDH antibody, Abcam, Cambridge, MA, USA) overnight at 4 °C. We used the enhanced chemiluminescence (Thermo Scientific, Waltham, MA) to detect protein bands and semi-quantified the band density with an ImageJ analysis software (National Institutes of Health).

Xenograft mouse model

The lentivirus particles carrying BANCR siRNA-1 sequences (si BANCR-1) were purchased from GenePharma Co. Ltd. We obtained the MKN45 cells with BANCR stably knock-down by infection with si BANCR-1 lentivirus. Then we subcutaneously injected 5×10^6 MKN45 cells with BANCR stably knock-down or control cells into the back flank of female nude mice (6–8 weeks old). Cisplatin (40 μM/kg) was administered intraperitoneally at day 5, 8, 12 and 15. Tumor size was measured at the indicated days by using a calliper. After 19 days, we isolated the xenograft tumor from mice, photographed the tumor and measured its weight.

Statistical analysis

All data were presented as the mean ± standard deviation (SD). We performed statistical analysis by one tail Student's t test. A p-value less than 0.05, 0.01 or 0.001 were significantly considered difference.

Results

BANCR expression was increased in GC patients and cell lines compared with normal cells

To evaluate BANCR expression in GC and normal cells, we utilized qPCR method for their relative levels. The resulted revealed that the expression of BANCR in 35 primary GC samples was much higher than corresponding normal tissues. Data was shown in Fig. 1A, ($P < 0.001$). Furthermore, the BANCR level was positively related to tumor node metastasis (TNM) stages ($P = 0.032$) and lymph node metastasis ($P = 0.045$) (Table 1).Moreover, it was also shown that BANCR expression was higher in GC cell lines (AGS, HGC27, SGC7901 and MKN45) than normal cells (GES-1) (Fig. 1B, $P < 0.05$).

Table 1
Association between BANCR expression and clinicopathological features

Clinicopathological Features	N	BANCR Expression		P Value
		Low	High	
Age (years)				
≤ 60	22	13	9	0.432
> 60	13	6	7	
Gender				
Female	16	7	9	0.257
Male	19	10	9	
Tumor size (cm)				
≤ 2	15	7	8	0.242
> 2	20	11	9	
TNM stage				
I + II	11	8	3	0.032*
III + IV	24	16	8	
Lymph node metastasis				
Positive	13	4	9	0.045*
Negative	22	14	8	
Abbreviations: TNM, tumor node metastasis.*statistically significant (*P < 0.05).				

BANCR overexpression induced GC cell resistance to cisplatin

In addition to surgery, chemotherapy frequently was used to treat gastric cancer patients those were in advantage and metastatic stages. Cisplatin and taxotere often were combined with the other drugs for chemotherapy (13). Unfortunately, therapeutic failure eventually occurred because of chemoresistance. To investigate the possible mechanism of BANCR in affecting the cisplatin resistance of GC cells, BANCR overexpression plasmid was transfected into AGS cells. As shown in Fig. 2A, BANCR overexpression plasmid could greatly increase BANCR expression in AGS cells. Then BANCR-overexpression AGS cells were treated with increasing concentrations of cisplatin (0 to 8 μM) for 24 hours. Cisplatin treatment reduced cell viability of AGS cells transfected with or without BANCR overexpression plasmid in a dose-dependent manner. However, BANCR-overexpression AGS cells showed higher cell viability (Fig. 2B, purple line) compared with control cells (Fig. 2B, black line). Moreover, the colony formation assay also showed

that BANCR -overexpression increased the number of colonies and protected cells from cisplatin killing (Fig. 2C).

BANCR knock-down reduced GC cell resistance to cisplatin

To further assess the functions of BANCR in GC cell resistance to cisplatin, we performed BANCR knock-down in MKN45 cells.As shown in Fig. 3A, BANCR siRNAs significantly inhibited BANCR expression in MKN45 cells with three different BANCR targeting sites. Cisplatin treatment reduced cell viability of MKN45 cells transfected with or without si-BANCR-1 in a dose-dependent manner. However, MKN45 cells treated with si-BANCR-1 showed the lower cell viability compared with control cells (Fig. 3B). Moreover, the colony formation assay also showed that BANCR knockdown decreased the number of colonies (Fig. 3C). These results indicated that BANCR promoted the chemoresistance of cisplatin in GC therapy.

BANCR regulated ERK1/2 signal pathway in GC cells

We next disclosed the molecular pathways involved in the BANCR-mediated resistance to cisplatin in GC cells. Previous studies have showed that BANCR regulate tumor biological behavior via ERK1/2 signal pathway (14, 15). Here, we analyzed the ERK1/2 pathway in BANCR-overexpression or BANCR-silenced cells. The results of western blotting showed that BANCR-overexpression markedly up-regulated the phosphorylation level of MEK1/2 and ERK1/2 in AGS cells (Fig. 4A). In contrast, BANCR-knockdown inhibited the phosphorylation of MEK1/2 and ERK1/2 in MKN45 cells (Fig. 4B).

BANCR overexpression induced GC cell resistance to cisplatin via ERK pathway

We further explored whether BANCR overexpression-induced cisplatin resistance was ERK1/2 signal pathway dependent. Cell viability analysis showed that BANCR-overexpression enhanced the cisplatin resistance of AGS cells (Fig. 5A); while Ly3214996, the inhibitor of ERK signal, treatment abolished the BANCR overexpression-mediated cisplatin resistance (Fig. 5A). Moreover, the colony formation assay also confirmed these results (Fig. 5B).

BANCR knockdown reduced GC cell resistance to cisplatin in vivo

To investigate the cisplatin resistance effect of BANCR on GC treatment *in vivo*, we established xenograft models of BANCR knockdown MKN45 tumors in nude mice and treated xenograft models with cisplatin. The images, tumor sizes, and mass showed that tumors from BANCR knockdown MKN45 cells were significantly less resistant to cisplatin than did tumors from control MKN45 cells (Fig. 6A–C). The expression of BANCR was much lower in BANCR knock-down MKN45 tumors than in control tumors (Fig. 6D). Moreover, the phosphorylation levels of MEK1/2 and ERK1/2 in BANCR knockdown MKN45 tumors were significantly decreased (Fig. 6E).

Discussion

LncRNAs have been reported to play essential roles in drug resistance in multiple cancers, such as breast cancer (16), gastric cancer (17), and lung cancer(18). For example, lnc-ATB/miR-200c promoted resistance to trastuzumab and then induced an invasion-metastasis cascade in breast cancer via upregulating ZEB1 and ZNF-217 (19). Fang et al. found that knockdown of LEIGC resulted in downregulated GC cells sensitivity to 5-fluorouracil (5-FU) (20). In lung cancer, the silencing of lncRNA UCA1 promoted geftinib resistance via targeting FOSL2 signal pathway(21). In this study, we found that BANCR promoted GC cell chemoresistance via activatin ERK1 pathway both *in vitro* and *in vivo.*

Previous studies have shown that aberrant BANCR expression is found in various malignant cancers, including melanoma, endometrial cancer, bladder cancer, and GC (11, 12, 22, 23). The upregulated BANCR expression was positively associated with clinical stage, tumor depth, lymph node metastasis and distant metastasis in GC patients (12). Here, we also found the expression of BANCR was obviously increased in primary GC samples and GC cell lines. BANCR was reported to participate in many biological behaviors of tumor. Shi et al. showed that BANCR down-regulation contributed to the inhibition of colorectal cancer cell growth through regulating P21 protein (24). Knockdown of BANCR expression in Huh7 cells using shRNA inhibited the proliferation, apoptosis, migration and invasion of hepatocellular carcinoma cells (25). Moreover, knockdown of BANCR in BGC-823 and MGC-803 cells inhibited cell growth and promoted cell apoptosis (26). In this study, BANCR overexpression induced, while BANCR knockdown reduced, GC cell resistance to cisplatin. In addition, BANCR knockdown reduced GC cell resistance to cisplatin *in vivo.* Previous study revealed that LncRNA BANCR was involved in chemotherapeutic resistance of colorectal cancer, which mediated sequestering miR-203 molecule (27). Our results showed that BANCR played a key role in drug resistance of GC via different pathway.

ERK1/2 pathway has been reported to be involved in cell growth, malignant transformation and drug resistance (28). Overexpression of amphiregulin in ovarian cancer induced tumor cell stemness and drug resistance through deregulating EGFR-ERK1/2 pathway (29). In pancreatic cancer, AGR2 knockdown inhibited the ERK1/2/AKT axis and further reduced pancreatic cancer cell viability, chemotherapy resistance, migration and invasion (30). Li et al found that psoriasin overexpression resulted in the decrease in sensitivity of GC cells to cisplatin by activating ERK1/2 signal (31). Therefore, ERK1/2 pathway plays pivotal role in tumor drug resistance. Overexpression of BANCR promoted epithelial-mesenchymal transition (EMT), migration and invasion of BCPAP cells by regulating the Raf/MEK/ERK1/2 signaling (14). BANCR regulated cancer stem cell markers in papillary thyroid cancer via the RAF/MEK/ERK1/2 signaling pathway (15). Moreover, the role of BANCR in regulating endometrial cancer cell proliferation, migration, and invasion in an ERK1/2/MAPK signaling pathway dependent manner (22). Therefore, we speculated that ERK1/2 signaling may be involved in the BANCR-induced drug resistance of GC cells. Indeed, our results showed that BANCR-overexpression markedly up-regulated the phosphorylation level of MEK and ERK1/2 in AGS cells. In contrast, BANCR-knockdown inhibited the phosphorylation of ERK1/2 in MKN45 cells. In addition, BANCR knockdown also reduced the phosphorylation level of ERK1/2 in MKN45 tumors *in vivo*. More importantly, Ly3214996, the inhibitor of

ERK signaling, treatment abolished the BANCR overexpression-mediated cisplatin resistance. These data confirmed that the chemotherapeutic resistance of BANCR in cisplatin treated GC cells was mediated via activating MER/ERK1 pathway.

Conclusion

BANCR was highly expressed in GC tissues and cell lines, and took part in the drug resistance of GC. Both *in vitro* and *in vivo* functional experimental analysis showed that BANCR promoted cisplatin resistance of GC cells via the ERK1/2 signaling pathway. This study described a cancer drug resistance property of BANCR and provided a potential therapeutic target of GC.

Abbreviations

BANCR, BRAF-activated non-coding RNA;CCK-8, cell counting kit-8; cDNA, complementary DNAs; 5-FU, 5-fluorouracil; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase ; GC, Gastric cancer; NS, no significant difference; LncRNAs, long non-coding RNAs; qPCR, quantity polymerase chain reaction; SD, standard deviation; TNM, tumor node metastasis ; VEC, vector.

Declarations

Ethics approval and consent to participate

All human gastric cancer sample and animal protocol were approved by the ethical committees of Lianyungang Municipal Oriental Hospital. All patients were given a written inform consent.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

All authors declare that there is no conflict of interest.

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Not applicable

Authors’ contributions

XM and HZ propagated the concept design. XM, YL, YF, and GW did the experiments and collected data. XM and HZ wrote animal experiment protocol.XM, YL, and HZ analyzed data and wrote manuscript. All authors were involved in final manuscript preparation and approval.

Acknowledhements

Not applicable

References

1. Carcas LP. Gastric cancer review. J Carcinog. 2014;13:14.
2. YiRen H, YingCong Y, Sunwu Y, Keqin L, Xiaochun T, Senrui C, et al. Long noncoding RNA MALAT1 regulates autophagy associated chemoresistance via miR-23b-3p sequestration in gastric cancer. Mol Cancer. 2017;16(1):174.
3. Goere D, Gras-Chaput N, Auperin A, Flament C, Mariette C, Glehen O, et al. Treatment of gastric peritoneal carcinomatosis by combining complete surgical resection of lesions and intraperitoneal immunotherapy using catumaxomab. BMC Cancer. 2014;14:148.
4. Kalmar A, Nagy ZB, Galamb O, Csabai I, Bodor A, Wichmann B, et al. Genome-wide expression profiling in colorectal cancer focusing on lncRNAs in the adenoma-carcinoma transition. BMC Cancer. 2019;19(1):1059.
5. Yang H, Jiang Z, Wang S, Zhao Y, Song X, Xiao Y, et al. Long non-coding small nucleolar RNA host genes in digestive cancers. Cancer Med. 2019;8(18):7693–704.
6. Barth DA, Slaby O, Klec C, Juracek J, Drula R, Calin GA, et al. Current Concepts of Non-Coding RNAs in the Pathogenesis of Non-Clear Cell Renal Cell Carcinoma. Cancers (Basel). 2019;11(10).
7. Bourguignon LYW. Matrix Hyaluronan-CD44 Interaction Activates MicroRNA and LncRNA Signaling Associated With Chemoresistance, Invasion, and Tumor Progression. Front Oncol. 2019;9:492.
8. Zebisch A, Hatzl S, Pichler M, Wolfler A, Sill H. Therapeutic Resistance in Acute Myeloid Leukemia: The Role of Non-Coding RNAs. Int J Mol Sci. 2016;17(12).
9. Y L, Y Y, B F, Y Q. Long Noncoding RNA lncARSR Promotes Doxorubicin Resistance in Hepatocellular Carcinoma via Modulating PTEN-PI3K/Akt Pathway. Journal of cellular biochemistry. 2017;118(12):4498–507.
10. X W YZ, B H, X D. Long noncoding RNA BLACAT1 modulates ABCB1 to promote oxaliplatin resistance of gastric cancer via sponging miR-361. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2018;99(undefined):832-8.

11. Flockhart RJ, Webster DE, Qu K, Mascarenhas N, Kovalski J, Kretz M, et al. BRAFV600E remodels the melanocyte transcriptome and induces BANCR to regulate melanoma cell migration. Genome Res. 2012;22(6):1006–14.
12. Li L, Zhang L, Zhang Y, Zhou F. Increased expression of LncRNA BANCR is associated with clinical progression and poor prognosis in gastric cancer. Biomed Pharmacother. 2015;72:109–12.
13. Wagner AD, Syn NL, Moehler M, Grothe W, Yong WP, Tai BC, et al. Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev. 2017;8:CD004064.
14. Wang Y, Gu J, Lin X, Yan W, Yang W, Wu G. lncRNA BANCR promotes EMT in PTC via the Raf/MEK/ERK signaling pathway. Oncology Letters. 2018;15(4):5865–70.
15. Wang Y, Lin X, Fu X, Yan W, Lin F, Kuang P, et al. Long non-coding RNA BANCR regulates cancer stem cell markers in papillary thyroid cancer via the RAF/MEK/ERK signaling pathway. Oncol Rep. 2018;40(2):859–66.
16. Chen Z, Pan T, Jiang D, Jin L, Geng Y, Feng X, et al. The lncRNA-GAS5/miR-221-3p/DKK2 Axis Modulates ABCB1-Mediated Adriamycin Resistance of Breast Cancer via the Wnt/beta-Catenin Signaling Pathway. Mol Ther Nucleic Acids. 2020;19:1434–48.
17. Wei L, Sun J, Zhang N, Zheng Y, Wang X, Lv L, et al. Noncoding RNAs in gastric cancer: implications for drug resistance. Mol Cancer. 2020;19(1):62.
18. Mondal P, Natesh J, Kamal MA, Meeran SM. Non-coding RNAs in Lung Cancer Chemoresistance. Curr Drug Metab. 2019;20(13):1023–32.
19. Shi S, Wang L, Yu B, Li Y, Jin Y, Bai X. LncRNA-ATB promotes trastuzumab resistance and invasion-metastasis cascade in breast cancer. Oncotarget. 2015;6(13):11652–63.
20. Fang X, Pan H, Leng R, Ye D. Long noncoding RNAs: novel insights into gastric cancer. Cancer Lett. 2015;356(2):357–66.
21. Chen X, Wang Z, Tong F, Dong X, Wu G, Zhang R. lncRNA UCA1 Promotes Gefitinib Resistance as a ceRNA to Target FOSL2 by Sponging miR-143 in Non-small Cell Lung Cancer. Mol Ther Nucleic Acids. 2019;19:643–53.
22. Wang D, Wang D, Wang N, Long Z, Ren X. Long Non-Coding. RNA BANCR Promotes Endometrial Cancer Cell Proliferation and Invasion by Regulating MMP2 and MMP1 via ERK/MAPK Signaling Pathway. Cell Physiol Biochem. 2016;40(3–4):644–56.
23. He A, Liu Y, Chen Z, Li J, Chen M, Liu L, et al. Over-expression of long noncoding RNA BANCR inhibits malignant phenotypes of human bladder cancer. J Exp Clin Cancer Res. 2016;35(1):125.
24. Shi Y, Liu Y, Wang J, Jie D, Yun T, Li W, et al. Downregulated Long Noncoding RNA BANCR Promotes the Proliferation of Colorectal Cancer Cells via Downregualtion of p21 Expression. PLoS One. 2015;10(4):e0122679.
25. Li J, Wang J, Zhou W, Zhang S, Le Y, He R. Downregulation of BRAF-activated non-coding RNA suppresses the proliferation, migration and invasion, and induces apoptosis of hepatocellular carcinoma cells. Oncol Lett. 2017;14(4):4751–7.

26. Zhang ZX, Liu ZQ, Jiang B, Lu XY, Ning XF, Yuan CT, et al. BRAF activated non-coding RNA (BANCR) promoting gastric cancer cells proliferation via regulation of NF-kappaB1. Biochem Biophys Res Commun. 2015;465(2):225–31.
27. Ma S, Yang D, Liu Y, Wang Y, Lin T, Li Y, et al. LncRNA BANCR promotes tumorigenesis and enhances adriamycin resistance in colorectal cancer. Aging. 2018;10(8):2062–78.
28. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta. 2007;1773(8):1263–84.
29. SL T, WC H, FC H, ZP Y, TH J. JW C, et al. miRNA-34c-5p inhibits amphiregulin-induced ovarian cancer stemness and drug resistance via downregulation of the AREG-EGFR-ERK pathway. Oncogenesis. 2017;6(5):e326.
30. QG L. YJ L, L Y. Knockdown of AGR2 induces cell apoptosis and reduces chemotherapy resistance of pancreatic cancer cells with the involvement of ERK/AKT axis. Pancreatology: official journal of the International Association of Pancreatology (IAP) [et al]. 2018;18(6):678–88.
31. Z Z, WG LLYCLY. J, J J. Psoriasin overexpression confers drug resistance to cisplatin by activating ERK in gastric cancer. Int J Oncol. 2018;53(3):1171–82.

Figures

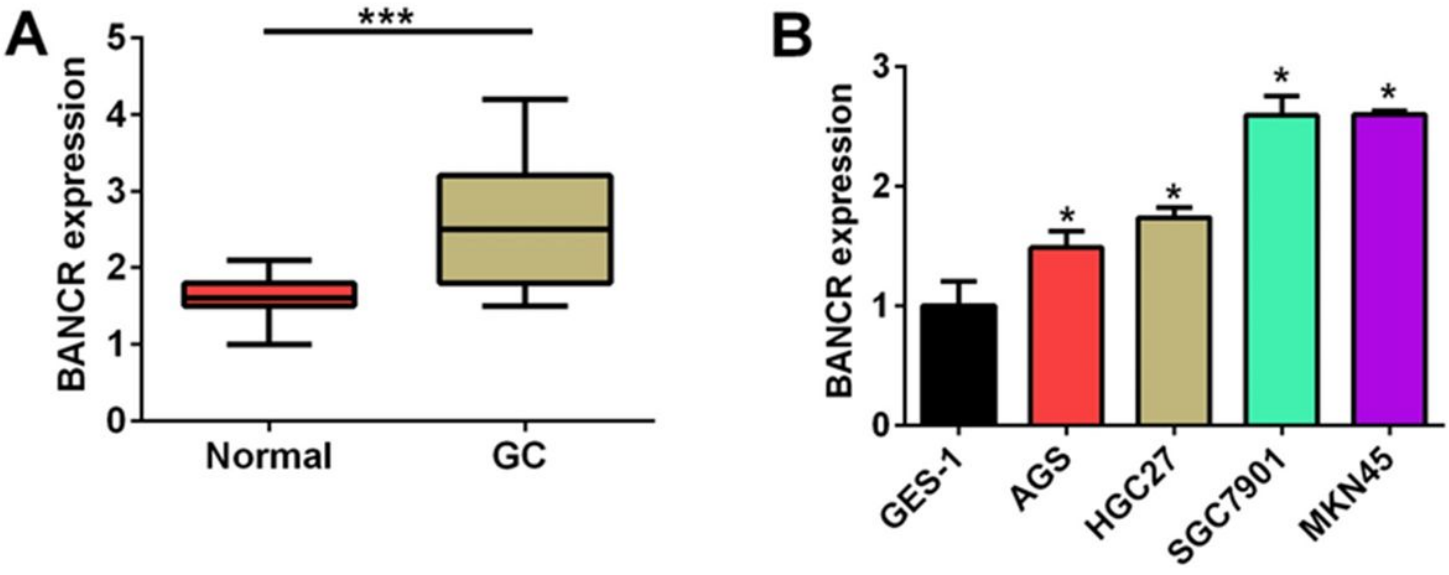

Figure 1

The expression of BANCR in GC tissues and cancer cells. (A) The BANCR level was increased in GC tissues (n=35) compared to adjacent normal tissues (n=35). (B) The BANCR level in the human non-malignant gastric epithelial cell line (GES-1) and cancer cell lines (AGS, HGC27, SGC7901 and MKN45)

were examined by qRT-PCR. Data are represented as the means ± SD from three independent experiments. *P < 0.05, ***P<0.001.

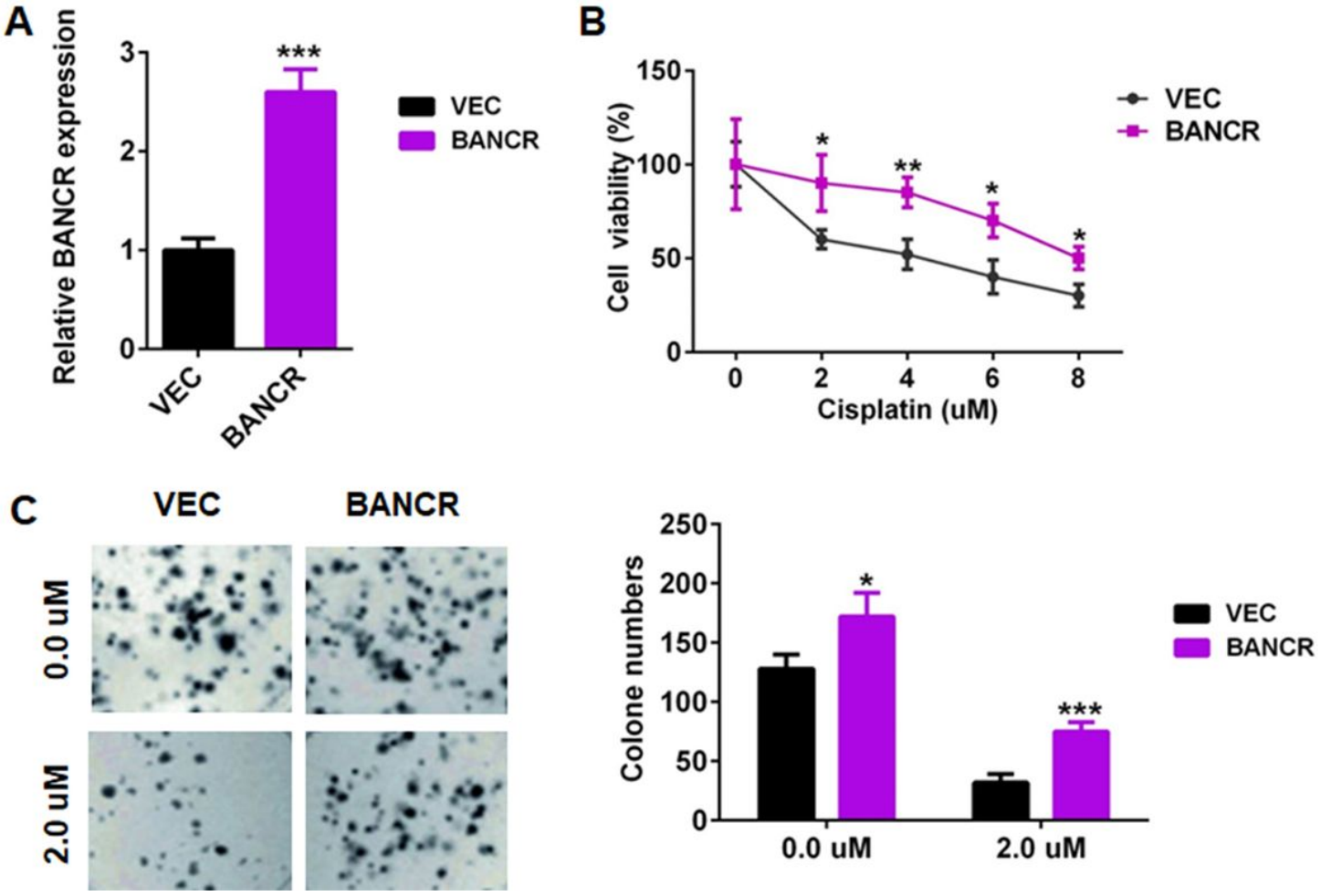

Figure 2

The effects of BANCR overexpression on GC cell proliferation. (A) The level of BANCR was tested in BANCR-overexpression AGS cells by qRT-PCR analysis. (B) BANCR overexpression increased the cell viability of AGS cells treated with cisplatin. (C) A clonogenic survival assay was performed using BANCR-overexpression AGS cells treated with cisplatin. Data are represented as the means ± SD from three independent experiments. VEC, only lentivirus vector infected AGS cells; BANCR, AGS cells with BANCR overexpression in lentivirus vector. *P<0.05, **P<0.01, ***P<0.001.

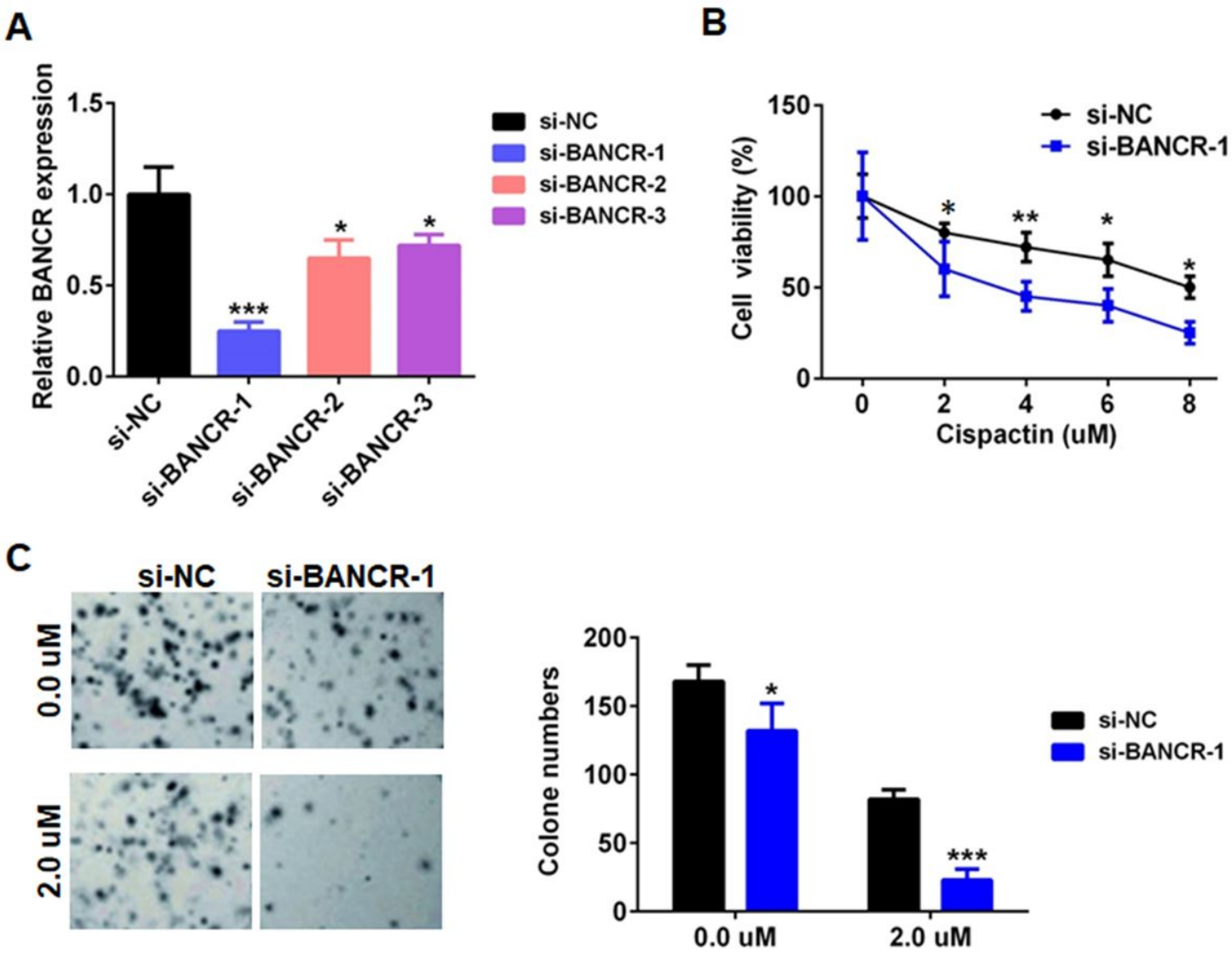

Figure 3

BANCR knockdown inhibited GC cell cisplatin resistance. (A) The level of BANCR was tested in MKN45 cells treated with BANCR siRNAs (BANCR siRNA-1, BANCR siRNA-2 or BANCR siRNA-3) by qRT-PCR analysis. (B) BANCR knockdown decreased the cell viability of MKN45 cells treated with cisplatin. (C) A clonogenic survival assay was performed using MKN45 cotreated with BANCR siRNA-1 and cisplatin. Data are represented as the means ± SD from three independent experiments. Si-NC,siRNA negative control vector, *P<0.05, **P<0.01, ***P<0.001.

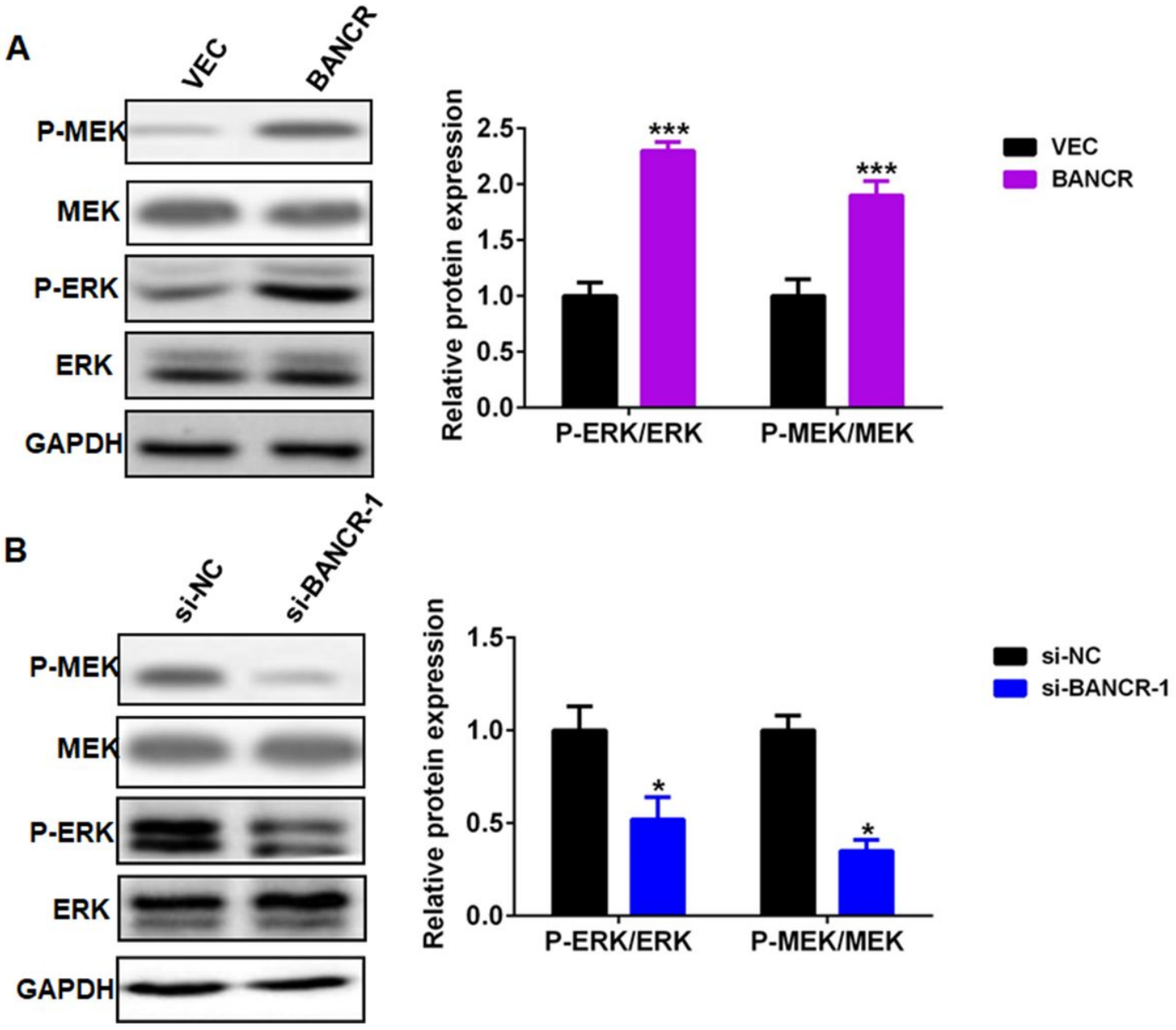

Figure 4

BANCR promoted cisplatin-induced ERK1/2 pathway in GC cells. (A) BANCR overexpression induced the phosphorylation of MEK1/2 and ERK1/2 in AGS cells treated with cisplatin. (B) BANCR knockdown reduced the phosphorylation of MEK1/2 and ERK1/2 in MKN45 cells treated with cisplatin. Data are represented as the means ± SD. *P<0.05, ***P<0.001.Vec, only lentivirus vector infected AGS cells; BANCR, AGS cells with BANCR overexpression in lentivirus vector.si-NC, negative control siRNA lentivirus; si-BANCR-1, BANCR-1 siRNA lentivirus.

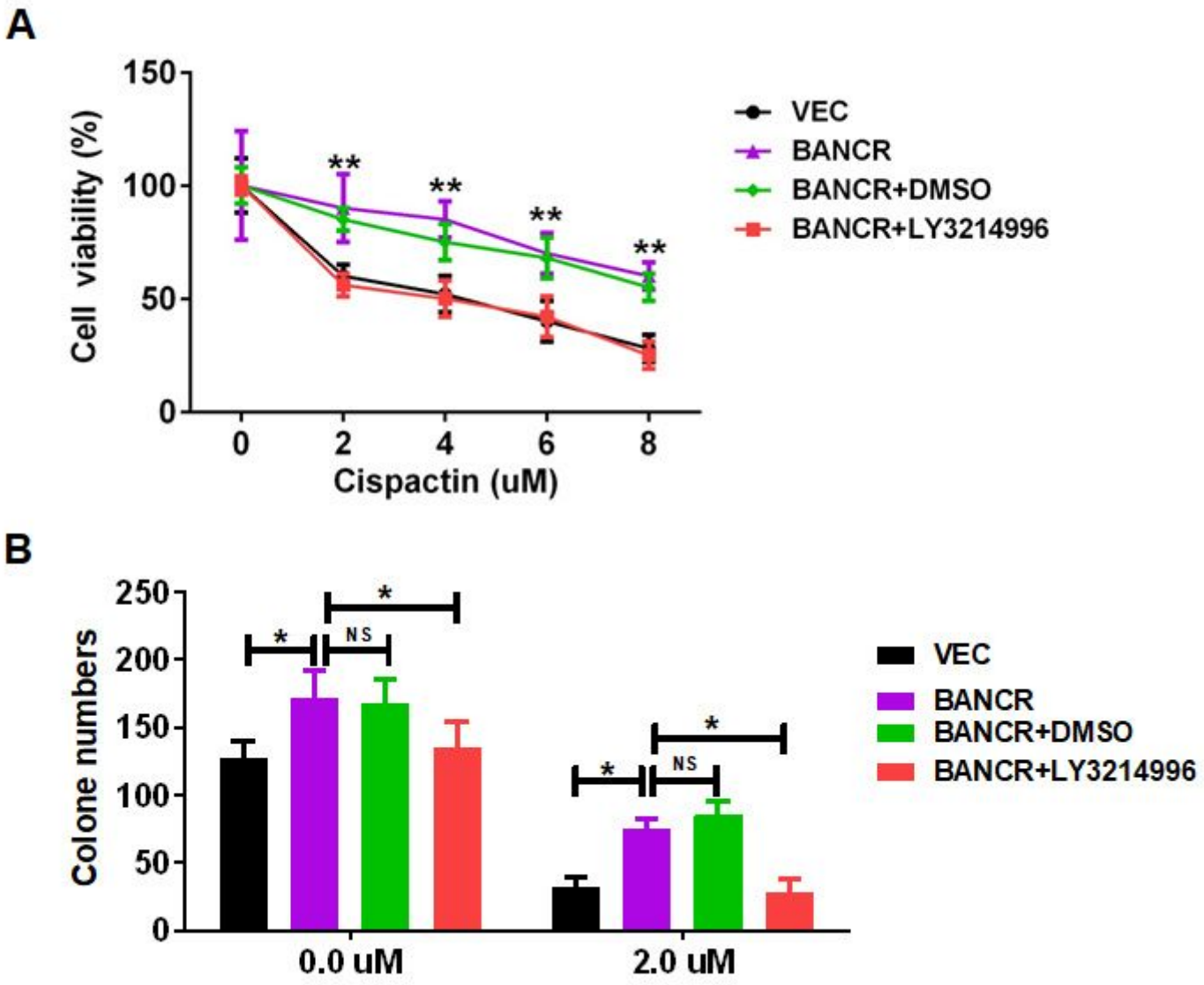

Figure 5

BANCR overexpression induced GC cell resistance to cisplatin via ERK pathway. (A) The increased cell viability of BANCR-overexpression AGS cells treated with cisplatin was abolished by LY3214996. (B) BANCR-overexpression increased the colony numbers of AGS cells treated with cisplatin, while LY3214996 treatment abolished the effect. Data are represented as the means ± SD. *P<0.05, **P<0.01.NS, no significant difference. Vec, only lentivirus vector infected AGS cells ; BANCR, AGS cells with BANCR overexpression in lentivirus vector.

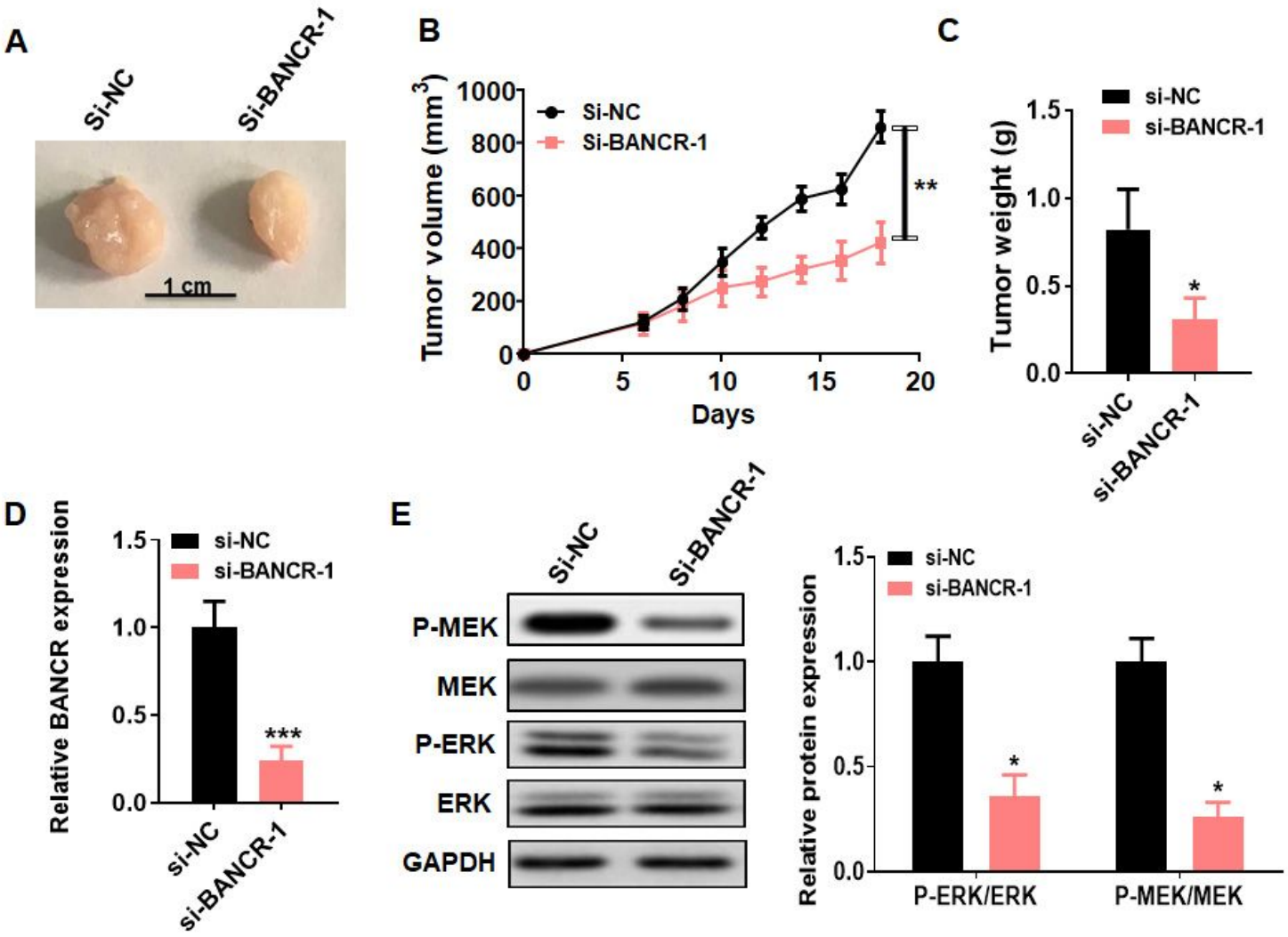

Figure 6

BANCR knockdown reduced GC cell resistance to cisplatin in vivo. (A-C) The tumor images (A), tumor sizes (B), and tumor mass (C) of BANCR knockdown MKN45 tumors treated with cisplatin. (D) The level of BANCR was tested in BANCR knockdown MKN45 tumors by qRT-PCR. (E) The phosphorylation level of MEK1/2 and ERK1/2 in BANCR knockdown MKN45 tumors was tested by western blot. Data are represented as the means ± SD. *P<0.05, **P<0.01, ***P<0.001. si-NC,negative control siRNA lentivirus infected MKN45 cells; si-BANCR-1, BANCR-1 siRNA lentivirus infected MKN45 cells.