IRE1α-targeting Downregulates ABC Transporters and Overcomes Drug Resistance of Colon Cancer Cells

Qiang Gao, Xiu-xiu Li, Yi-ming Xu, Jin-zhao Zhang, Shi-di Rong, Yan-qing Qin, Jing Fang

PII: S0304-3835(20)30069-0

DOI: https://doi.org/10.1016/j.canlet.2020.02.007

Reference: CAN 114689

To appear in: *Cancer Letters*

Received Date: 25 September 2019

Revised Date: 6 February 2020

Accepted Date: 7 February 2020

Please cite this article as: Q. Gao, X.-x. Li, Y.-m. Xu, J.-z. Zhang, S.-d. Rong, Y.-q. Qin, J. Fang, IRE1α-targeting Downregulates ABC Transporters and Overcomes Drug Resistance of Colon Cancer Cells, *Cancer Letters*, https://doi.org/10.1016/j.canlet.2020.02.007.

Abstract

Drug resistance is a big problem in cancer treatment and one of the most prominent mechanisms underlain is overexpression of ATP-binding cassette (ABC) transporters, particularly ABCB1, ABCC1 and ABCG2. Inhibition of ABC transporters is an important approach to overcome drug resistance. The inositol-requiring enzyme 1α (IRE1α), an arm of unfolded protein response (UPR), splices *XBP1* mRNA to generate an active transcription factor XBP1s. UPR is implicated in drug resistance. However, the underlying mechanism is unclear. We found that the anticancer drugs such as 5-fluorouracil (5-FU) activated the IRE1α-XBP1 pathway to induce the expression of ABCB1, ABCC1 and ABCG2 in colon cancer cells. Inhibition of IRE1α RNase activity with small molecule 4μ8c suppressed the drug-induced expression of these ABC transporters and sensitized 5-FU-resistant colon cancer cells to drug treatment. *In vivo* xenograft assay indicates that administration of 4μ8C substantially enhanced the efficacy of 5-FU chemotherapy on 5-FU-resistant colon cancer cells. These results suggest that IRE1α-targeting might be a strategy to cope with drug resistance of colon cancer.

Keywords: IRE1α; ABC transporter; Drug resistance; Colon cancer.

IRE1α-targeting Downregulates ABC Transporters and Overcomes Drug Resistance of Colon Cancer Cells

Qiang Gao[1], Xiu-xiu Li[1], Yi-ming Xu[1], Jin-zhao Zhang[1],
Shi-di Rong[1], Yan-qing Qin[1] and Jing Fang[2,3,*]

[1] Shanghai Institute of Nutrition and Health, Shanghai Institutes of Biological Sciences, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai 200031.

[2] Cancer Institute, the Affiliated Hospital of Qingdao University, Qingdao, 266061;

[3] Cancer Institute, Qingdao University, Qingdao, 266061.

Running title: IRE1α promotes cancer drug resistance via ABC transporters

*** Corresponding author**: Dr. Jing Fang. Email: jfang2018@163.com; jfang@sibs.ac.cn.

Funding

This work was supported by Science and Technology Commission of Shanghai Municipality Grant (16JC1406100), Ministry of Science and Technology of China (2017YFD0400206) and National Natural Science Foundation of China (31470769).

Disclosure of potential conflicts of interest

The authors declare that there are no potential conflicts of interest.

Abstract

Drug resistance is a big problem in cancer treatment and one of the most prominent mechanisms underlain is overexpression of ATP-binding cassette (ABC) transporters, particularly ABCB1, ABCC1 and ABCG2. Inhibition of ABC transporters is an important approach to overcome drug resistance. The inositol-requiring enzyme 1α (IRE1α), an arm of unfolded protein response (UPR), splices *XBP1* mRNA to generate an active transcription factor XBP1s. UPR is implicated in drug resistance. However, the underlying mechanism is unclear. We found that the anticancer drugs such as 5-fluorouracil (5-FU) activated the IRE1α-XBP1 pathway to induce the expression of ABCB1, ABCC1 and ABCG2 in colon cancer cells. Inhibition of IRE1α RNase activity with small molecule 4μ8c suppressed the drug-induced expression of these ABC transporters and sensitized 5-FU-resistant colon cancer cells to drug treatment. *In vivo* xenograft assay indicates that administration of 4μ8C substantially enhanced the efficacy of 5-FU chemotherapy on 5-FU-resistant colon cancer cells. These results suggest that IRE1α-targeting might be a strategy to cope with drug resistance of colon cancer.

Keywords: IRE1α; ABC transporter; Drug resistance; Colon cancer.

1. Introduction

Cancers have the ability to develop resistance to chemotherapy, and the increasing prevalence of these drug resistant cancers necessitates further research and treatment development. Multidrug resistance is a phenomenon by which, after exposure to a chemotherapeutic agent, cancer cells develop resistance, and simultaneous cross-resistance, to a wide range of functionally and structurally unrelated chemotherapeutic drugs (1, 2). Intrinsic or acquired multidrug resistance is one of the main reasons for chemotherapy failure, leading to the recurrence of malignant tumors and ultimately, patient relapse or death (3). A variety of mechanisms have been attributed to multidrug resistance, such as enhanced drug efflux, increased DNA damage repair, reduced apoptosis, elevated autophagy, and/or altered drug metabolism (4-6).

Previous studies have confirmed that multidrug resistance both in cancer cell lines and human tumor tissues is most often associated with the overexpression of the ATP-binding cassette (ABC) transporters (4, 7, 8). These ABC transporters are efflux pumps that transport various structurally unrelated and potentially dangerous substances out of the cells. They hydrolyze ATP for energy and actively pump drug components out of cells, leading to drug resistance. In these transporters, ABCB1 (also known as MDR1 or P-gp), ABCC1 (MRP1) and ABCG2 (BCRP1) play an important role in drug resistance *in vivo* (4, 7). Developing inhibitors of ABC transporters is an important approach to overcoming drug resistance. Extensive efforts have been made to develop inhibitors targeting ABC transporters, particularly ABCB1. However, no such agents have been developed successfully as was initially hoped. Inhibition of expression of these ABC transporters is also an effective approach to overcome drug

resistance of cancer cells (9).

The unfolded protein response (UPR) is a cellular stress response related to the endoplasmic reticulum (ER). It allows cells to manage ER stress resulting from accumulation of unfolded and/or misfolded proteins in the lumen of ER (10). Three ER-localized proteins, inositol-requiring enzyme 1 (IRE1α), pancreatic ER kinase (PERK), and activating transcription factor 6 (ATF6) constitute the three arms of the UPR to resolve ER stress. IRE1α is the most evolutionally conserved among the three UPR arms (11, 12). IRE1α is ubiquitously expressed and possesses both endoribonuclease and protein kinase activities. Upon activation, IRE1α removes a 26-bp nucleotide intron from the mRNA encoding X-box binding protein (XBP) 1 to generate a spliced active form of this transcription factor (XBP1s). XBP1s controls the expression of genes involved in protein folding, ER-associated degradation, protein quality control and phospholipid synthesis (13).

Sustained activation of the UPR has been implicated in cancers (14, 15), and it is believed to contribute to oncogenic processes (16, 17). Chemotherapeutic agents trigger ER stress (18, 19). UPR is activated in response to ER stress and it may play an important role in tumor chemotherapy resistance (14, 20, 21). Inhibition of GRP78, an unfolded protein response regulator, could enhance the sensitivity of malignant gliomas (22), breast cancer cells (23) and renal cell carcinoma cells (24) to drugs. While, GRP78 overexpression was found to promote drug resistance of cancer cells (22, 25). Activation of ATF4, a downstream target of PERK, led to multidrug resistance (26, 27). A recent study showed that XBP1 was upregulated in tamoxifen-insensitive breast cancer MCF-7 cells and inhibition of XBP1 splicing reestablished tamoxifen sensitivity to tamoxifen-resistant MCF-7 cells (28), suggest

that the IRE1α-XBP1 axis is involved in tamoxifen resistance of breast cancer cells. Though the UPR contributes to drug resistance of cancers, the underlying mechanism remains unclear.

Drug resistance is an obstacle to a successful chemotherapy for cancers including colon cancer. Colon cancer is the third most common malignancy worldwide (29). Chemotherapy is a standard treatment for this disease (30) and drug resistance develops in nearly all patients with colon cancer (31). Upregulated expression of ABC transporters such as ABCB1, ABCC1 and ABCG2 is one of the most commonly observed mechanisms contributing to drug resistance in colon cancer cells (32). To data, there are little efficient methods to overcome drug resistance of colon cancer. Hence, studying the underlying mechanism and developing new effective approach to overcoming drug resistance are urgently needed. Here we report that the anticancer drugs upregulated the expression of ABCB1, ABCC1 and ABCG2 through IRE1α-XBP1 axis in colon cancer cells. Inhibition of IRE1α RNase activity with small molecule 4μ8C inhibited the drug-induced expression of these ABC transporters and enhanced efficacy of drug chemotherapy on 5-FU-resistant colon cancer cells. Our results suggest that targeting of IRE1α is an approach to overcome drug resistance of colon cancer.

2. Materials and Methods

2.1. Cell culture and reagents

Human colon cancer cells RKO and HCT116 were grown in DMEM and M5A medium, respectively. Human embryonic kidney 293T cells were cultured in DMEM medium. All medium were supplemented with 10% fetal bovine serum, 100 u/ml penicillin, and 100 μg/ml

streptomycin. The cells were cultured at 37°C in a 5% CO_2 incubator. 5-FU was a product from Sigma. Capecitabine and Oxaliplatin were from Selleck and MedChemExpress, respectively. The IRE1α RNase inhibitor 4μ8C was a product of Selleck Chemicals. To establish 5-FU-resistant colon cancer cells, the cells were incubated initially in medium containing 5-FU at 0.25 μg/ml (1.92 μM). The concentration of 5-FU was gradually increased till 5-FU reached at 8 μM for RKO cells and 30 μM for HCT116 cells.

2.2. Antibodies

Antibodies against IRE1α(14C10) ABCB1(E1Y7B) and ABCC1(D5C1X) were from Cell signaling Technology. ABCG2(ab108312) and XBP1 antibodies were was from Abcam and BioLegend, respectively. Beta-actin(A3854) antibody was a product of Sigma.

2.3. Quantitative real-time PCR (qPCR)

qPCR was performed as described (33). Beta-actin was used as the internal control. The primers used in this work are as follows.

ABCB1: 5'AAGCCACGTCAGCTCTGGAT3'(F); 5'CTGCATTCTGGATGGTGGAC3'(R);

ABCC1: 5'CACGACGCCTTCATGTTCTC3'(F), 5'GGCTGGACAGGAGGAACAAC3'(R);

ABCG2: 5'AGCAGCAGGTCAGAGTGTGG3'(F), 5'CTGAAGCCATGACAGCCAAG3'(R);

IRE1α: 5'CTCCACTCCCTCAACATCGT3'(F), 5'CTTCTTGCAGAGGCCAAAGT3'(R);

XBP1: 5'CTTGTAGTTGAGAACCAGGAGT3'(F), 5'CCCAACAGGATATCAGACTCTG3'(R);

β-actin: 5'GATCATTGCTCCTCCTGAGC3'(F), 5'ACTCCTGCTTGCTGATCCAC3'(R).

2.4. Short hairpin RNA (shRNA) and small interference RNA (siRNA)

To inhibit the expression of human IRE1α, siRNA oligos or shRNA knockdown viruses were employed. The pLKO.1 vector was used to construct shRNA virus against human IRE1α. To

construct the hairpin siRNA expression cassette, complementary DNA nucleotides of IRE1α RNA interference were synthesized, annealed and inserted into pLKO.1. The control virus has a scrambled sequence. The targeting sequence for IRE1α and scrambled sequence are as described (34). The sequences of siRNA oligos against IRE1α are as follows:

siIRE1α-1: 5'GCGUAAAUUCAGGACCUAU3';

siIRE1α-2: 5'GGAGAGAAGCAGCAGACUU3';

siXBP1-1: 5'GGAACAGCAAGUGGUAGAUTT3';

siXBP1-2: 5'CCAGUCAUGUUCUUCAAAUTT3';

Control: 5'UUCUCCGAACGUGUCACGUTT3'.

2.5. Vector construction

The *ABCB1* promoter sequence from 793 bp upstream to 113 bp downstream of the human *ABCB1* gene transcription start site (–793 to +113) was cloned by genomic PCR using human genomic DNA as a template. The *ABCB1* promoter (–793~+113) luciferase reporter plasmid (ABCB1-Luc) was constructed into pGL3 vector (Promega). A series of mutated ABCB1-Luc plasmids derived from ABCB1-Luc were also constructed. The construct encoding XBP1s was generously provided by Dr Yong Liu at Wuhan University (35).

2.6. Chromatin immunoprecipitation (ChIP) assay

ChIP assay was carried out with Millipore EZ-ChIP™ kit (Upstate) according to the manufacturer's instruction. The XBP1-specific antibody was used for the ChIP assay. qPCR was done to quantitate the ChIP-enriched DNA. Two pairs of primers were used. The primers for *ABCB1* promoter enrichment are as follows.

(1) 5'ACAGAATTGGAGAGGTCGGAGT3'(F), 5'GGGCAAGTAGAGAAACGCGC3'(R);

(2) 5'CCTGGAAAAAACACGGGCATTGA3'(F), 5'GGTCTTTCTTCAGCATGCTTGACA3'(R).

2.7. Cell viability assay

Colon cancer cells were plated in 96-well plates (3000/well). The next day, anticancer drug was added and the cells were incubated for indicated time. The cells were stained for 4 h with thiazolyl blue tetrazolium bromide (MTT) dissolving in DMSO. The OD at 570 nm was read and cell viability was calculated as a ratio of OD values of drug-treated samples to those of controls. The cell viability of control cells at 24 h was designated as 1.

2.8. Xenograft growth of colon cancer cells

In vivo xenograft growth was performed as described (36). In brief, the 4-week-old male nude mice (BALB/cA-nu/nu) from Shanghai Experimental Animal Center were maintained in pathogen-free conditions. The mice were subcutaneously injected at each flank with colon cancer cells (3×10^6). Tumor volumes were measured with a caliper and calculated using the following equation: volume = $a\times b^2\times0.5326$, where a is the longer dimension, and b is the shorter one. All animals were maintained and used in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Institute of Nutrition and Health.

2.9. Statistic analysis

Statistical analysis was made using the unpaired two-tailed Student's *t*-test or two-way analysis of variance (ANOVA) with GraphPad Prism 5.0. The data are mean ± standard deviation (SD). *P* value < 0.05 is considered statistically significant.

3. Results

3.1. 5-FU induces ABCB1 expression and stimulates XBP1 splicing

5-FU-resistant colon cancer RKO (RKO/5-FU/R) cells were selected as described in Methods. We found that the transcript level of *ABCB1* was elevated in RKO/5-FU/R cells, as compared to parent RKO cells (Fig. 1A). Immunoblotting shows that the protein level of ABCB1 was also increased in RKO/5-FU/R cells (Fig. 1B). We treated RKO cells with 5-FU and found that both mRNA (Fig. 1C) and protein levels (Fig. 1D) of ABCB1 were increased. We also treated colon cancer HCT116 cells with 5-FU and similar results were obtained (Fig. 1C and D). These results imply that 5-FU induces the expression of ABCB1.

Interestingly, we found that the splicing of XBP1 was substantially enhanced in RKO/5-FU/R cells (Fig. 1E). We treated RKO and HCT116 cells with 5-FU and determined XBP1 splicing (Fig. 1F). The results show that 5-FU treatment also enhanced the splicing of XBP1 in these cells (Fig. 1F). These results suggest that 5-FU activates IRE1α.

3.2. IRE1α is involved in 5-FU-induced expression of ABCB1

The aforementioned results show that 5-FU induced ABCB1 expression and activated IRE1α (Fig. 1). This drove us to determine whether IRE1α was involved in the induction of ABCB1 by 5-FU. To this end, we employed 4μ8C, a specific inhibitor of IRE1α RNase in our work (37). We found that 4μ8C treatment decreased the expression of ABCB1 in RKO/5-FU/R cells (Fig. 2A). This inhibitor also blocked the induction of ABCB1 in RKO cells treated with 5-FU for 48 hr (Fig. 2B). These results imply that IRE1α is involved in the induction of ABCB1 by 5-FU. To solidify this, we inhibited the expression of IRE1α by means of RNA interference. We found that knockdown of IRE1α decreased ABCB1 in RKO/5-FU/R cells (Fig. 2C) and inhibited the induction of ABCB1 in RKO cells treated with 5-FU for 48 hr (Fig. 2D). These data suggest that IRE1α is required for 5-FU-induced expression of ABCB1.

3.3. XBP1s is a transcription factor of ABCB1

Upon activation, IRE1α splices XBP1 mRNA to produce an active transcription factor XBP1s. Our data show that 5-FU stimulated the splicing of XBP1 and enhanced the transcript level of *ABCB1* (Fig. 1). We therefore conjectured that 5-FU might activate the expression of ABCB1 through XBP1s. To know this, we treated RKO cells with 5-FU and determined the effect of knockdown of XBP1 on ABCB1 induction. The results show that knockdown of XBP1 prevented 5-FU from inducing the expression of ABCB1 (Fig. 3A), implying that 5-FU induces the expression of ABCB1 through XBP1s. We found that overexpression of XBP1s induced the expression of ABCB1 (Fig. 3B), providing an evidence that XBP1s acts as a transcription factor of ABCB1.

To investigate whether XBP1s is a transcription factor of *ABCB1*, we constructed an *ABCB1* promoter luciferase reporter plasmid ABCB1(−793~113)-Luc (Fig. 3C). We found that overexpression of XBP1s stimulated the luciferase activities of this reporter in RKO, HCT116 and 293T cells (Fig. 3D). Subsequently, we constructed three truncated *ABCB1* promoter luciferase reporter vectors ABCB1(−606~113)-Luc, ABCB1(−402~113)-Luc and ABCB1(−166~113)-Luc (Fig. 3C). Overexpression of XBP1s stimulated significantly the activities of ABCB1(−606~113)-Luc and ABCB1(−402~113)-Luc reporters (Fig. 3E). When the ABCB1(−166~113)-Luc reporter was examined, overexpression of XBP1s had minor effect on its luciferase activity. These results suggest that the XBP1s binding sites are possibly located within the region of −793~−167. We analyzed this region and found that it had three sites ($^{-601}$ACGT^{-598}, $^{-307}$ACGT^{-304} and $^{-260}$ACGT^{-257}) that were similar to XBP1s DNA binding motif (38). We constructed ABCB1(−793~−167)-Luc reporter (Fig. 3F) and found

that overexpression of XBP1s stimulated the luciferase activity of this reporter (Fig. 3G). Then we constructed mutated ABCB1(−793~−167)-Luc reporter plasmids at the possible XBP1s binding site (ACGT→ ACTT) . Overexpression of XBP1s stimulated the activities of ABCB1(−793~−167)-Luc reporter with single ($G^{-599}T$, $G^{-305}T$ or $G^{-258}T$) or double mutations ($G^{-599}T/G^{-305}T$) (Fig. 3G). If all these three sites were mutated ($G^{-599}T/G^{-305}T/G^{-258}T$), overexpression of XBP1s could not activate the reporter activity (Fig. 3G). These results suggest that XBP1s may bind these sites to activate *ABCB1* transcription.

We found that 5-FU treatment stimulated ABCB1 luciferase reporter activity which was suppressed by 4μ8C (Fig. 3H). We also did ChIP assay in 293T and HCT116 cells to solidify that XBP1s could bind the *ABCB1* promoter. The immunoprecipitation experiment was performed using XBP1s antibody or IgG. The *ABCB1* promoter was amplified by two pairs of primer. We found that immunoprecipitation by XBP1s antibody substantially enriched *ABCB1* promoter, but not by the IgG control antibody (Fig. 3I). These results imply that XBP1s binds *ABCB1* promoter.

Taken together, our results suggest that 5-FU induces the expression of ABCB1 through IRE1α/XBP1 axis.

3.4. 5-FU induces the expression of ABCC1 and ABCG2 through IRE1α-XBP1 axis

The ABC transporters ABCC1 and ABCG2 also play a crucial role in drug-resistance (4, 7). We found that ABCC1 and ABCG2 were also upregulated in RKO/5-FU/R cells (Fig. 4A). We treated RKO and HCT116 cells with 5-FU for 48 hr and found that the expression of ABCC1 and ABCG2 was increased (Fig. 4B). The results indicate that 5-FU induces not only ABCB1 but also ABCC1 and ABCG2.

We determined whether 5-FU induced ABCC1 and ABCG2 through IRE1α-XBP1 axis. Inhibition of IRE1α with 4μ8C decreased the expression of ABCC1 and ABCG2 in RKO/5-FU/R cells (Fig. 4C). This inhibitor also suppressed 5-FU-induced expression of ABCC1 and ABCG2 in RKO cells (Fig. 4D). Knockdown of IRE1α decreased ABCC1 and ABCG2 in RKO/5-FU/R cells (Fig. 4E) and repressed the induction of ABCC1 and ABCG2 by 5-FU in RKO cells (Fig. 4F). These data suggest that 5-FU induces the expression of ABCC1 and ABCG2 through IRE1α. To know whether XBP1 is involved in the induction of these two transporters by 5-FU, we knocked down XBP1. We found that knockdown of XBP1 prevented 5-FU from inducing ABCC1 and ABCG2 (Fig. 4G). Together, these results suggest that 5-FU induces the expression of ABCC1 and ABCG2 through IRE1α-XBP1 axis.

3.5. Capecitabine and oxaliplatin induce the expression of ABCB1, ABCC1 and ABCG2

Our results show that 5-FU induced the expression of ABC transporters through IRE1α-XBP1 axis. This drove us to determine whether other anticancer drugs had the similar effect. To know this, we examined capecitabine and oxaliplatin that were often used to treat colon cancers clinically. We treated RKO cells with these drugs and found that both capecitabine and oxaliplatin enhanced the splicing of XBP1, indicating that they activate IRE1α (Fig. 5A). Treatment of RKO cells with these drugs induced the transcription of *ABCB1*, ABCC*1* and *ABCG2* (Fig. 5B). And the induction of expression of these genes by 5-FU was suppressed by 4μ8C (Fig. 5B). These results suggest that capecitabine and oxaliplatin induce the expression of *ABCB1*, ABCC*1* and *ABCG2* through IRE1α.

3.6. Inhibition of IRE1α overcomes drug resistance

We determined the sensitivity of RKO and RKO/5-FU/R cells to capecitabine and oxaliplatin.

These cells were treated with capecitabine or oxaliplatin for different times and cell survival was determined by MTT assay. We found that the survivability of RKO/5-FU/R cells is much higher than that of RKO cells (Fig. 5C), implying that the 5-FU-resistant RKO cells also developed resistance to capecitabine and oxaliplatin.

We speculated that inhibition of IRE1α might overcome drug resistance as IRE1α was involved in the induction of ABCB1, ABCC1 and ABCG2 by drugs. To this end, we inhibited IRE1α with 4μ8C and determined the survival of RKO/5-FU/R cells treated with 5-FU. As expected, 4μ8C decreased substantially the survival of the cells challenged with 5-FU (Fig. 5D). We then challenged RKO/5-FU/R cells with capecitabine and oxaliplatin and found that 4μ8C also sensitized the cells to these two drugs (Fig. 5E). These results suggest that inhibition of IRE1α RNase activity enhances the efficacy of drugs chemotherapy on 5-FU-resistant cancer cells.

3.7. Inhibition of IRE1α enhances 5-FU efficacy on 5-FU-resistant HCT116 cells in vivo

Finally, we determined whether inhibition of IRE1α could enhance the efficacy of chemotherapy on xenograft growth of drug-resistant colon cancer cells in nude mice. We employed 5-FU-resistant HCT116 cells (HCT116/5-FU/R) in our work. The HCT116/5-FU/R cells were selected as described in Methods. Compared to HCT116 cells, the 5-FU-resistant HCT116 cells had elevated splicing of XBP1 (Fig. 6A) and increased expression of ABCB1, ABCC1 and ABCG2 (Fig. 6B). Treatment of HCT116/5-FU/R cells with 4μ8C decreased the protein levels of ABCB1, ABCC1 and ABCG2 (Fig. 6C). Similar to RKO/5-FU/R cells, the HCT116/5-FU/R cells became sensitive to 5-FU in the presence of 4μ8C (Fig. 6D).

The HCT116/5-FU/R cells were implanted into nude mice at each flank. Tumors were

allowed to grow for 16 days before drug treatment. Drug administration to the mice was as described in Methods. Administration of 5-FU alone had minor inhibitory effect on xenograft growth (Fig. 6E). Administration of 4μ8C alone had moderate inhibitory effect. The combination of 5-FU and 4μ8C had a strong inhibitory effect on xenograft growth. These results suggest that inhibition of IRE1α enhances the efficacy of 5-FU chemotherapy on tumor growth of 5-FU-resistant HCT116 cells *in vivo*. Administration of 4μ8C decreased the protein levels of ABCB1, ABCC1 and ABCG2 in tumors (Fig. 6F) and this might be an important reason why the HCT116/5-FU/R cells were sensitive to 5-FU treatment *in vivo*. A working model is proposed (Fig. 6G)

4. Discussion

In this manuscript, we demonstrate that the anticancer drugs induce the expression of ABCB1, ABCC1 and ABCG2 through IRE1α-XBP1 axis. Inhibition of IRE1α RNase activity with 4μ8C enhanced efficacy of 5-FU chemotherapy on 5-FU-resistant colon cancer cells *in vitro* and *in vivo*. Our results suggest that the IRE1α contributes drug resistance of colon cancer cells through upregulating these ABC transporters and IRE1α-targeting might be a strategy to cope with drug resistance of cancers.

A few mechanisms have been attributed to drug resistance and the extrusion of anticancer drugs by ABC transporters is one of the most widely recognized one (2, 7, 39). UPR is also shown to contribute to drug resistance (14, 20, 21). However, the underlying molecular mechanism remains unclear. We found that the anticancer drug 5-FU, capecitabine and

oxaliplatin stimulated XBP1 splicing and induced the expression of *ABCB1*, *ABCC1* and *ABCG2*. The drug-induced expression of these genes was blocked by IRE1α inhibitor 4μ8C. Our findings reveal a new mechanism underlying the regulation of expression of ABCB1, ABCC1 and ABCG2 by drugs.

Drug resistance is a key determinant of cancer chemotherapy failure and one of the major causes of drug resistance is the enhanced efflux of drugs by membrane ABC transporters. Targeting ABC transporters projects a promising approach to eliminating or suppressing drug resistance in cancer treatment (4). Extensive efforts have been made to target ABC transporters, particularly ABCB1, for overcoming drug resistance. However, no such inhibitors have been developed successfully for clinical use (40). Inhibition of expression of ABC transporters is another approach to cope with drug resistance of cancers. Our results show that inhibition of IRE1α attenuated drug-induced expression of ABCB1, ABCC1 and ABCG2 (Fig. 2-5). Thus, targeting of IREα might be a way to overcome drug resistance. In fact, our results show that inhibition of IRE1α with 4μ8C sensitized the 5-FU-resistant colon cancer cells to drug treatment *in vitro* and *in vivo* (Fig. 5 and 6). These results suggest that targeting of IRE1α overcomes drug resistance through downregulating the expression of these ABC transporters.

It has been demonstrated that IRE1α is involved in tumor progression (41). We reported recently that IRE1α plays an important role in development of colon and liver cancers (34, 42). We found that inhibition of IRE1α RNase activity inhibited xenograft growth of colon cancer cells (34) and loss of IRE1α reduced the occurrence of diethylnitrosamine-induced hepatocellular carcinoma (42). Logue et al (43) demonstrated in triple-negative breast cancer

(TNBC) that IRE1α RNase activity contributed paclitaxel-mediated expansion of tumor-initiating cells and inhibition of IRE1α RNase activity increases paclitaxel- mediated tumor suppression in a xenograft model of TNBC. Zhao et al (44) showed in breast cancer cells that inhibition of IRE1α RNase activity suppressed the MYC- overexpressing tumor growth *in vivo* and IRE1α-targeting substantially enhanced the efficacy of docetaxel chemotherapy. These results suggest that IRE1α contributes cancer development and it may serve as a target for cancer treatment.

In this manuscript, we have demonstrated that the anticancer drugs 5-FU, capecitabine and oxaliplatin activate IRE1α-XBP1 axis to induce the expression of ABCB1, ABCC1 and ABCG2 in colon cancer cells. The IRE1α RNase inhibitor 4μ8C suppressed the drug-induced expression of these ABC transporters and enhanced efficacy of drug treatment on drug-resistance colon cancer cells. Our results reveal the role and molecular mechanism of IRE1α-XBP1 axis in drug resistance of colon cancer cells. These findings suggest that targeting of IRE1α might be a strategy to cope with drug resistance of colon cancers.

Author contribution

QG, XL, Y X, JZ, SR, data acquisition; YQ, technique support; JF, work design, supervision and paper writing.

Conflicts of interest statement

The authors declare that there are no potential conflicts of interest.

Acknowledgements

This work was supported by Science and Technology Commission of Shanghai Municipality Grant (16JC1406100), National Natural Science Foundation of China (31470769) and Ministry of Science and Technology of China (2017YFD0400206).

References

1. Yusuf RZ, Duan Z, Lamendola DE, Penson RT, Seiden MV. Paclitaxel resistance: molecular mechanisms and pharmacologic manipulation. *Curr Cancer Drug Targets* 2003; 3:1-19.
2. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov* 2006; 5:219-234.
3. Liang XJ, Chen C, Zhao Y, Wang PC. Circumventing tumor resistance to chemotherapy by nanotechnology. *Methods Mol Biol* 2010; 596:467–488.
4. Li W, Zhang H, Assaraf YG, Zhao K, Xu X, Xie J, Yang DH, Chen ZS. Overcoming ABC transporter-mediated multidrug resistance: Molecular mechanisms and novel therapeutic drug strategies. *Drug Resist Updat* 2016; 27:14-29.
5. Kachalaki S, Ebrahimi M, Mohamed Khosroshahi L, Mohammadinejad S, Baradaran B. Cancer chemoresistance; biochemical and molecular aspects: a brief overview. *Eur J Pharm Sci* 2016; 89:20–30.
6. Salehan MR, Morse HR. DNA damage repair and tolerance: a role in chemotherapeutic drug resistance. *Br J Biomed Sci* 2013; 70:31–40.
7. Fletcher JI, Williams RT, Henderson MJ, Norris MD, Haber M. ABC transporters as mediators of drug resistance and contributors to cancer cell biology. *Drug Resist Update* 2016; 26:1-9.
8. Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, Hu T, Jiang L, Li J. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade. *Cancer Lett* 2016; 370:153-64.

9. Szakács G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov* 2006; 5:219-234.
10. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; 8:519-529.
11. Todd DJ, Lee AH, Glimcher LH. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* 2008; 8: 663-674.
12. Cox JS, Shamu CE, Walter P. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* 1993; 73: 1197-1206.
13. Morl K, Ma W, Gething MJ, Sambrook J. A transmembrane protein with a cdc2+CDC28-related kinase activity is required for signaling from the ER to the nucleus. *Cell* 1993; 74:743-756.
14. Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. *Nat Rev Cancer* 2014; 14:581-597.
15. Oakes SA, Papa FR. The Role of Endoplasmic Reticulum Stress in Human Pathology. *Annu Rev Pathol* 2015; 10:173-194.
16. Ma Y, Hendershot, LM. The role of the unfolded protein response in tumour development: friend or foe? *Nat Rev Cancer* 2004; 4:966-977.
17. Chevet E, Hetz C, Samali A. Endoplasmic Reticulum Stress–Activated Cell Reprogramming in Oncogenesis. *Cancer Discov* 2015; 5:586-597.
18. Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. *Nat Rev Drug Discov* 2008; 7:1013–30.
19. Schwarz DS, Blower MD. The endoplasmic reticulum: structure, function and response to cellular signaling. *Cell Mol Life Sci* 2016; 73:79–94.
20. Avril T, Vauléon E, Chevet E. Endoplasmic reticulum stress signaling and chemotherapy resistance in solid cancers. *Oncogenesis* 2017; 6(8):e373.
21. Nagelkerke A, Bussink J, Sweep FC, Span PN. The unfolded protein response as a target for cancer therapy. *Biochim Biophys Acta* 2014; 1846:277-284.
22. Pyrko P, Schönthal AH, Hofman FM, Chen TC, Lee AS. The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant

gliomas. *Cancer Res* 2007; 67:9809-9816.

23. Dong D, Ko B, Baumeister P, Swenson S, Costa F, Markland F, Stiles C, Patterson JB, Bates SE, Lee AS. Vascular targeting and antiangiogenesis agents induce drug resistance effector GRP78 within the tumor microenvironment. *Cancer Res* 2005; 65:5785-5791.
24. Lin JA, Fang SU, Su CL, Hsiao CJ, Chang CC, Lin YF, Cheng CW. Silencing glucose-regulated protein 78 induced renal cell carcinoma cell line G1 cell-cycle arrest and resistance to conventional chemotherapy. *Urol Oncol* 2014; 32:29 (e21-11).
25. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. *J Biol Chem* 2003; 278:20915-20924.
26. Tanabe M., H. Izumi, T. Ise, S. Higuchi, T. Yamori, K. Yasumoto, K. Kohno, Activating transcription factor 4 increases the cisplatin resistance of human cancer cell lines. *Cancer Res* 2003; 63:8592–8595.
27. Igarashi T, Izumi H, Uchiumi T, Nishio K, Arao T, Tanabe M, Uramoto H, Sugio K, Yasumoto K, Sasaguri Y, Wang KY, Otsuji Y, Kohno K. Clock and ATF4 transcription system regulates drug resistance in human cancer cell lines. *Oncogene* 2007; 26:4749-4760.
28. Ming J, Ruan S, Wang M, Ye D, Fan N, Meng Q, Tian B, Huang T. A novel chemical, STF-083010, reverses tamoxifen-related drug resistance in breast cancer by inhibiting IRE1/XBP1. *Oncotarget* 2015; 6:40692-40703.
29. Davies RJ, Miller R, Coleman N. Colorectal cancer screening: prospects for molecular stool analysis. *Nat Rev Cancer* 2005; 5:199-209.
30. Wu G, Wilson G, George J, Liddle C, Hebbard L, Qiao L. Overcoming treatment resistance in cancer: Current understanding and tactics. *Cancer Lett* 2017; 387:69-76.
31. Dallas NA, Xia L, Fan F, Gray MJ, Gaur P, van Buren G, Samuel S, Kim MP, Lim SJ, Ellis LM. Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. *Cancer Res* 2009; 69:1951-1957.
32. Hu T, Li Z, Gao CY, Cho CH. Mechanisms of drug resistance in colon cancer and its

therapeutic strategies. *World J Gastroenterol* 2016; 22:6876-89.

33. Xue J, Li X, Jiao S, Wei Y, Wu G, Fang J. Prolyl Hydroxylase-3 Is Down-regulated in Colorectal Cancer Cells and Inhibits IKKβ Independent of Hydroxylase Activity. *Gastroenterology* 2010; 138:606-615.
34. Li XX, Zhang HS, Xu YM, Zhang RJ, Chen Y, Fan L, Qin YQ, Liu Y, Li M, Fang J. Knockdown of IRE1α inhibits colonic tumorigenesis through decreasing β-catenin and IRE1α targeting suppresses colon cancer cells. *Oncogene* 2017; 36:6738-6746.
35. Shao M, Shan B, Liu Y, Deng Y, Yan C, Wu Y, Mao T, Qiu Y, Zhou Y, Jiang S, Jia W, Li J, Li J, Rui L, Yang L, Liu Y. Hepatic IRE1α regulates fasting-induced metabolic adaptive programs through the XBP1s-PPARα axis signaling. *Nat Commun* 2014; 5:3528.
36. Zhang R, Li X, Wei L, Qin Y, Fang J. Lemur tyrosine kinase 2 acts as a positive regulator of NF-κB activation and colon cancer cell proliferation. *Cancer Lett* 2019; 454:70-77.
37. Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, Silverman RH, Neubert TA, Baxendale IR, Ron D, Harding HP. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. *Proc Natl Acad Sci U S A* 2012; 109:E869-878.
38. Yamamoto K, Suzuki N, Wada T, Okada T, Yoshida H, Kaufman RJ, Mori K. Human HRD1 promoter carries a functional unfolded protein response element to which XBP1 but not ATF6 directly binds. *J Biochem* 2008; 144:477-86.
39. Kartal-Yandim M, Adan-Gokbulut A, Baran Y. Molecular mechanisms of drug resistance and its reversal in cancer. *Crit Rev Biotechnol* 2016; 36:716-726.
40. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer* 2018; 18:452-464.
41. Madden E, Logue SE, Healy SJ, Manie S, Samali A. The role of the unfolded protein response in cancer progression: From oncogenesis to chemoresistance. *Biol Cell* 2019; 111:1-17.
42. Wu Y, Shan B, Dai J, Xia Z, Cai J, Chen T, Lv S, Feng Y, Zheng L, Wang Y, Liu J, Fang J, Xie D, Rui L, Liu J, Liu Y. Dual role for inositol-requiring enzyme 1α in promoting the development of hepatocellular carcinoma during diet-induced obesity in mice.

Hepatology 2018; 68:533-546.

43. Logue SE, McGrath EP, Cleary P, Greene S, Mnich K, Almanza A, Chevet E, Dwyer RM, Oommen A, Legembre P, Godey F, Madden EC, Leuzzi B, Obacz J, Zeng Q, Patterson JB, Jäger R, Gorman AM, Samali A. Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. *Nat Commun* 2018; 9:3267.
44. Zhao N, Cao J, Xu L, Tang Q, Dobrolecki LE, Lv X, Talukdar M, Lu Y, Wang X, Hu DZ, Shi Q, Xiang Y, Wang Y, Liu X, Bu W, Jiang Y, Li M, Gong Y, Sun Z, Ying H, Yuan B, Lin X, Feng XH, Hartig SM, Li F, Shen H, Chen Y, Han L, Zeng Q, Patterson JB, Kaipparettu BA, Putluri N, Sicheri F, Rosen JM, Lewis MT, Chen X. Pharmacological targeting of MYC-regulated IRE1/XBP1 pathway suppresses MYC-driven breast cancer. *J Clin Invest* 2018; 128:1283-1299.

Figure legends

Figure 1. 5-FU induced ABCB1 expression and XBP1 splicing.

(A) Determination of the relative *ABCB1* mRNA levels in 5-FU-resistant RKO cells. (B) Determination of ABCB1 protein in 5-FU-resistant RKO cells. (C) Determination of relative *ABCB1* mRNA levels in RKO and HCT116 cells treated with 5-FU (0, 10, 20 μM) for 48 hr. (D) Determination of ABCB1 protein levels in RKO and HCT116 cells treated with 5-FU (0, 10, 20 μM) for 48 hr. (E) Determination of XBP1 splicing in RKO and RKO/5-FU-R cells by regular PCR. The primers used are 5'AGCAAGTGGTGGATTTGGAAGAAG3'(F) and 5'AGGGTCCAACTTGTCCAGAATG3'(R). (F) RKO and HCT116 cells were treated with 5-FU (10 μM for 48 hr). * $P<0.05$; ** $P<0.01$.

Figure 2. 5-FU activates IRE1α-XBP1 signaling pathway.

(A) RKO/5-FU-R cells were treated with 4μ8C (20 μM) for 24 hr. followed by determination

of mRNA (left panel) and protein (right panel) of ABCB1. (B) RKO cells were treated with 5-FU (20 μM) or 5-FU (20 μM) plus 4μ8C (20 μM) for 48 hr, followed by determination of mRNA (left panel) and protein (right panel) of ABCB1. (C) RKO/5-FU-R cells were transfected with control or siIRE1α oligos as indicated. After 48 hr, the cells were harvested for determination of mRNA (left panel) and protein (right panel) of ABCB1. (D) RKO cells were transfected with control or siIRE1α oligos as indicated. After 24 hr, the cells were treated with 5-FU (20 μM) for 48 hr, followed by qPCR (left panel) and westernblot (right panel). *, P<0.05; **, P<0.01; ***, P<0.001.

Figure 3. XBP1s is a transcription factor of ABCB1.

(A) RKO cells were transfected with siXBP1 oligos as indicated. After 24 hr, the cells were treated with 5-FU (20 μM) for 48 hr followed by qPCR (left panel) and westernblot (right panel). (B) Overexpression of XBP1s induced the expression of ABCB1. (C) Construction of *ABCB1* promoter luciferase reporter plasmids. (D) RKO, HCT116 and 293T cells were transfected with ABCB1(−793~113)-Luc reporter with or without the vector encoding XBP1s. After 24 hr, the cells were harvested for luciferase activity assay. (E) 293T cells were transfected with ABCB1(−793~113)-Luc or truncated reporters with or without XBP1s vector. After 24 hr, the cells were harvested for luciferase activity assay. (F) Construction of ABCB1(−793~−167)-Luc and mutated ABCB1(−793~−167)-Luc reporter vectors. (G) 293T cells were transfected with ABCB1(−793~−167)-Luc or mutated reporter vectors with or without XBP1s plasmid. After 24 hr, the cells were harvested for luciferase activity assay. (H) RKO cells were transected with ABCB1 luciferase reporter construct. After 24 h, 5-FU (20 μM) or 5-FU+4μ8C (20 μM) were added and the cells were incubated for another 24 h before being harvested for luciferase activity assay. (I) ChIP assay was performed in 293T and

HCT116 cells as described in Methods. *, P<0.05; **, P<0.01; ***, P<0.001.

Figure 4. 5-FU induces ABCC1 and ABCG2 via IRE1α-XBP1 axis.

(A) Expression of ABCC1 and ABCG2 in RKO and RKO/5-FU-R cells. Messenger RNA levels (left panel); Protein levels (right panel). (B) RKO and HCT116 cells were treated with 5-FU (0, 10, 20 μM) for 48 hr. ABCC1 and ABCG2 mRNA (left panel) and protein levels (right panel). (C) RKO/5-FU/R cells were treated with 4μ8C (20 μM) for 24 hr, followed by qPCR (left panel) and westernblot (right panel). (D) RKO cells were incubated with 5-FU (20 μM) in the presence or absence of 4μ8C (20 μM) for 48 hr, followed by qPCR (left panel) and westernblot (right panel). (E) RKO/5-FU/R cells were transfected with control or IRE1α siRNA oligos. After 48 hr the cells were harvested for qPCR (left) and westernblot (right). (F) RKO cells were transfected with control or IRE1α siRNA oligos. After 24 hr 5-FU (20 μM) was added and the cells were incubated for another 48 hr. The cells were harvested for qPCR (left) and westernblot (right). (G) RKO cells were transfected with XBP1 siRNA oligos as indicated. After 24 hr, the cells were incubated with 5-FU (20 μM) for 48 hr. *, P<0.05; **, P<0.01; ***, P<0.001.

Figure 5. Inhibition of IRE1α sensitizes RKO/5-FU/R cells to drug treatment.

(A) RKO cells were treated with capecitabine (10 μM) or oxaliplatin (10 μM) for 48 hr. XBP1 splicing was determined by regular PCR as described above. (B) RKO cells were treated with capecitabine (10 μM) or oxaliplatin (10 μM) in the presence or absence of 4μ8C (20 μm) for 48 hr. The cells were harvested for determining the expression of *ABCB1*, *ABCC1* and *ABCG2* by qPCR. (C) RKO and RKO/5-FU/R cells were incubated in medium containing capecitabine (10 μM) or oxaliplatin (10 μM). Cell survival was determined by MTT assay. (D) RKO/5-FU/R cells were treated with 5-FU (8 μM) in the presence or absence of 4μ8C.

Survival of the cells was determined as described above. (E) RKO/5-FU/R cells were treated with capecitabine (10 μM) or oxaliplatin (10 μM) in the presence or absence of 4μ8C. *, P<0.05; **, P<0.01.

Figure 6. Inhibition of IRE1α sensitizes HCT116/5-FU/R cells to 5-FU *in vivo*.

(A) The splicing of XBP1 was increased in HCT116/5-FU/R cells. (B) Expression of ABCB1, ABCC1 and ABCG2 was increased in HCT116/5-FU/R cells. (C) HCT116/5-FU/R cells were treated with 4μ8C (20 μM) for 24 h, followed by westernblot. (D) HCT116/5-FU/R cells were treated with 5-FU (20 μM) in the absence or presence of 4μ8C (5, 10, 20 μM). Cell survival was determined at different time intervals by MTT assay. (E) 4μ8C enhanced the efficacy of 5-FU chemotherapy *in vivo*. HCT116/5-FU/R cells were implanted onto nude mice at each flank (3×10^6). After 16 days, the mice were administrated i.p. with 5-FU (25 mg/kg, every two days), 4μ8C (5 μg/g, twice a week) or combination of 5-FU and 4μ8C. (F) Determination of the expression of ABCB1, ABCC1 and ABCG2 in tumors. (G) A proposed working model. *, P<0.05; **, P<0.01; ***, P<0.001.

Figure 1

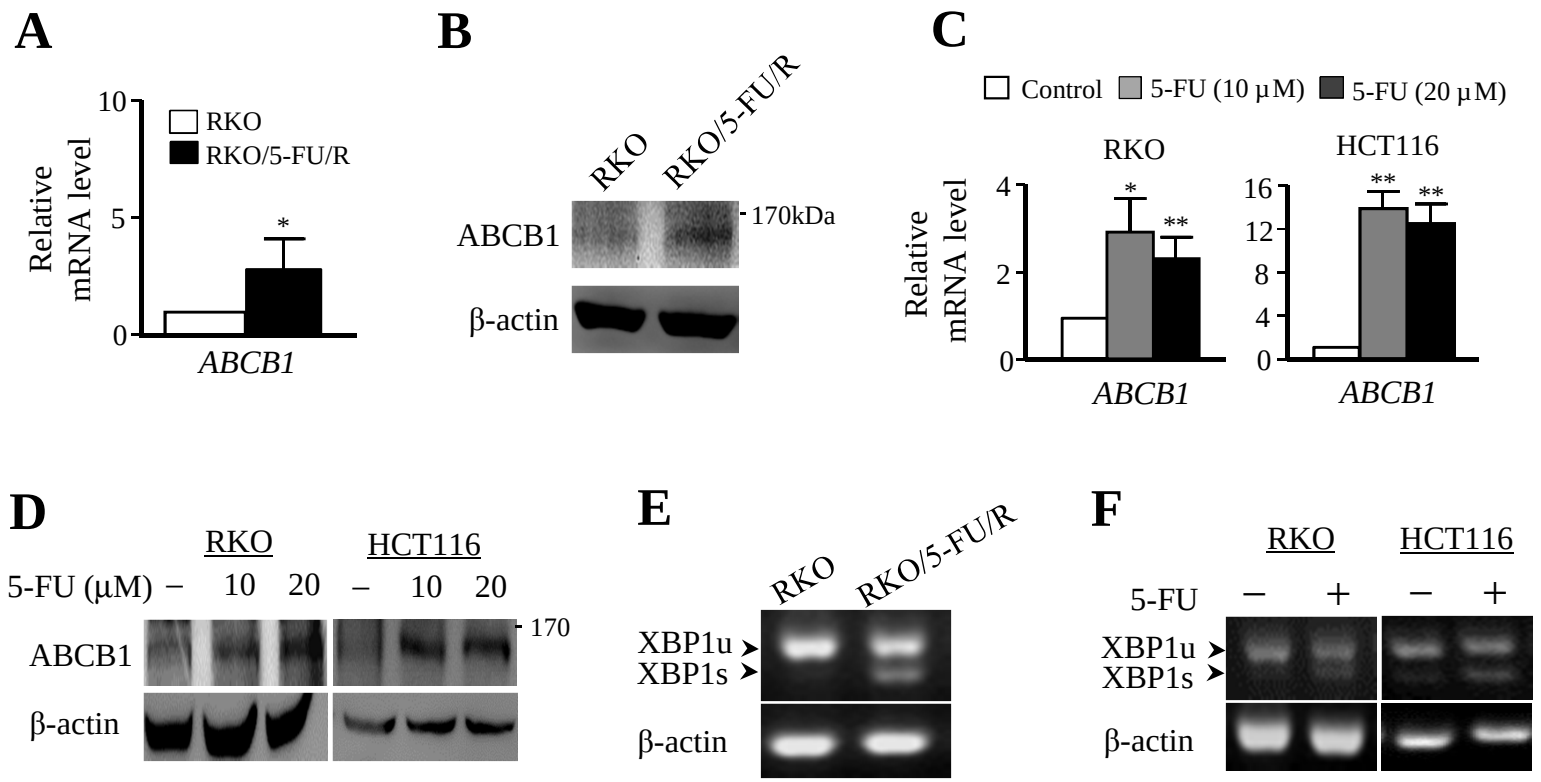

A
10
5
0
Relative mRNA level
RKO
RKO/5-FU/R
*
ABCB1
B
RKO
RKO/5-FU/R
ABCB1
170kDa
β-actin
C
Control
5-FU (10 μM)
5-FU (20 μM)
RKO
HCT116
Relative mRNA level
4
2
0
16
12
8
4
0
*
**
**
**
ABCB1
ABCB1
D
RKO
HCT116
5-FU (μM) − 10 20 − 10 20
ABCB1
170
β-actin
E
RKO
RKO/5-FU/R
XBP1u
XBP1s
β-actin
F
RKO
HCT116
5-FU − + − +
XBP1u
XBP1s
β-actin

Figure 2

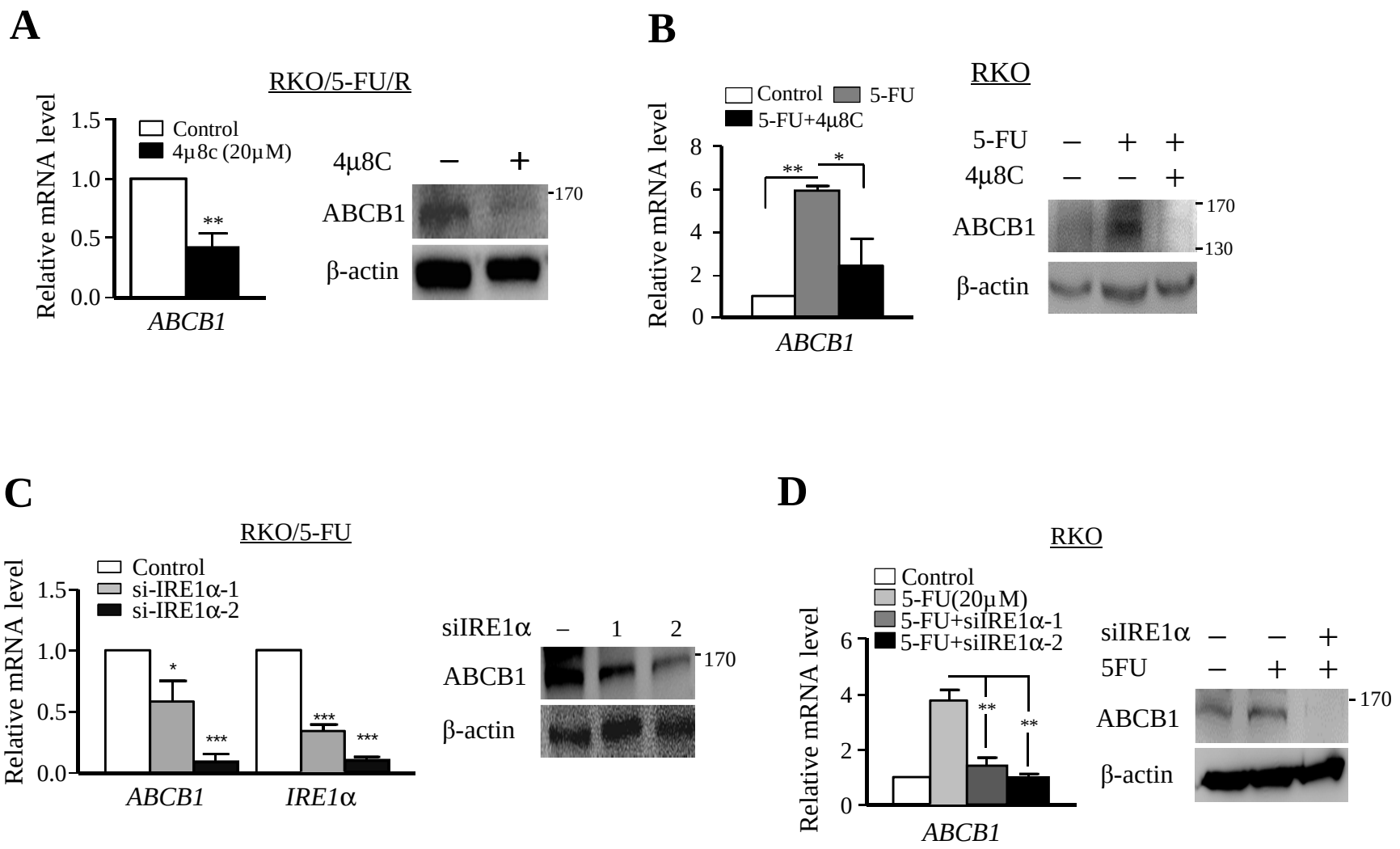

A
RKO/5-FU/R
Relative mRNA level
Control
4μ8c (20μM)
1.5
1.0
0.5
0.0
**
ABCB1
4μ8C – +
ABCB1
β-actin
170
B
RKO
Control
5-FU
5-FU+4μ8C
Relative mRNA level
8
6
4
2
0
**
*
ABCB1
5-FU – + +
4μ8C – – +
ABCB1
β-actin
170
130
C
RKO/5-FU
Control
si-IRE1α-1
si-IRE1α-2
Relative mRNA level
1.5
1.0
0.5
0.0
*
***
***
***
ABCB1
IRE1α
siIRE1α – 1 2
ABCB1
β-actin
170
D
RKO
Control
5-FU(20μM)
5-FU+siIRE1α-1
5-FU+siIRE1α-2
Relative mRNA level
6
4
2
0
**
**
ABCB1
siIRE1α – – +
5FU – + +
ABCB1
β-actin
170

Figure 3

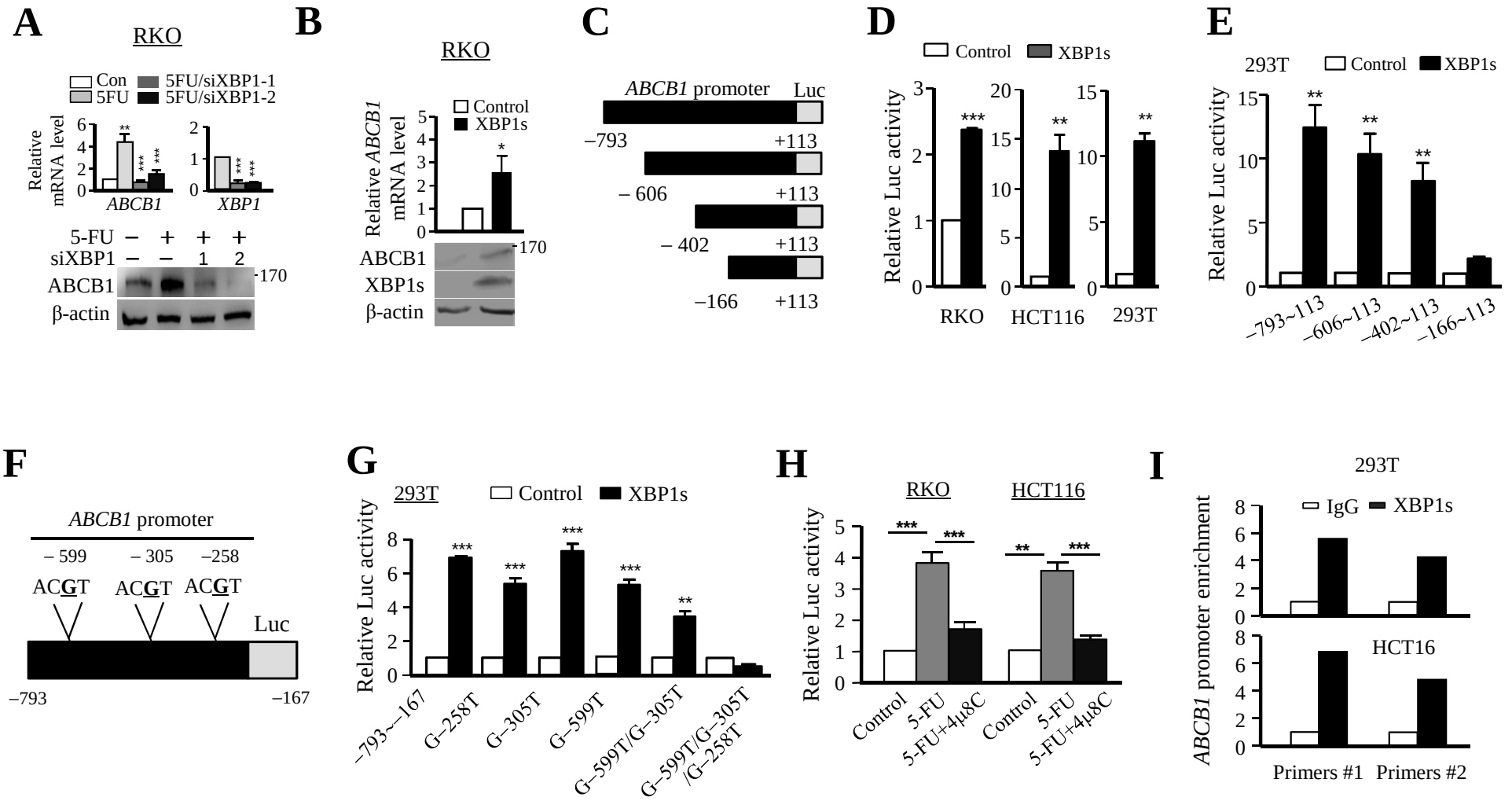

A
RKO
Con
5FU
5FU/siXBP1-1
5FU/siXBP1-2
Relative mRNA level
ABCB1
XBP1
5-FU
siXBP1
ABCB1
β-actin
170
B
RKO
Control
XBP1s
Relative ABCB1 mRNA level
ABCB1
XBP1s
β-actin
170
C
ABCB1 promoter
Luc
−793
+113
− 606
− 402
−166
D
Control
XBP1s
Relative Luc activity
RKO
HCT116
293T
E
293T
Control
XBP1s
Relative Luc activity
−793~113
−606~113
−402~113
−166~113
F
ABCB1 promoter
− 599
− 305
−258
ACGT
Luc
−793
−167
G
293T
Control
XBP1s
Relative Luc activity
−793~−167
G−258T
G−305T
G−599T
G−599T/G−305T
G−599T/G−305T /G−258T
H
RKO
HCT116
Relative Luc activity
Control
5-FU
5-FU+4μ8C
I
293T
IgG
XBP1s
ABCB1 promoter enrichment
HCT16
Primers #1
Primers #2

Figure 4

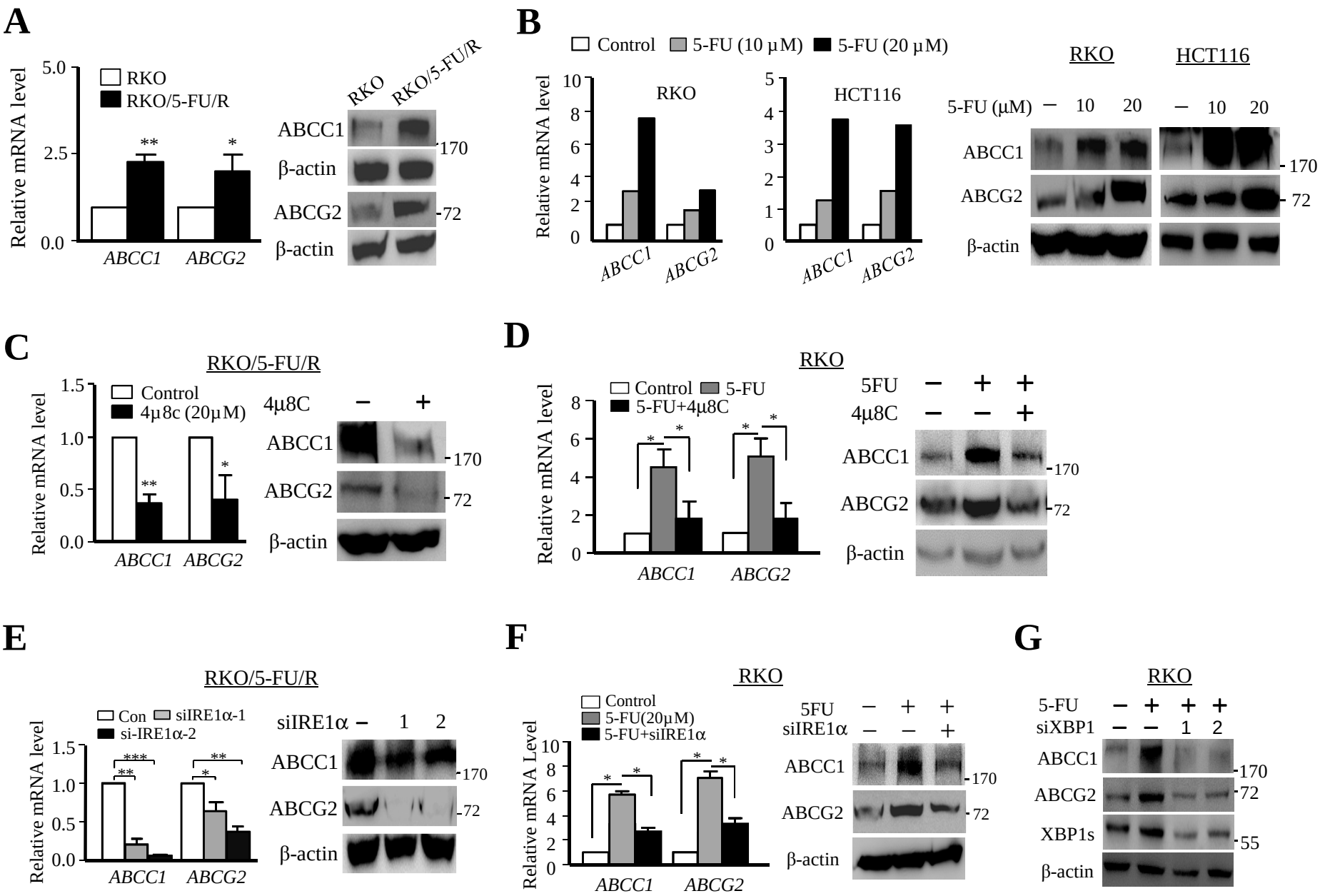

A
Relative mRNA level
RKO
RKO/5-FU/R
ABCC1
ABCG2
β-actin
B
Control
5-FU (10 μM)
5-FU (20 μM)
RKO
HCT116
5-FU (μM)
C
RKO/5-FU/R
Control
4μ8c (20μM)
4μ8C
D
RKO
Control
5-FU
5-FU+4μ8C
5FU
E
RKO/5-FU/R
Con
siIRE1α-1
si-IRE1α-2
siIRE1α
F
RKO
Control
5-FU(20μM)
5-FU+siIRE1α
G
RKO
5-FU
siXBP1
XBP1s
170
72
55

Figure 5

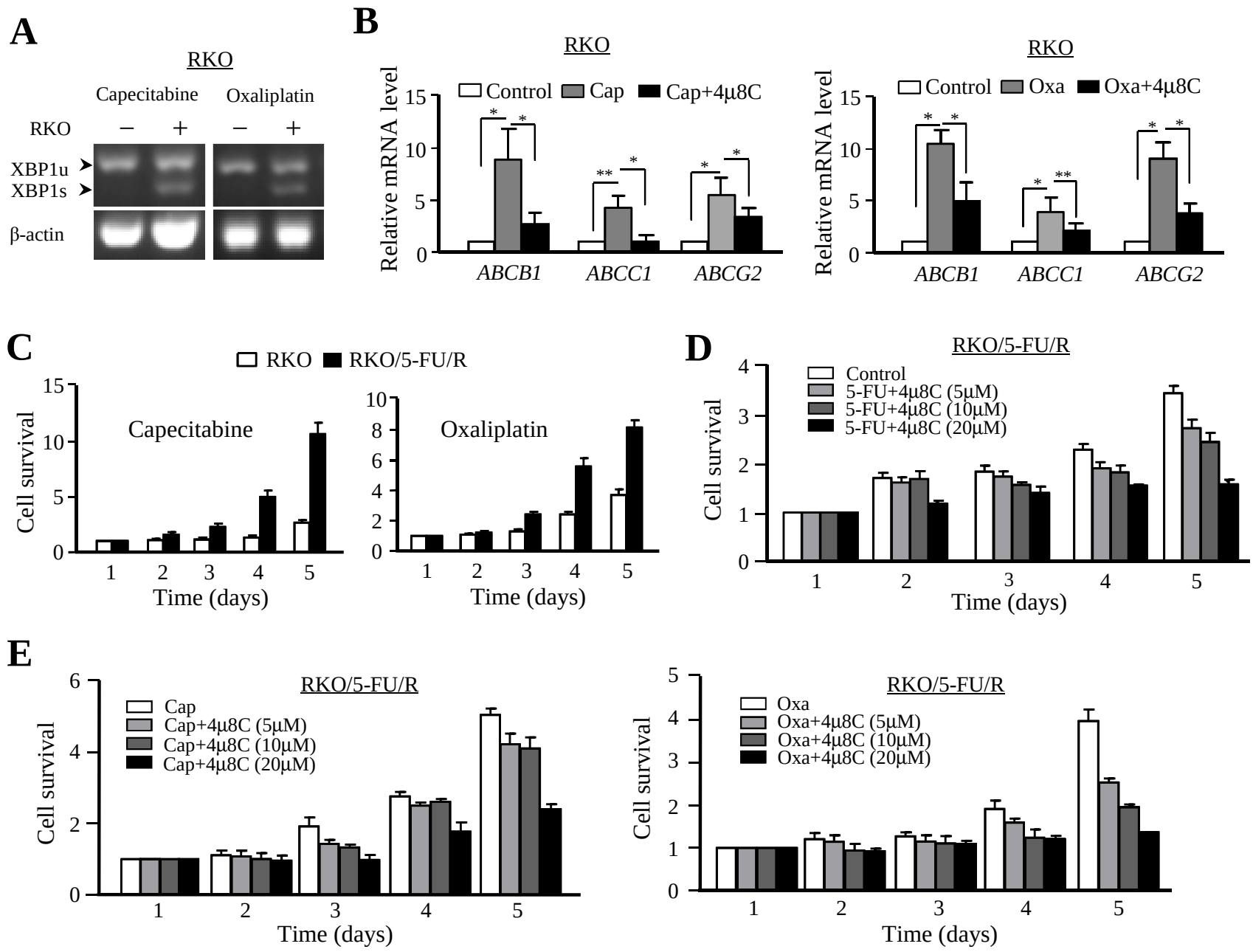
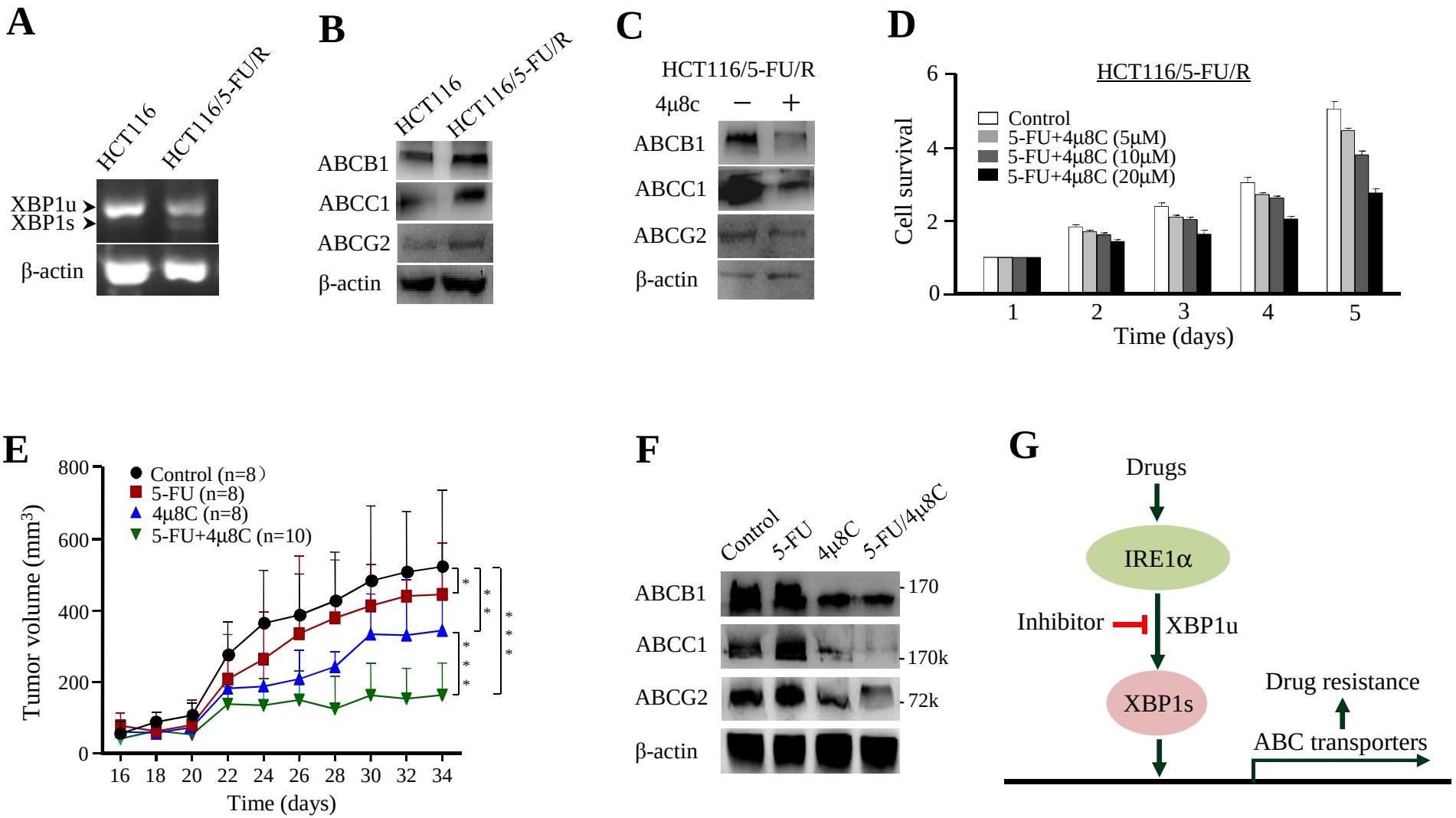

A
RKO
Capecitabine
Oxaliplatin
RKO
−
+
−
+
XBP1u
XBP1s
β-actin
B
RKO
Relative mRNA level
Control
Cap
Cap+4μ8C
ABCB1
ABCC1
ABCG2
RKO
Control
Oxa
Oxa+4μ8C
C
RKO
RKO/5-FU/R
Capecitabine
Oxaliplatin
Cell survival
Time (days)
D
RKO/5-FU/R
Control
5-FU+4μ8C (5μM)
5-FU+4μ8C (10μM)
5-FU+4μ8C (20μM)
Cell survival
Time (days)
E
RKO/5-FU/R
Cap
Cap+4μ8C (5μM)
Cap+4μ8C (10μM)
Cap+4μ8C (20μM)
Cell survival
Time (days)
RKO/5-FU/R
Oxa
Oxa+4μ8C (5μM)
Oxa+4μ8C (10μM)
Oxa+4μ8C (20μM)
Cell survival
Time (days)

Figure 6


A
HCT116
HCT116/5-FU/R
XBP1u
XBP1s
β-actin
B
HCT116
HCT116/5-FU/R
ABCB1
ABCC1
ABCG2
β-actin
C
HCT116/5-FU/R
4μ8c − +
ABCB1
ABCC1
ABCG2
β-actin
D
HCT116/5-FU/R
Control
5-FU+4μ8C (5μM)
5-FU+4μ8C (10μM)
5-FU+4μ8C (20μM)
Cell survival
Time (days)
E
Control (n=8）
5-FU (n=8)
4μ8C (n=8)
5-FU+4μ8C (n=10)
Tumor volume (mm³)
Time (days)
F
Control
5-FU
4μ8C
5-FU/4μ8C
ABCB1
ABCC1
ABCG2
β-actin
170
170k
72k
G
Drugs
IRE1α
Inhibitor
XBP1u
XBP1s
Drug resistance
ABC transporters

Highlights

1. The anticancer drugs activate IRE1α-XBP1 axis and induce ABCB1, ABCC1 and ABCG2
2. XBP1 acts as a transcription factor of drug resistance gene *ABCB1*, *ABCC1* and *ABCG2*
3. IRE1α inhibitor 4μ8C suppresses the expression of ABCB1, ABCC1 and ABCG2
4. Targeting of IRE1α with 4μ8C overcomes drug resistance of colon cancer cells

Statement

The authors declare that there is no conflict of interest.