Cancer Chemotherapy and Pharmacology (2019) 84:1279–1288
https://doi.org/10.1007/s00280-019-03958-4

ORIGINAL ARTICLE

BATF2 inhibits chemotherapy resistance by suppressing AP-1 in vincristine-resistant gastric cancer cells

Wei Yang[1] · Sihui Zhao[1] · Bian Wu[1] · Jianbiao Xu[1] · Zaozao Wu[1] · Jianhui Guo[1] · Rong Zeng[2]

Received: 23 May 2019 / Accepted: 4 September 2019 / Published online: 23 September 2019

Abstract

Purpose Chemotherapy remains the primary treatment used to improve overall survival and quality of life for patients with gastric cancer (GC); however, multidrug resistance is a major reason underlying failure of chemotherapy. Drug resistance (DR) can arise because of molecular changes inhibiting drug–target interactions; for example, overexpression of drug efflux pumps, such as P-gp, mediated by the activation of AP-1. BATF2 is a suppressor of AP-1; therefore, this study aimed to determine how *BATF2* interacts with AP-1to inhibit DR in GC cells.

Methods Expression of BATF2 in drug-responsive and non-responsive GC tumor tissues was evaluated by quantitative PCR and western blotting. Further, expression levels of BATF2- and AP-1-related genes were confirmed in vincristine-resistant SGC7901/VCR cells treated with cisplatin or 5-fluorouracil. A BATF2 overexpression system was established in SGC7901/VCR cells, and then AP-1 also overexpressed in the cells with upregulated BATF2 levels. Further, an AP-1 knockdown system was generated in SGC7901/VCR cells. MTT and flow cytometry assays were performed in the BATF2/AP-1 overexpression system, to evaluate cell proliferation, cell cycle effects, and apoptosis, and the expression of various proteins was detected by western blotting in AP-1/BATF2 overexpression cells. Finally, the effects of BATF2 overexpression in an in vivo nude mouse GC model were evaluated.

Results We found that BATF2 was overexpressed in tissues from patients with non-responsive GC and the VCR resistance cell line, SGC7901/VCR, while levels of c-Fos and c-Jun were reduced in the SGC7901/VCR cell line. BATF2 overexpression suppressed levels of AP-1 and P-gp. Further, our data demonstrate that cell proliferation is suppressed, and the cell cycle and apoptosis are induced in SGC7901/VCR cells overexpressing both AP-1 and BATF2. Overexpression of AP-1 restored levels of genes downstream of AP-1 in BATF2 overexpressing cells. Compared with controls, tumor growth of SGC7901/VCR cells in nude mice was suppressed in the BATF2 overexpression group.

Conclusion AP-1 down-regulation by BATF2 overexpression or AP-1 knockdown can inhibit DR in GC cells. These findings suggest that BATF2 inhibits DR in SGC7901/VCR GC cells by down-regulating AP-1 expression.

Keywords BATF2 · Inhibit · Overexpression · AP-1 · Knockdown · Drug resistance

Introduction

The incidence and mortality rates from gastric cancer (GC) are significantly higher in China than those in other countries [1]. Chemotherapy, a main treatment measure for patients with cancer, can effectively prevent metastasis and tumor recurrence after surgery [2]; however, tumors may recur, or even progress, after long-term use of chemotherapy drugs in patients with GC. To optimize treatment for patients with GC, overcoming multidrug resistance (MDR) to chemotherapy drugs is an urgent problem [3].

Chemotherapeutic drug resistance (DR) involves complex mechanisms. The overexpression of P-glycoprotein (P-gp),

✉ Rong Zeng
huand99@126.com

[1] Second Department of General Surgery, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunming 650032, Yunnan, China

[2] Department of Medical Oncology, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, No. 157 Jinbi Road, Kunming 650032, Yunnan, China

a membrane-localized drug efflux protein pump, encoded by the human multidrug resistance 1 (*MDR1*) gene, causes MDR; P-gp is expressed at extremely high levels on hyperactivation of the NFκB, AP-1, and Nrf2 transcription factors [4]. Anti-tumor agents, such as doxorubicin and paclitaxel, are absorbed intracellularly to exert their anti-cancer effects. When P-gp is overexpressed, the concentration of anti-cancer drugs in the cell is lowered, resulting in DR [5]. Jun, Fos, and activating transcription factor (ATF) subunits recognize the same common DNA-binding site, referred to as the activator protein-1 (AP-1) binding site, and are all dimeric AP-1 transcription factors. AP-1 regulates important processes, such as cell proliferation, differentiation, apoptosis, and inflammation [6].

BATF2, also known as MDA-D-74, activating transcription factor-like 2 (ATF-like 2), or SARI, has a basic leucine zipper structure, similar to those of BATF and BATF3, and acts as an inhibitor of AP-1. Su et al. reported that *BATF2* was stably expressed at the mRNA level in a variety of normal cells, while its expression was dysregulated in tumor cells. Further, overexpression of BATF2 suppresses cell proliferation and stimulates apoptosis in cancer cells, but not in normal cells [7].

Based on these data, in this study we evaluated the relationship between BATF2 and AP-1 in SGC7901/VCR GC cells, including the function of AP-1, and how BATF2 inhibits chemotherapy resistance.

Materials and methods

Patients and clinical tissue specimens

In total, 67 tumor tissues were collected from patients with GC after surgery at the First People's Hospital of Yunnan Province from 2015 to 2017. Each patient signed a written notice of informed consent. Among collected samples, 31 tumor tissues were sensitive to chemotherapeutic drugs, while the remaining were non-sensitive. Samples were preserved at − 80 °C until use.

Cells culture and animals

The human GC cell line, SGC7901, and its VCR-resistant form, SGC7901/VCR, were purchased from Shanghai Institutes for Biological Sciences. Cells were cultured in RPMI-1640 (Gibco, Grand Island, NY, USA) medium with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), and kept in a constant humidity chamber at 37 °C under 5% CO_2.

Female BALB/c nude mice (5–6 weeks old) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China) and housed in a specific pathogen-free environment. According to international recognized guidelines on Animal Welfare, the experimental protocol was carried out under the supervision of the Ethics Committee of the First People's Hospital of Yunnan Province.

Drugs and reagents

Cisplatin (DDP) and 5-Fluorouracil (5-Fu) were purchased from QILU PHARMA (Jinan, Shandong, China). Vincristine sulfate (VCR) was purchased from Sigma Aldrich (#V0400000). Dilutions of all reagents were freshly prepared before each experiment. An Annexin V-FITC flow cytometry detection kit was purchased from Nanjing Key GEN Biotech. Co., Ltd. (Nanjing, Jiangsu, China). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco (Gibco, Grand Island, NY, USA).

Transient transfection of BATF2 and AP-1

The plasmids, pcDNA3.1-BATF2 and pcDNA3.1-AP-1, were purchased from Obio Technology (Shanghai, China). Before transfection, SGC7901/VCR seed cell culture media were replaced with RPMI-1640 supplemented with 10% FBS, without antibiotics. Plasmids and Lipofectamine™ 2000 were separately diluted in RPMI-1640 without FBS. The treated plasmid and transfection reagent were mixed together, and the mixture was added into the seeded cells. Transfected cells were collected at 24, 48, and 72 h post-transfection and used to determine cell viability and the expression of related genes at the mRNA and protein levels.

Transfection with AP-1 siRNAs

Cells, transfected with pcDNA3.1-BATF2-SGC7901/VCR and pcDNA3.1-SGC7901/VCR, were plated in 6-well plates and, after culture for 24 h, cells were transfected with siRNAs targeting AP-1 using Lipofectamine RNAi MAX Reagent, based on the manufacturer's protocol. Silencing was evaluated by western blotting 24 h after transfection. siRNAs were synthesized by Genepharma (Shanghai, China).

Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was extracted using Trizol (Invitrogen) and genomic DNA residues were removed from RNA using Rnase-free DNaseI (Promega). After the purity and integrity of RNA were evaluated, RNA was reverse transcribed into cDNA (Takara, D6110A). Then, using the resulting cDNA as the template, qRT-PCR was performed using Power SYBR green PCR master mix (Applied Biosystems) on an ABI 7500 series PCR machine (Applied Bio systems). Cycle threshold (CT) values were normalized to those generated by evaluation of *GAPDH* expression. Primers for amplification

of *BATF2*, *AP-1*, *MDR1*, *c-Fos*, *c-Jun*, *P-gp*, and *NADPH* were synthesized by Generay (Shanghai, China).

Western blot assay

Protein samples were extracted from tissues or cells and their concentrations were quantified using the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). SDS-PAGE (10%) was used to separate the proteins, which were then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). Subsequently, the samples were blocked for 2 h with 5% skim milk in Tris-buffered saline (TBS) at room temperature (RT). Blots were then incubated overnight at 4 °C with primary antibodies against BATF2 (Abcam, ab157466), c-Fos (Abcam, ab134122), c-Jun (Abcam, ab32137), P-gp (Abcam,ab129450), CDK4 (Abcam, ab199728), Cyclin D1 (Abcam, ab16663), Caspase3 (Abcam, ab32351), and NADPH (Santa Cruz Biotechnology, sc-48388), washed, and probed again with the appropriate horseradish-peroxidase-conjugated secondary antibody (goat anti-rabbit, sc-2004; Santa Cruz Biotechnology) diluted 1:5000. Protein expression levels were detected using an enhanced chemiluminescence reagent (ECL buffer; Beyotime Biotechnology, Haimen, China) by X-ray film.

Analysis of cell viability

Cell viability was measured using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Then, 1×10^4 SGC7901/VCR or SGC7901 cells were added into each well of 96-well plates (Corning Incorporated, Corning, NY, USA) and cultured in a fully humidified incubator at 37 °C under an atmosphere enriched with 5% CO_2. After 24 h, cells were treated with DDP at a range of concentrations from 0.25 to 6 μg/mL, as well as 5-Fu at concentrations from 1 to 12 μg/mL, in RPMI-1640 medium containing 10% FBS. MTT (50 μL, 5 mg/mL; Sigma Aldrich) was added into each well 72 h later and incubated for 4 h. Then, supernatants were gently removed and 150 μL dimethylsulfoxide (Sigma Aldrich) added to each well, followed by oscillation at low speed for 10 min. Absorbance at 490 nm was measured using a spectrophotometer (MPR-2100, Syntron, Carlsbad, CA, USA). Cell viability was determined by MTT reduction. The 50% inhibitory concentration of drugs (IC50) was defined as the drug concentration that caused a 50% reduction in the number of cells [5].

Measurement of apoptosis and the cell cycle

Adherent cells were detached using 0.25% trypsin and trypsin removed from the supernatant by centrifugation. For analysis of apoptosis, cells were washed twice with cold PBS and collected by centrifugation (2000×*g*) for 5 min. Then, cells were re-suspended in 1 × binding buffer and incubated with Annexin V-phycoerythrin at room temperature for 15 min in the dark. Immediately before analysis by flow cytometry, 7-amino-actinomycin was added to each sample, and apoptotic cells detected using a CytoFLEX flow cytometer (Beckman Coulter, Inc., Brea, CA, USA).

For cell cycle analysis, cells were fixed in cold 70% methanol in PBS for 1 h after they were detached. Then, cells were washed once with PBS and re-suspended in 1 mg/mL Propidium Iodide (Sigma) and 0.5 mg/mL RNaseA (Thermo Scientific) in PBS for 30 min at 37 °C. Approximately, 1.5×10^4 cells were analyzed using the FL3-A channel on a CyFlow® flow cytometer (Beckman Coulter, Inc.). Apoptosis rates were calculated as the percentage of the sub-G1 peak in the cell proliferation process.

Tumor xenograft models

Female BALB/c nude mice ($n=8$; 5–6 weeks old) were divided into two groups (4 mice/group) and injected subcutaneously into the left flank, with either pcDNA3.1/BATF2-SGC7901/VCR or pcDNA3.1-SGC7901/VCR (5×10^6 cells in 200 μL PBS). Tumor growth was assessed by measuring the maximum tumor diameter every three days and calculating the tumor volume (V) using the formula $V=(4\pi/3)\times(D/2)^3$, where D is the diameter of the tumor. Several weeks after tumor inoculation, all mice were killed and tumor weights determined.

Statistical analysis

All data were analyzed using SPSS 17.0 software (SPSS, Chicago, IL, USA) and were expressed as mean ± SD. Differences between groups were analyzed using the Student's t test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of BATF2 is down-regulated in tissue samples from non-drug-responsive GC tumors

BATF2 expression was determined at the mRNA and protein levels in tissue samples from patients with chemotherapy-responsive and non-responsive GC tumors. Analysis by qRT-PCR and western blotting demonstrated that BATF2 expression at the mRNA (Fig. 1a) and protein (Fig. 1b) levels were significantly lower in non-responding than in responding tissues.

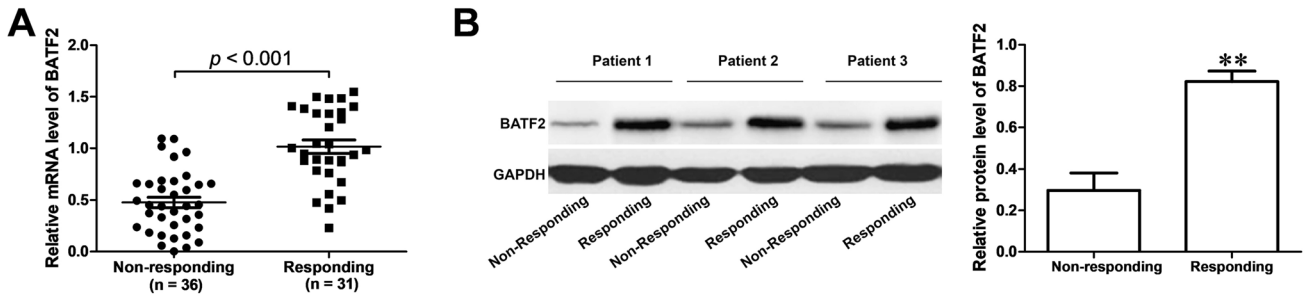

Fig. 1 Expression of BATF2 was downregulated in tumor samples from patients with non-responding GC. **a** *BATF2* mRNA expression levels in tumor samples from patients with non-responding and responding GC were evaluated by qRT-PCR; **b** Western blotting analysis of BATF2 in tumor samples from patients with non-responding and responding GC and quantification of relative BATF2 protein levels

The IC50 values for DDP and 5-Fu were increased in SGC7901/VCR compared with SGC7901 cells

MTT assays were performed to evaluate the sensitivity of the SGC7901 and SGC7901/VCR cell lines to anti-cancer drugs, and curves illustrating cell viability according to drug concentration drawn (Fig. 2a). As shown in Fig. 2b, the IC50 values for DDP and 5-Fu in SGC7901/VCR cells were markedly higher than those in SGC7901 cells.

Expression of BATF2 is down-regulated, while that of AP-1-related genes is up-regulated, in SGC7901/VCR GC cells

To explore the role of BATF2 in GC DR, qRT-PCR and western blotting were used to assess BATF2 expression in SGC7901 and SGC7901/VCR cells. Compared with SGC7901 cells, levels of BATF2 were significantly lower in SGC7901/VCR cells (Fig. 2c). Furthermore, the expression of AP-1-related genes, including c-Fos, c-Jun, and P-gp, were higher at both the mRNA and protein levels in SGC7901/VCR cells compared with those in SGC7901 cells (Fig. 2d). These results demonstrated that there is a negative correlation between the expression of BATF2- and AP-1-related genes in GC cells.

Overexpression of BATF2 inhibits cell proliferation, induces cell cycle arrest at the G_0/G_1 phase, and stimulates apoptosis in SGC7901/VCR GC cells

SGC7901/VCR cells were transfected with pcDNA3.1-BATF2 or control (pcDNA3.1) plasmids. Twenty-four hours after transfection, cells were observed by fluorescence microscopy to confirm successful transfection and calculate transfection efficiency, which was 80% (Fig. 3a). Western blotting showed that BATF2 protein was strikingly increased in BATF2-overexpressing cells compared with controls (Fig. 3b), indicating that the BATF2 overexpression system was successfully established. Further, we found that overexpression of BATF2 led to a significant decrease in the viability of SGC7901/VCR cells (Fig. 3c, $P < 0.05$ or $P < 0.01$) compared with controls. Moreover, expression levels of CDK4 and cyclin D1 were lower in the BATF2-overexpressing group than that in the vector control group (Fig. 3d) and increased BATF2 levels promoted apoptosis by increasing levels of cleaved caspase-3 protein (Fig. 3d).

To further explore the effects of BATF2 up-regulation, we performed flow cytometry and western blotting to investigate the distribution in specific phases of the cell cycle of SGC7901/VCR cells overexpressing BATF2. More BATF2 overexpressing cells were in the G_0 phase and they were strikingly arrested at the G_1 phase of the cell cycle, compared with control cells (Fig. 3e). The data presented in Fig. 3f demonstrate that BATF2 overexpression significantly induced early apoptosis of SGC7901/VCR cells.

Overexpression of BATF2 inhibits expression of the c-Fos, c-Jun, MDR1, and AP-1 genes

The data presented in Fig. 4a show that mRNA levels of *c-Fos*, *c-Jun*, *MDR1*, and *AP-1* were clearly reduced in cells overexpressing BATF2. Further, levels of AP-1 related proteins in cells overexpressing both AP-1 and BATF2 were strikingly higher in the AP-1 transfected group, compared with controls (Fig. 4b and c).

AP-1 knockdown regulates cell proliferation, the cell cycle, and apoptosis

The c-Fos and c-Jun protein levels were significantly lower in cells treated with siRNA targeting AP-1 (siAP-1 group) than those in the negative control siRNA (siNC) group (Fig. 5a). MTT assays revealed that cell viability was reduced significantly at 24, 48, and 72 h after transfection compared with controls (Fig. 5b). Further siAP-1 cells were suppressed at the G_0 and strikingly arrested at the G_1 phase

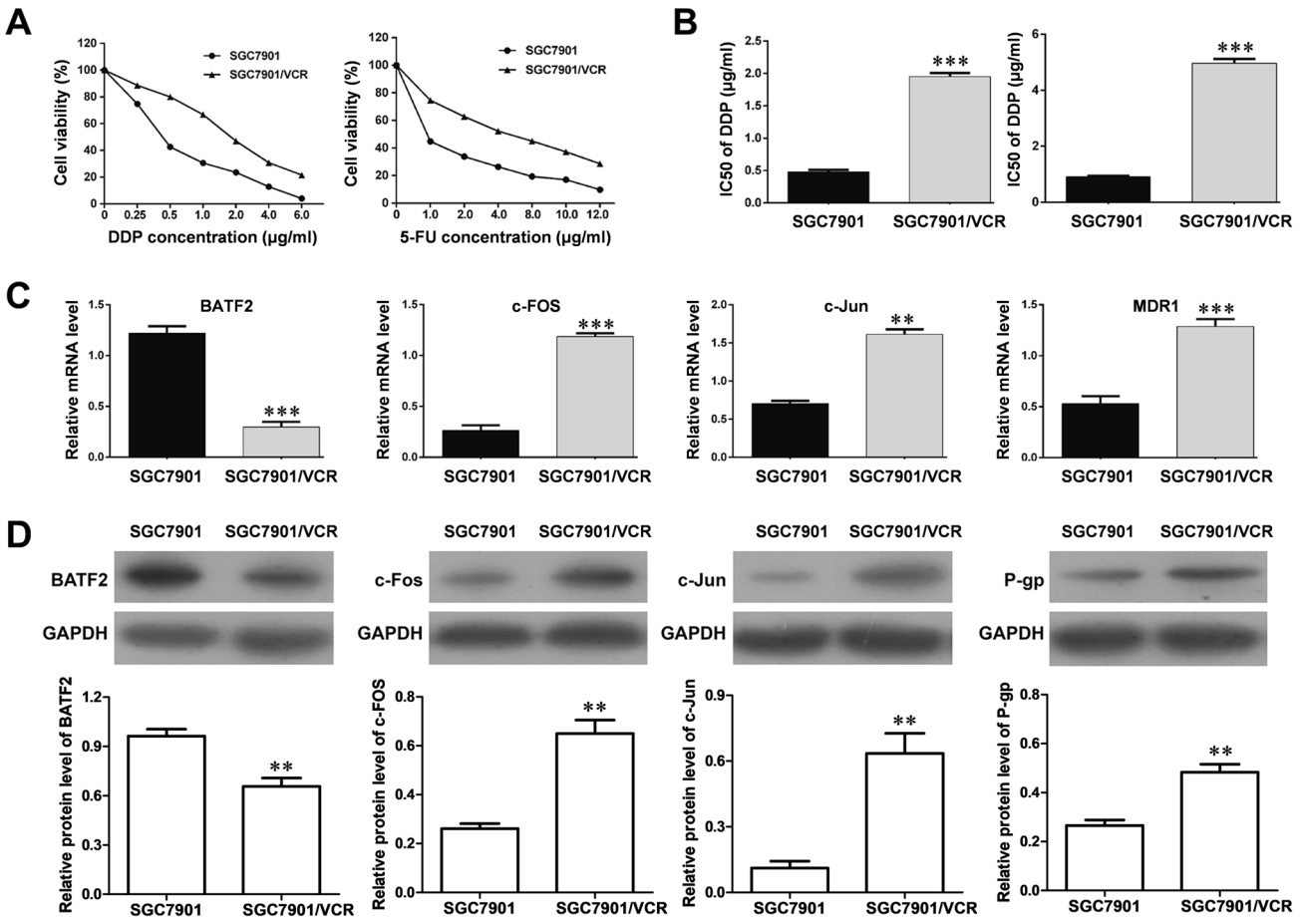

Fig. 2 Changes in IC50 for DDP and 5-Fu and expression of BATF2- and AP-1-related genes in SGC7901/VCR cells. **a** Anticancer drug dose–effect curves; **b** IC50 values; **c** Expression of *BATF2*- and AP-1-related genes at the mRNA level in SGC7901 and SGC7901/VCR cells determined by quantitative PCR; **d** Expression of BATF2- and AP-1-related genes at the protein level in SGC7901 and SGC7901/VCR cells determined by western blotting and quantification of relative protein levels (*$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. SGC7901 cells)

of the cell cycle compared with siNC cells (Fig. 5c). Similarly, flow cytometry analysis demonstrated that more GC cells were induced in both early and late apoptosis in the group transfected with siAP-1, with an overall significant increase in apoptotic cells compared with controls (Fig. 5d).

Overexpression of BATF2 inhibits GC in vivo

Next, we investigated whether BATF2 overexpression could arrest tumor xenograft growth in nude mice injected with BATF2-SGC7901/VCR or pcDNA3.1-SGC7901/VCR cells. Tumors in mice transfected with BATF2-SGC7901/VCR cells were much smaller than those transfected with pcDNA3.1-SGC7901/VCR (Fig. 6a). For the first few days after the cells were injected, there was no difference in tumor volume between the BATF2 overexpression and control groups; however, on the 27th day post-injection, the tumor volume in the BATF2 group was significantly less than that in the vector control group (Fig. 6b). In addition, tumor weight in the BATF2-SGC7901/VCR group was significantly lower than that in the pcDNA3.1-SGC7901/VCR group (Fig. 6c).

Discussion

Tumors exhibit various mechanisms of DR, including reducing the intake of water-soluble anti-cancer drugs and increasing the outflow of hydrophobic anti-tumor agents; the latter is a major cause of resistance to chemotherapy drugs. An intricate molecular network controls the sensitivity of tumor cells to chemotherapeutic agents, including diverse genetic changes that can result in drug-resistant cell lines. The vincristine-resistant cell line, SGC7901/VCR, which is derived from the human GC cell line, SGC7901, by in vitro stepwise selection using vincristine, also exhibits

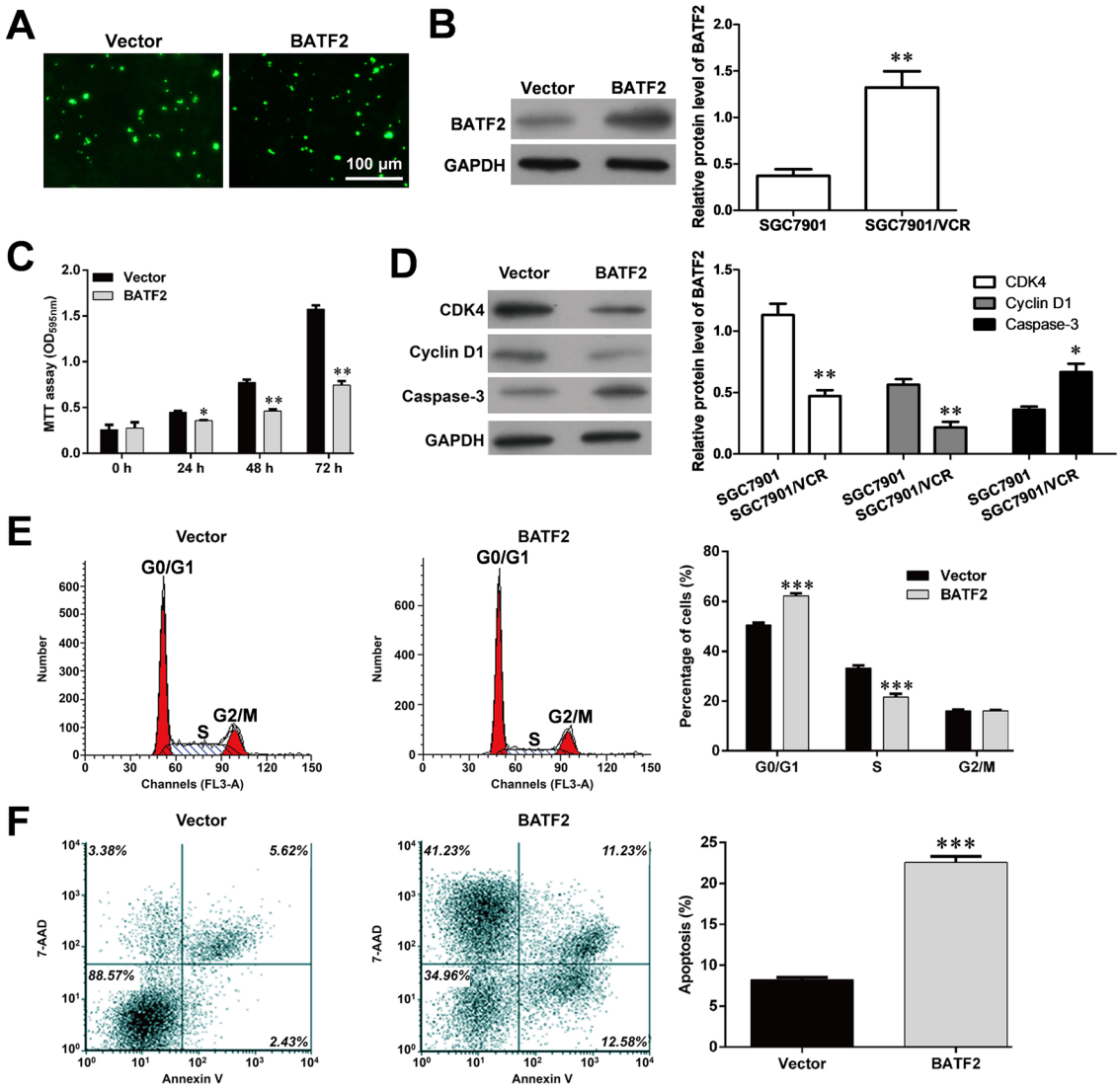

Fig. 3 Overexpression of BATF2 inhibits cell proliferation, and induces cell cycle arrest at G_0/G_1 phase and apoptosis in SGC7901/VCR gastric cancer cells. GC7901/VCR cells were transfected with pcDNA3.1-BATF2 and control pcDNA3.1. **a** GC7901/VCR cells transfected with pcDNA3.1-BATF2 or pcDNA3.1 were observed by fluorescence microscopy at 24 h post-transfection; **b** Western blotting analysis to determine whether the over-expression BATF2 system was successful; **c** Cell viability determined by MTT assay; **d** Expression of cell cycle regulators and AP-1-related proteins evaluated by western blotting and protein quantification; **e** Flow cytometry to evaluate the cell cycle; **f** Flow cytometry to detect apoptosis (*$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. vector)

cross-resistance to other anticancer drugs, including cisplatin, etoposide, mitomycin C, and 5-fluorouracil (5-FU). Therefore, the SGC7901/VCR cell line was selected for use in this study [8, 9].

A previous study [10] showed that excessive production of P-glycoprotein (P-gp) is one mechanism underlying MDR. P-gp is encoded by the *MDR1* gene and functions as a protein efflux pump, which in drug metabolism acts to pump substrates out of cancer cells in an ATP-dependent manner, conducive to reducing intracellular drug accumulation, resulting in resistance to multiple drugs [11]. Recent studies [12–14] have shown that *MDR1* transcription is inhibited by NF-κB, which can be targeted as a measure to reverse DR. AP-1 is a factor upstream of *MDR1* and has an important role in stimulating full MDR1 promoter activity. The AP-1 transcription factor complex comprises c-Jun and c-Fos and is related to cell proliferation, apoptosis, and tumor development and progression [11]. Therefore, we established an AP-1 overexpression system that resulted in excessive expression of P-gp, c-Jun, and c-Fos (Fig. 4b). In addition, a siRNA targeting AP-1 was used to repress AP-1 expression, resulting in markedly decreased expression of c-Jun and c-Fos, as well as AP-1 (Fig. 5a). Further, cell proliferation (Fig. 5b) and cell cycle progression (Fig. 5c) were also

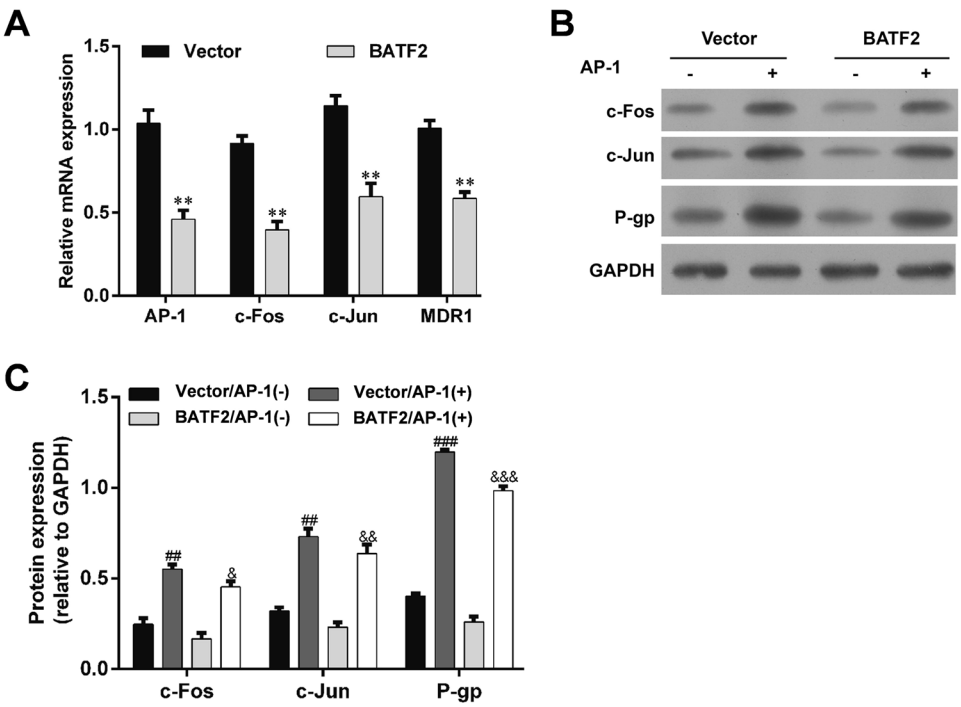

Fig. 4 The AP-1 overexpression system promoted *c-Fos*, *c-Jun*, *MDR1*, and *AP-1* gene expression. pcDNA 3.1-BATF2-SGC7901/VCR and DNA3.1 cells were transfected with pcDNA3.1-AP-1. **a** Detection of the AP-1-related proteins, AP-1, c-Fos, c-Jun, and MDR1, in SGC7901/VCR cells overexpressing BATF2 without AP-1 overexpression. **b** Twenty-four hours after AP-1 transfection, expression of proteins downstream of AP-1 were analyzed by western blotting; **c** Quantification of proteins downstream of AP-1 (**$P<0.01$ vs. vector; $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. vector + NC; $^{\&}P<0.05$, $^{\&\&}P<0.01$, $^{\&\&\&}P<0.001$ vs. vector + AP-1)

inhibited, and apoptotic cells (Fig. 5d) markedly increased in SGC7901/VCR GC cells with AP-1 knocked down. These results suggested that P-gp, encoded by *MDR1*, is positively regulated by the expression of AP-1, and that AP-1 influences cell proliferation, the cell cycle, and apoptosis in SGC7901 GC cells, consistent with the results published by Zhao et al. [11].

The gene encoding BATF2 maps to chromosome 11q12–11q13 and has three coding exons, encoding a putative 274 amino acid residue protein [15]. There is good evidence that BATF2 is a tumor suppressor with roles in numerous types of cancer, including lung adenocarcinoma [16], colorectal cancer [17, 18], and esophageal squamous cell carcinoma [19], among others. Initially, we found that expression of BATF2 was significantly lower in non-responding GC tumor tissue samples than in those from responding clinical cases (Fig. 1). To explore the expression of BATF2 in drug-resistant cells, drug sensitivity assays were conducted in SGC7901/VCR GC cells. The results showed that BATF2 levels were lower in SGC7901/VCR cells than in SGC7901 cells, while the expression levels of c-Fos, c-Jun, and P-gp were higher in SGC7901/VCR than SGC7901 cells (Fig. 2). Su et al. reported that BATF2 interacts with c-Jun in the nucleus and reduces AP-1 complex DNA-binding activity, thereby inhibiting AP-1-related gene transcription [7], consistent with our findings.

Furthermore, a BATF2 overexpression system in SGC7901 cells was constructed in this study, to investigate how BATF2 suppresses AP-1, resulting in clear inhibition of cell proliferation and cell cycle progression, while apoptosis was remarkably enhanced (Fig. 3), consistent with the results of AP-1 interference, and resulting in reversal of anti-cancer DR. Wen et al. reported that overexpression of BATF2 could suppress tumor growth and cause apoptosis in malignant glioma, melanoma, and prostate cancer cell lines, in accordance with our findings [20]. Moreover, in vivo experiments using BATF2-overexpressing GC cells were performed in nude mice, demonstrating that BATF2 overexpression significantly reduced tumor volume and weight compared with controls (Fig. 6). These results indicate that BATF2 suppresses GC DR by inhibiting AP-1 expression.

Overall, we found that BATF2 overexpression leads to reduced expression of P-gp, c-Jun, and c-Fos, inhibits cell proliferation, cell cycle progression, and tumor growth, while enhancing apoptosis both in vitro and in vivo. Furthermore, knockdown of AP-1 caused down-regulation of c-Jun and c-Fos, and suppressed cell proliferation and cell cycle progression, while enhancing apoptosis in vitro. It is established that overexpression of P-gp leads to resistance to anti-cancer agents. Hyperactivation of AP-1 causes P-gp overexpression and BATF2 may reverse anti-cancer DR by suppressing AP-1 expression. Hence, both BATF2 and AP-1 are of great potential value for development as anti-cancer drug targets.

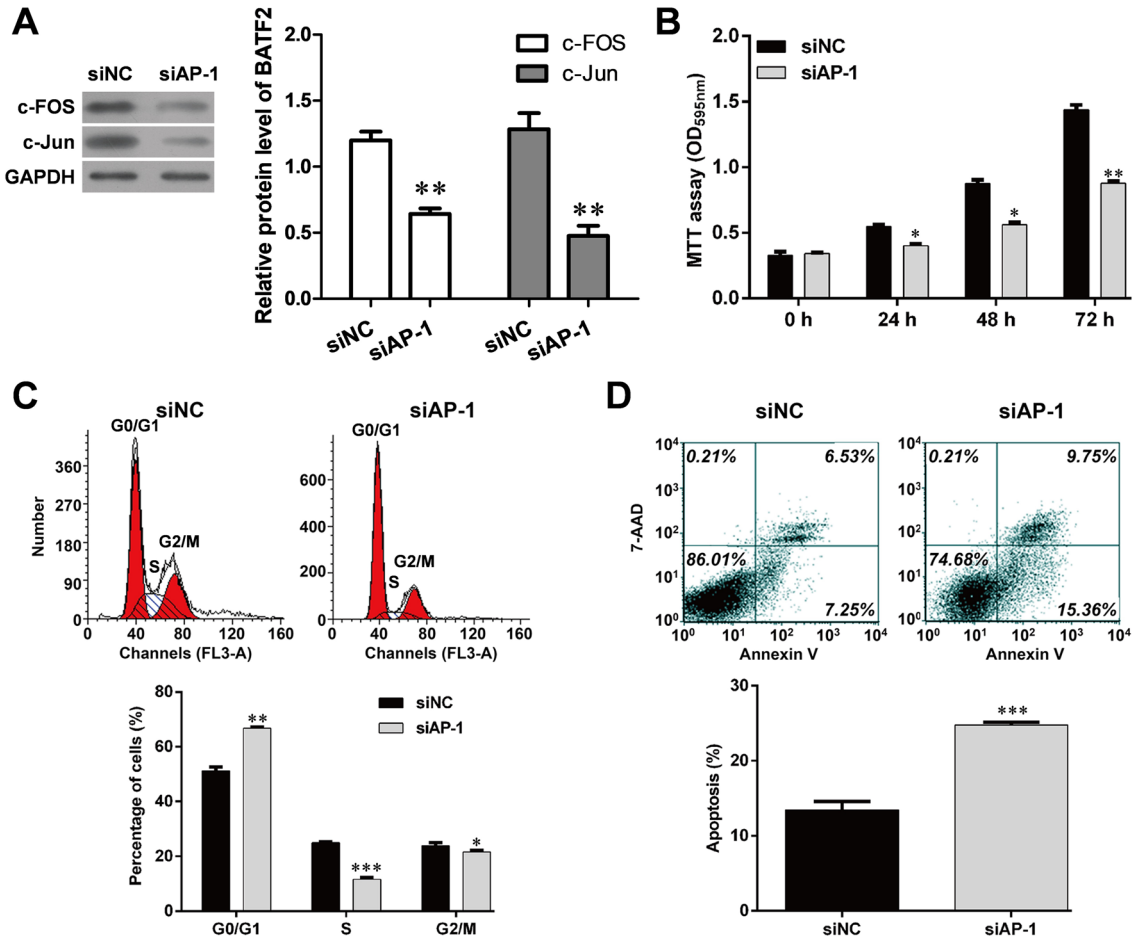

Fig. 5 Effects of AP-1 knockdown on cell proliferation, the cell cycle, and apoptosis. **a** Western blotting was conducted to analyze AP-1 knockdown. **b** MTT assay was used to evaluate cell proliferation after the cells were treated with siAP-1 **c**–**d** Flow cytometry was used to assess cell cycle progression and apoptosis after AP-1 knockdown (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. siNC)

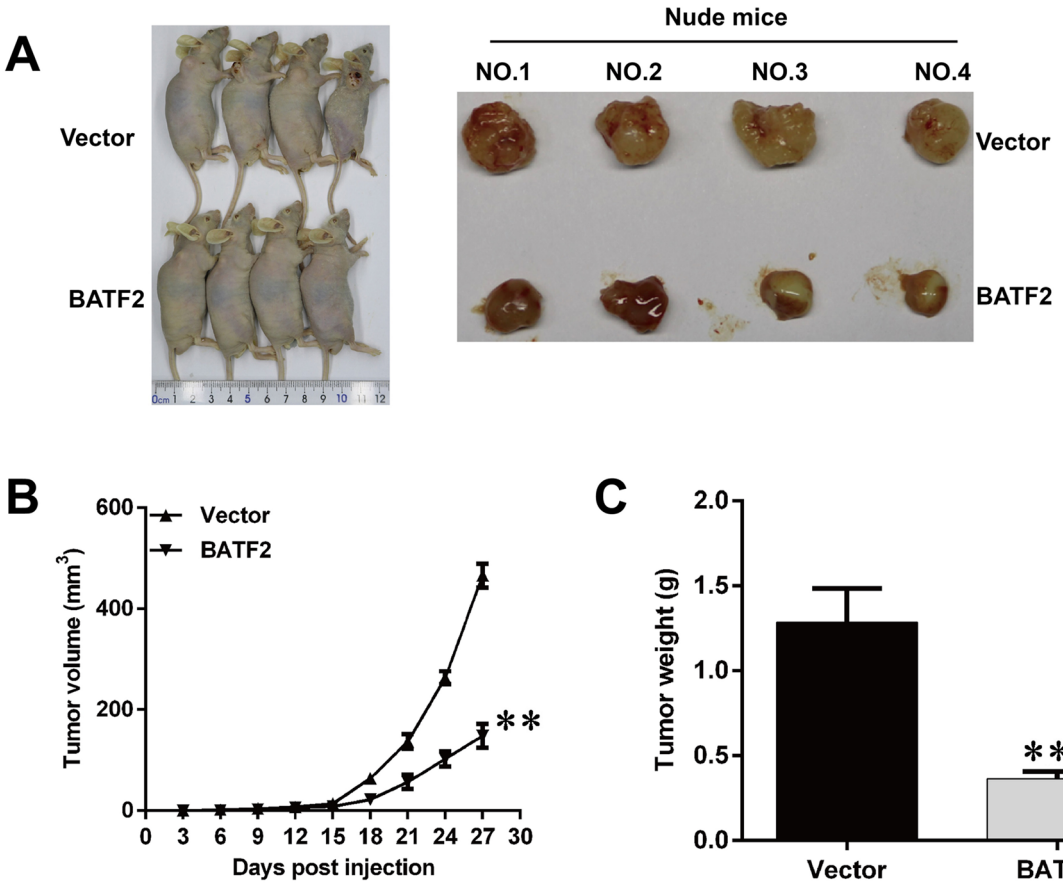

Fig. 6 Overexpression of BATF2 inhibited gastric cancer tumor growth in vivo. Tumor growth curve and weight: **a** Tumors were removed on the 27th day post-implantation; **b** Tumor volumes were measured every 3 days after BATF2-SGC7901/VCR and pcDNA3.1-SGC7901/VCR cells were injected; **c** Tumor weight in the BATF2 overexpression and empty vector groups (**$P<0.01$, ***$P<0.001$ vs. siNC)

Acknowledgements This study was supported by the applied Basic Research Joint Fund Project of Yunnan Provincial Science and Technology Department-Kunming Medical University (Grant No.: 2017FE468(-108); 2015FB099) and Scientific Research Fund Project of Yunnan Provincial Education Department (Grant No.: 2016ZDX020).

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

References

1. Li WB, Li Y, Yu C, He YM (2015) Reversal of multidrug resistance by the Chinese medicine Yiqi Jianpi Huaji Decoction and the mechanism of action in human gastric cancer SGC7901/VCR cells. Evid Based Complement Alternat Med 2015:390812. https://doi.org/10.1155/2015/390812
2. Sun F, Lu X, Li H, Peng Z, Wu K, Wang G, Tong Q (2012) Special AT-rich sequence binding protein 1 regulates the multidrug resistance and invasion of human gastric cancer cells. Oncol Lett 4(1):156–162. https://doi.org/10.3892/ol.2012.681
3. Yang X, Cai H, Liang Y, Chen L, Wang X, Si R, Qu K, Jiang Z, Ma B, Miao C, Li J, Wang B, Gao P (2015) Inhibition of c-Myc by let-7b mimic reverses mutidrug resistance in gastric cancer cells. Oncol Rep 33(4):1723–1730. https://doi.org/10.3892/or.2015.3757
4. Zhang H, Zhang XF, Wu XJ, Li WW, Su P, Cheng HX, Lei X, Gao P, Zhou GY (2012) Interference of Frizzled 1 (FZD1) reverses multidrug resistance in breast cancer cells through the Wnt/b-catenin pathway. Cancer Lett 323:106–113
5. Suttana W, Mankhetkorn S, Poompimon W, Palagani A, Zhokhov S, Gerlo S, Haegeman G, Berghe W (2010) Differential chemosensitization of P-glycoprotein overexpressing K562/Adr cells by withaferin A and Siamois polyphenols. Mol Cancer 9:99
6. Papoudou-Bai A, Hatzimichael E, Barbouti A, Kanavaros P (2017) Expression patterns of the activator protein-1 (AP-1) family members in lymphoid neoplasms. Clin Exp Med 17(3):291–304. https://doi.org/10.1007/s10238-016-0436-z
7. Su ZZ, Lee SG, Emdad L, Lebdeva IV, Gupta P, Valerie K, Sarkar D, Fisher PB (2008) Cloning and characterization of SARI (suppressor of AP-1, regulated by IFN). Proc Natl Acad Sci U S A 105(52):20906–20911. https://doi.org/10.1073/pnas.0807975106

8. Yang YX, Xiao ZQ, Chen ZC, Zhang GY, Yi H, Zhang PF, Li JL, Zhu G (2006) Proteome analysis of multidrug resistance in vincristine-resistant human gastric cancer cell line SGC7901/VCR. Proteomics 6(6):2009–2021. https://doi.org/10.1002/pmic.200402031
9. Chen Z, Zhang L, Xia L, Jin Y, Wu Q, Guo H, Shang X, Dou J, Wu K, Nie Y, Fan D (2014) Genomic analysis of drug resistant gastric cancer cell lines by combining mRNA and microRNA expression profiling. Cancer Lett 350(1–2):43–51. https://doi.org/10.1016/j.canlet.2014.04.010
10. Nobili S, Landini I, Mazzei T, Mini E (2012) Overcoming tumor multidrug resistance using drugs able to evade P-glycoprotein or to exploit its expression. Med Res Rev 32(6):1220–1262. https://doi.org/10.1002/med.20239
11. Zhao P, Wang S, Jiang J, Liu H, Zhu X, Zhao N, Li J, Yin Y, Pan X, Yang X, Guo J, Xu W (2018) TIPE2 sensitizes osteosarcoma cells to cis-platin by down-regulating MDR1 via the TAK1- NF-kappaB and - AP-1 pathways. Mol Immunol 101:471–478. https://doi.org/10.1016/j.molimm.2018.08.010
12. Huang J, Zhao M, Li X, Ma L, Zhang J, Shi J, Li B, Fan W, Zhou Y (2015) The cytotoxic effect of the benzene metabolite hydroquinone is mediated by the modulation of MDR1 expression via the NF-kappaB signaling pathway. Cell Physiol Biochem 37(2):592–602. https://doi.org/10.1159/000430379
13. Zhang R, Lu M, Zhang Z, Tian X, Wang S, Lv D (2016) Resveratrol reverses P-glycoprotein-mediated multidrug resistance of U2OS/ADR cells by suppressing the activation of the NF-kappaB and p38 MAPK signaling pathways. Oncol Lett 12(5):4147–4154. https://doi.org/10.3892/ol.2016.5136
14. Requenez-Contreras JL, Lopez-Castillejos ES, Hernandez-Flores R, Moreno-Eutimio MA, Granados-Riveron JT, Martinez-Ruiz GU, Aquino-Jarquin G (2017) MiR-138 indirectly regulates the MDR1 promoter by NF-kappaB/p65 silencing. Biochem Biophys Res Commun 484(3):648–655. https://doi.org/10.1016/j.bbrc.2017.01.168
15. Guler R, Roy S, Suzuki H, Brombacher F (2015) Targeting Batf2 for infectious diseases and cancer. Oncotarget 6(29):26575–26582. https://doi.org/10.18632/oncotarget.5576
16. Wang C, Su Y, Zhang L, Wang M, You J, Zhao X, Zhang Z, Liu J, Hao X (2012) The function of SARI in modulating epithelial-mesenchymal transition and lung adenocarcinoma metastasis. PLoS One 7(9):e38046. https://doi.org/10.1371/journal.pone.0038046
17. Dai L, Cui X, Zhang X, Cheng L, Liu Y, Yang Y, Fan P, Wang Q, Lin Y, Zhang J, Li C, Mao Y, Wang Q, Su X, Zhang S, Peng Y, Yang H, Hu X, Yang J, Huang M, Xiang R, Yu D, Zhou Z, Wei Y, Deng H (2016) SARI inhibits angiogenesis and tumour growth of human colon cancer through directly targeting ceruloplasmin. Nat Commun 7:11996
18. Liu Z, Wei P, Yang Y, Cui W, Cao B, Tan C, Yu B, Bi R, Xia K, Chen W, Wang Y, Zhang Y, Du X, Zhou X (2015) BATF2 deficiency promotes progression in human colorectal cancer via activation of HGF/MET signaling: a potential rationale for combining MET inhibitors with IFNs. Clin Cancer Res 21(7):1752–1763. https://doi.org/10.1158/1078-0432.CCR-14-1564
19. Han T, Wang Z, Yang Y, Shu T, Li W, Liu D, Li P, Qi R, Ren Y, Li L, Liu H, Zhang S, Zhang L (2015) The tumor-suppressive role of BATF2 in esophageal squamous cell carcinoma. Oncol Rep 34(3):1353–1360. https://doi.org/10.3892/or.2015.4090
20. Wen HJ, Chen YJ, Hu ZD, Mo Q, Tang JY, Sun CZ (2014) Decreased expression of BATF2 is significantly associated with poor prognosis in oral tongue squamous cell carcinoma. Oncol Rep 31(1):169–174