5-Fluorouracil enhances the chemosensitivity of gastric cancer to TRAIL via inhibition of the MAPK pathway

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Primary research

Abstract

Background

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has the ability to selectively trigger cancer cell apoptosis and can be used as a target for tumor therapy. However, gastric cancer cells are usually insensitive to TRAIL so reducing this drug resistance may improve the treatment of gastric cancer.

Methods

In this study, we used Cell Counting Kit-8 (CCK-8) and 5-ethynyl-2'-deoxyuridine (EdU) experiments to determine the effects of 5-fluorouracil (5-FU) and TRAIL on the proliferation of gastric cancer cells. An Annexin V/propidium iodide (PI) staining experiment was used to detect apoptosis, and Western blotting was used to analyze the expression levels of apoptosis-related proteins and mitogen-activated protein kinase (MAPK) pathway proteins. The antitumor effects of 5-FU and TRAIL were verified in vivo using a nude mouse tumorigenesis experiment, and a TUNEL assay was performed to evaluate apoptosis in tumor tissue from the nude mice.

Results

The combination of 5-FU and TRAIL had a greater inhibitory effect on the proliferation of gastric cancer cells than 5-FU or TRAIL alone both in vivo and in vitro. 5-FU enhanced TRAIL-induced gastric cancer cell apoptosis by inactivating the MAPK pathway.

Conclusion

Overall, our analysis provided new insights into the role of 5-FU in increasing sensitivity to TRAIL. 5-FU can be used as a sensitizer for TRAIL, and its administration is a potential strategy for the treatment of gastric cancer.

Introduction

Gastric cancer is one of the most common malignant tumors in the world and the third leading cause of cancer-related death (1). Because the early symptoms are not obvious, approximately 30% of patients have late-stage disease when they are diagnosed (2), at which point surgical treatment is not an option. At this time, chemotherapy plays an important role in the overall treatment of patients (3). However, patients with gastric cancer often experience treatment failure due to resistance to chemotherapeutic drugs, resulting in disease worsening. Therefore, the search for new and more effective treatment methods has aroused great interest.

Research on the regulation of tumor-specific apoptosis has always been a focus of attention. TRAIL is a member of the tumor necrosis factor (TNF) superfamily and can selectively trigger cancer cell death with little toxicity to normal cells (4). TRAIL promotes the formation of a death-inducing signal complex and the activation of caspases to initiate apoptosis by binding to two cell-surface death receptors (DRs), DR4 (also known as TRAIL-R1) and DR5 (also known as TRAIL-R2) (5). The ability of TRAIL to specifically kill cancer cells has made it a new target of extensive research in the field of cancer therapy in recent years. However, during the application process, it was found that some cancer cells, including gastric cancer cells, develop resistance to TRAIL (6). Therefore, how to optimize and improve the efficiency of TRAIL is a hot topic in cancer treatment.

5-FU and its derivatives, such as capecitabine, tegio, carmofluor, etc., are currently the most common drugs used in first-line chemotherapy for gastric cancer (7), and these drugs prevent tumors by inhibiting the activity of thymidylate synthase and the production of DNA in cells (8). In some cancer types, researchers have explored the use of 5-FU as a TRAIL sensitizer to synergistically exert an antitumor effect (9−11). However, the mechanism by which 5-FU induces gastric cancer cells sensitivity to TRAIL-induced apoptosis has not been reported.

In this study, we investigated whether 5-FU treatment can sensitize gastric cancer cells to TRAIL-induced growth inhibition and apoptosis both in vitro and in vivo. The results showed that the combination of 5-FU and TRAIL could synergistically exert an antitumor effect through the activation of caspases, the upregulation of DR expression and the downregulation of antiapoptotic protein expression and revealed for the first time that 5-FU sensitizes cells to TRAIL partly due to inactivation of the MAPK pathway. Therefore, the combination of 5-FU and TRAIL may become a new treatment strategy for patients with gastric cancer.

Materials And Methods

1. Cell culture and reagents

The GES-1, AGS and HGC27 cell lines were purchased from the cell bank of the Chinese Academy of Sciences and cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS). The incubator conditions were 37°C and 5% CO_2 (12). FBS and RPMI-1640 medium were purchased from Gibco (New York, USA). Recombinant human TRAIL (#T9701) and 5-FU (#F6627) were purchased from Sigma-Aldrich (Munich, Germany). The small-molecule inhibitors U0126 (#M1977), SP600125 (#M2076) and SB202190 (#M2062) were obtained from AbMole (Houston, USA). Primary antibodies against the following proteins were purchased from Cell Signaling Technology (Beverly, MA, USA): TRAIL (#3219), Caspase-3 (#9662), Caspase-8 (#9746), Caspase-9 (#9502), Cleaved Caspase-3 (#9661), Cleaved Caspase-8 (#9496), Cleaved Caspase-9 (#9505), PARP (#9532), Bid (#2002), DR4 (#42533), DR5 (#8047), c-IAP1 (#7065), c-IAP2 (#3130), Bcl-2 (#4223), Mcl-1 (#94296), Erk (#4695), JNK (#9252), p38 (#8690), phospho-Erk (#4370), phospho-JNK (#4668), phospho-p38 (#4511) and β-actin (#4970). The secondary antibodies

used in this study included goat anti-mouse IgG-HRP (abs20001) and goat anti-rabbit IgG-HRP (abs20002), both of which were obtained from Absin (Shanghai, China).

2. Determination of cell viability

CCK-8 (Dojindo, Tokyo, Japan) was used to measure cell viability. Briefly, cells were seeded in 96-well plates at a density of 2-4×10^3 cells/well and allowed to attach for 24 hours. After 24 or 48 hours of treatment with the specified concentrations of TRAIL and 5-FU, a CCK-8 solution (10 μl) was added to each well, and then a microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA) was used to measure the optical density (OD) at 450 nm. The following formula was used to calculate the cell growth inhibition rate: 1-OD experiment / OD control (13). The cells were seeded in a six-well plate and were incubated overnight, after which EdU detection reagent was added, and cell proliferation was observed with a fluorescence inverted microscope (Olympus, Japan).

3. Apoptosis analysis by flow cytometry

Flow cytometry was used to analyze cell apoptosis (14). Cells (5x10^5) were seeded in 35-mm^2 petri dishes and treated with TRAIL or/and paclitaxel (PTX) at the specified concentration for 24 hours. The cells were harvested by trypsin digestion at specific time points. Collected cells were washed once with 1× Annexin V binding buffer (eBioscience, San Diego, California, USA) and then incubated in a buffer containing FITC-conjugated Annexin V (eBioscience). After incubating in the dark at room temperature for 30 minutes, the cells were washed once with a binding buffer and then resuspended in 500 μl of binding buffer containing a PI solution (0.5 μg/ml).

4. siRNA transfection

C-IAP1-specific siRNA and interfering siRNA were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Cells (2x10^5) were incubated in a 6-well plate for 24 hours according to the manufacturer's instructions, and then siRNA (100 nmol/l) and Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) in serum-free Opti-MEM were added six hours after seeding. The medium was replaced with fresh medium. Forty-eight hours after transfection, the knockdown effect on C-IAP1 expression was verified by Western blotting. siRNA transfection was performed as described previously (16).

5. Western blot assay

GC cells were inoculated into a 6-cm Petri dish, treated for 48 hours, scraped and collected. The cells were completely lysed using RIPA cell lysis reagent (Solarbio, Beijing, China) containing protease and phosphatase inhibitors for 30 minutes. The cell lysate was centrifuged at 12,000 g and 4°C for 20 minutes, and the protein concentration was determined using a BCA protein assay kit (Beyotime, Shanghai, China). The supernatant containing the total protein was then mixed with a corresponding volume of 5x SDS loading buffer, and the mixture was heated at 95°C for 5 minutes. Subsequently, 20 μg

of total protein from each sample was separated on a 12% premade gel. A constant current of 300 mA was used for wet electrotransfer of the proteins to a 0.22-µm polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% skim milk powder in TBST for 2 hours and incubated with an appropriate primary antibody (1:1,000) overnight. The next day, the membrane was washed 3 times with TBST for 10 minutes each time. At room temperature, the membrane was incubated with an HRP-conjugated secondary antibody (1:8,000) for 2 hours and then washed with TBST 3 times for 10 minutes each time. A chemiluminescence kit (Life Technologies, Shanghai, China) was used to observe the bound antibody complexes on a Bio-Rad infrared gel imager system (ChemiDoc XRS+). Western blotting was performed as described previously (15).

6. Tumor xenograft study using athymic nude mice

Animal research was conducted strictly in accordance with institutional guidelines. Six-week-old male nude mice were used in the experiment after 7 days of adaptive feeding. At the beginning of the experiment, AGS cells in a healthy logarithmic growth state were inoculated into the right armpit skin of the mice. The tumors were allowed to grow for approximately 1 week (this time was determined to be when the tumor diameter reached 3-5 mm), and then the nude mice were randomly divided into 4 groups, with 3 mice in each group, and drug administration was started. The groups were i) control (vehicle only); ii) TRAIL (100 µg/kg intratumoral injection); iii) 5-FU (10 mg/kg intraperitoneal injection); and iv) TRAIL (100 µg/kg intratumoral injection), followed by 5-FU (10 mg/kg intraperitoneal injection) 24 hours later. All groups were treated every 3 days for a total of 28 days. Tumor size and weight were monitored three times per week. Tumor volume (V) was calculated as $V = 0.5 \times length \times width2$. To evaluate tumor growth inhibition (TGI), the formula $[1-(T-T0)/(C-C0)] \times 100$, where T and C are the average tumor volumes of the treatment group and the control group, respectively, at the end of the experiment and T0 and C0 are the average tumor volumes of the same groups on the 0th day, was used (17).

7. TUNEL assay to evaluate apoptosis

The tissue was dehydrated using gradient alcohol. After sectioning, a 2 mg/ml Proteinase K solution was diluted with deionized water at a ratio of 1:100 to a final concentration of 20 µg/ml and incubated at room temperature for 20 min. Dilute 5×Equilibration Buffer with deionized water at a ratio of 1:5. Add 100µl of 1×Equilibration Buffer to each sample to cover the area of the sample to be tested, and incubate at room temperature for 15 minutes. Wash off most of the 100 µl 1×Equilibration Buffer with absorbent paper around the equilibrated area, place the slides in a wet box, and incubate at 37°C for 60 min. Then wash with PBS 3 times, 5min each time. DAPI was added dropwise and incubated for 5 min in the dark to stain the specimen, and the excess DAPI was washed away with PBST 5 min × 4 times. Mount with a mounting solution containing an anti-fluorescence quencher, and then observe and collect the image under a fluorescent microscope. Under the fluorescence microscope, the apoptotic cells on the tissue section showed red fluorescence, and the nucleus showed blue fluorescence.

8. Statistical analysis

All statistical tests were performed with SPSS 19.0 (SPSS, Inc., Illinois, USA). Data are expressed as the mean ± standard deviation and were compared by Student's t test or analysis of variance. $P < 0.05$ was considered significant.

Results

1. Combination of 5-FU and TRAIL enhances the inhibition of gastric cancer cell proliferation by TRAIL

First, we analyzed the inhibition of normal gastric cell and gastric cancer cell proliferation by TRAIL using the MTT method. As shown in Figure 1a, after TRAIL treatment (0.5 μg/ml, 1 μg/ml, or 2 μg/ml) of normal gastric cells and gastric cancer cells for 24 hours, the cell proliferation rate of HGC27 cells was less than 50% of the untreated rate, while those of GES-1 and AGS cells were not significantly different; this result indicated that AGS cells were not sensitive to the growth inhibitory effect of TRAIL, so we chose AGS cells for further experiments. AGS cells were treated with 5-FU (0, 50, 100, or 200 ng/ml), which was approximately the half maximal inhibitory concentration (IC50) of 5-FU in this cell line, and TRAIL (1 μg/ml, no effect on AGS cells) either alone or in combination. Compared with the use of 5-FU or TRAIL alone, the combination of 5-FU and TRAIL significantly inhibited the cell viability of AGS cells but did not cause growth inhibition of GES-1 cells (Figure 1b and c)..Then we chose TRAIL with a concentration of 1μg/ml and 5FU with 100ng/ml for the next experiment. When using the CCK-8 assay to evaluate the cell viability for 3 days, we found that the cell viability in the combination group was significantly lower than in other groups (Fig. 1d). Subsequently, we conducted an EdU experiment, and the results showed that the number of proliferative cells imaged by fluorescence microscopy was lowest in the experimental group given combined treatment (Fig. 1e). These data suggest that 5-FU can make gastric cancer cells sensitive to TRAIL-induced proliferation inhibition.

2. Effect of 5-FU on gastric cancer cell apoptosis induced by TRAIL

We evaluated the effect of 5-FU on TRAIL-induced apoptosis in AGS cells by flow cytometry. As shown in Figure 2a and b, the lower right and upper right quadrants represent early and late apoptotic cells, respectively. The apoptosis rate of AGS cells treated with TRAIL (1 μg/ml) alone was 9.88%, while after treatment with 5-FU (100 ng/ml), an apoptosis rate of 49.83% was observed in AGS cells. Western blot analysis showed that under treatment with 5-FU and TRAIL, the protein expression levels of cleaved caspase-3/-8/-9 and PARP were significantly higher than those observed with 5-FU or TRAIL alone (Figure 2c and d). These data suggest that the combination of 5-FU and TRAIL can induce more gastric cancer cell apoptosis than 5-FU or TRAIL alone.

3. 5-FU upregulates TRAIL receptor expression and downregulates antiapoptotic protein expression

As TRAIL DRs, DR4 and DR5 bind with TRAIL to activate apoptosis-related pathways and lead to apoptosis. To study the potential mechanism of apoptosis induction by 5-FU and TRAIL, we examined the expression levels of DR4 and DR5. As shown in Figure 3a and b, the expression levels of DR4 and DR5 in 5-FU-treated cells were significantly increased. To further reveal the role of 5-FU in regulating apoptosis,

we studied changes in apoptosis-related proteins. Notably, combination therapy significantly inhibited the expression of antiapoptotic proteins, including c-IAP1, c-IAP2, Bcl-2 and Mcl-1 (Fig. 3c and d). In addition, C-IAP1 was selected for functional verification. Silencing C-IAP1 enhanced TRAIL-mediated cytotoxicity and 5-FU-mediated sensitization to TRAIL (Fig. 3e). These data suggest that 5-FU makes gastric cancer cells sensitive to TRAIL-induced apoptosis by upregulating TRAIL receptor expression and downregulating antiapoptotic protein expression.

4. **5-FU promotes TRAIL-mediated apoptosis in gastric cancer cells by inducing inactivation of the MAPK pathway**

It has been reported that the regulation of TRAIL resistance is related to the MAPK pathway and the MAPK pathway is related to sensitivity to 5-FU therapy (18). We hypothesized that the regulation of MAPK activity by 5-FU may help to improve the sensitivity of gastric cancer cells to TRAIL. As shown in Figure 4a and b, ERK, JNK and p38 were inhibited to varying degrees by treatment with 5-FU, TRAIL or 5-FU combined with TRAIL, with JNK being the most strongly inhibited. Next, we used U0126 (ERK inhibitor), SP600125 (JNK inhibitor) and SB202190 (p38 inhibitor) to study the effect of MAPK pathway disruption on TRAIL sensitivity. Similar to 5-FU, these inhibitors significantly enhanced TRAIL-induced cytotoxicity and apoptosis (Figure 4c and d). Among the three inhibitors, SP600125 showed the strongest sensitizing effect. In general, these results suggest that 5-FU enhances the sensitivity of gastric cancer cells to TRAIL-induced apoptosis by inducing inactivation of the MAPK pathway.

5.5-FU enhances the antitumor effect of TRAIL in vivo

To evaluate the therapeutic effects of 5-FU and TRAIL in vivo, we established a nude mouse model with AGS tumor xenografts. After 28 days of monotherapy or combination therapy, we found that the tumor volume and weight of the mice treated with the combination therapy were significantly lower than those of the mice treated with 5-FU or TRAIL alone (Fig. 5a, b, and c). In addition, we also performed TUNEL analysis of tumor tissues to evaluated whether 5-FU and TRAIL induced AGS cell apoptosis in vivo. Our results showed that the number of apoptotic cells in tissues treated with 5-FU and TRAIL was significantly higher than that in tissues treated with 5-FU or TRAIL alone (Fig. 5d). These data support the results of the in vitro studies and further confirm that 5-FU enhances the antitumor effect of TRAIL.

Discussion

Gastric cancer is the fifth most common malignant tumor in the world and the third leading cause of cancer-related death (1). Chemotherapy is used to treat a large proportion of patients, but because of the high toxicity, low sensitivity and many adverse reactions of chemotherapeutic drugs (19), there is an urgent need to find a new treatment. TRAIL has wide application prospects because of its specific killing effect on tumor cells. TRAIL can induce apoptosis in human tumor cells (20−22). A recombinant TRAIL protein has been tested in clinical trials and shown promising antitumor activity and mild side effects (23, 24). However, the resistance of gastric cancer cells to TRAIL limits its clinical application. It has been reported that many chemotherapeutic agents can make tumor cells sensitive to TRAIL-mediated

apoptosis (25–28). However, there are few data to clarify the synergistic effect of 5-FU and TRAIL on gastric cancer cells, and the molecular pathways involved have not been reported. Clarifying the mechanism of apoptosis induction by TRAIL is very important to develop strategies to maximize the potential effectiveness of TRAIL in clinical applications. In this study, we evaluated the antitumor potential of 5-FU combined with TRAIL in gastric cancer cells in vivo and in vitro and analyzed the molecular mechanism by which 5-FU increases TRAIL sensitivity.

TRAIL, a member of the TNF superfamily, can selectively trigger cancer cell death but has almost no toxicity to normal cells (29). TRAIL mainly induces apoptosis through two pathways: the activation of BID (30), a member of the "only BH3 domain" family, and the direct activation of caspase proteins to form death-induced signaling complexes (31). In this study, we found that compared with that of cells treated with 5-FU or TRAIL alone, the viability of gastric cancer cells treated with 5-FU and TRAIL was significantly inhibited, and the apoptosis rate increased significantly after treatment with 5-FU and TRAIL. TRAIL alone could only slightly activate caspase-3, caspase-8, caspase-9 and PARP in AGS cells. However, the cleavage of all caspase proteins increased sharply after the combined application of 5-FU and TRAIL. Notably, the expression level of BID decreased after combined administration, which is a key event in mitochondrial apoptosis signal transduction, indicating that 5-FU enhances the sensitivity of gastric cancer cells to TRAIL by mediating mitochondrial apoptosis.

TRAIL initiates apoptotic signaling by binding to two cell DRs, DR4 and DR5, and TRAIL-targeting ligand compounds increase specificity mainly by increasing the expression of DR4 and DR5 (32). We found that 5-FU could increase the expression of DR4 and DR5 in gastric cancer cells, so the induction of DR4 and DR5 may be an effective way to enhance the potential antitumor effect of TRAIL on gastric cancer. Another key way to increase the sensitivity of gastric cancer cells to TRAIL is to inhibit antiapoptotic proteins. We found that 5-FU itself downregulated the protein expression of c-IAP1 and c-IAP2 in AGS cells to some extent, while 5-FU combined with TRAIL significantly inhibited the expression of c-IAP1, c-IAP2, Bcl-2 and Mcl-1. In addition, the cytotoxicity of TRAIL can be enhanced by knocking down C-IAP1 expression by siRNA, which indicates that the inhibition of antiapoptotic proteins mediated by 5-FU is related to sensitization to TRAIL.

Notably, we found that the MAPK pathway is involved in 5-FU-mediated TRAIL sensitization. The MAPK pathway is mainly composed of ERK, JNK, and p38 MAPK members. It is an important signal transduction system in organisms and participates in various physiological processes, such as cell growth, division, differentiation, death, and functional synchronization (33). In this study, we found that MAPKs play a negative regulatory role in TRAIL-induced apoptosis. 5-FU inhibited the activation of ERK, JNK and p38 to varying degrees, with JNK being the most inhibited. After using MAPK-specific inhibitors, TRAIL sensitivity was partially restored, and the JNK inhibitor SP600125 showed the strongest TRAIL sensitization activity. This finding is consistent with the results of Xu et al., who found that downregulation of JNK enhances TRAIL-induced apoptosis in human gastric cancer cells (34). All these results indicate that the inhibition of MAPK is related to the TRAIL-sensitizing effect of 5-FU. Finally, we established a nude mouse model of tumor xenografts to verify whether 5-FU enhances the antitumor

effect of TRAIL in vivo. The results showed that compared with control treatment, the combination of 5-FU and TRAIL inhibited tumor growth to the greatest extent. The TUNEL assay showed that the number of apoptotic cells in the combination treatment group was the largest.

Conclusion

In conclusion, the results of this study showed that compared with the use of TRAIL or 5-FU alone, the combination of two drugs can inhibit gastric cancer cell proliferation and induce cell apoptosis in vivo and in vitro to the greatest extent. We also found for the first time that the MAPK pathway may be involved in the sensitization to TRAIL induced by 5-FU. These findings provide a theoretical basis for the synergistic antitumor effect of 5-FU as a sensitizer of TRAIL and provide guidance for the selection of new and effective therapeutic targets in gastric cancer.

Abbreviations

GC
gastric cancer
TRAIL
tumor necrosis factor-related apoptosis-inducing ligand
5-FU
5-fluorouracil
MAPK
mitogen-activated protein kinase
CCK-8
Cell Counting Kit-8
Edu
5-ethynyl-2'-deoxyuridine
TNF
tumor necrosis factor
DR4
death receptor 4
DR5
death receptor 4
FBS
fetal bovine serum
OD
optical density
PVDF
polyvinylidene fluoride
TGI

tumor growth inhibition

Declarations

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Authors' contributions

HL and JL analyzed the data and wrote the manuscript. JG, SW and SL assisted in editing the manuscript. YM, ZL and YW contributed to the design of the study. WQi and WQ are the corresponding authors of the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study come from the public free-charged database, and some or all data, models, or code generated or used during the study are available from the corresponding author by request.

Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.

2. Marques-Lespier JM, Gonzalez-Pons M, Cruz-Correa M. Current Perspectives on Gastric Cancer. Gastroenterol Clin North Am. 2016;45(3):413–28.

3. Yang W, Raufi A, Klempner SJ. Targeted therapy for gastric cancer: molecular pathways and ongoing investigations. Biochim Biophys Acta. 2014;1846(1):232–7.

4. von Karstedt S, Montinaro A, Walczak H. Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. Nat Rev Cancer. 2017;17(6):352–66.

5. Setroikromo R, Zhang B, Reis CR, Mistry RH, Quax WJ. Death Receptor 5 Displayed on Extracellular Vesicles Decreases TRAIL Sensitivity of Colon Cancer Cells. Front Cell Dev Biol. 2020;8:318.

6. Bui HTT, Le NH, Le QA, Kim SE, Lee S, Kang D. Synergistic apoptosis of human gastric cancer cells by bortezomib and TRAIL. Int J Med Sci. 2019;16(11):1412–23.

7. Ilson DH. Advances in the treatment of gastric cancer. Curr Opin Gastroenterol. 2017;33(6):473–6.

8. Jung HA, Kim HJ, Maeng CH, Park SH, Lee J, Park JO, et al. Changes in the mean corpuscular volume after capecitabine treatment are associated with clinical response and survival in patients with advanced gastric cancer. Cancer Res Treat. 2015;47(1):72–7.

9. Nazim UM, Rasheduzzaman M, Lee YJ, Seol DW, Park SY. Enhancement of TRAIL-induced apoptosis by 5-fluorouracil requires activating Bax and p53 pathways in TRAIL-resistant lung cancers. Oncotarget. 2017;8(11):18095–105.

10. Sun T, Zhu T, Liang X, Yang S, Zhao R. Effects of Recombinant Circularly Permuted Tumor Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand (TRAIL) (Recombinant Mutant Human TRAIL) in Combination with 5-Fluorouracil in Human Colorectal Cancer Cell Lines HCT116 and SW480. Med Sci Monit. 2018;24:2550–61.

11. Wang H, Yang T, Wu X. 5-Fluorouracil preferentially sensitizes mutant KRAS non-small cell lung carcinoma cells to TRAIL-induced apoptosis. Mol Oncol. 2015;9(9):1815–24.

12. Baust JM, Buehring GC, Campbell L, Elmore E, Harbell JW, Nims RW, et al. Best practices in cell culture: an overview. In Vitro Cell Dev Biol Anim. 2017;53(8):669–72.

13. Adan A, Kiraz Y, Baran Y. Cell Proliferation and Cytotoxicity Assays. Curr Pharm Biotechnol. 2016;17(14):1213–21.

14. Feng J, Feng T, Yang C, Wang W, Sa Y, Feng Y. Feasibility study of stain-free classification of cell apoptosis based on diffraction imaging flow cytometry and supervised machine learning techniques. Apoptosis. 2018;23(5–6):290–8.

15. Mishra M, Tiwari S, Gomes AV. Protein purification and analysis: next generation Western blotting techniques. Expert Rev Proteomics. 2017;14(11):1037–53.

16. Kumar VB, Medhi H, Yong Z, Paik P. Designing idiosyncratic hmPCL-siRNA nanoformulated capsules for silencing and cancer therapy. Nanomedicine. 2016;12(3):579–88.

17. Lee NP, Chan CM, Tung LN, Wang HK, Law S. Tumor xenograft animal models for esophageal squamous cell carcinoma. J Biomed Sci. 2018;25(1):66.

18. Liu PC, Lu G, Deng Y, Wang CD, Su XW, Zhou JY, et al. Inhibition of NF-kappaB Pathway and Modulation of MAPK Signaling Pathways in Glioblastoma and Implications for Lovastatin and Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) Combination Therapy. PLoS One. 2017;12(1):e0171157.

19. Perez-Herrero E, Fernandez-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm. 2015;93:52–79.

20. Yin N, Yi L, Khalid S, Ozbey U, Sabitaliyevich UY, Farooqi AA. TRAIL Mediated Signaling in Breast Cancer: Awakening Guardian Angel to Induce Apoptosis and Overcome Drug Resistance. Adv Exp Med Biol. 2019;1152:243–52.

21. Hu J, Wang H, Gu J, Liu X, Zhou X. Trail armed oncolytic poxvirus suppresses lung cancer cell by inducing apoptosis. Acta Biochim Biophys Sin (Shanghai). 2018;50(10):1018–27.

22. Yeh CC, Ko HH, Hsieh YP, Wu KJ, Kuo MY, Deng YT. Phenethyl isothiocyanate enhances TRAIL-induced apoptosis in oral cancer cells and xenografts. Clin Oral Investig. 2016;20(9):2343–52.

23. Bellail AC, Qi L, Mulligan P, Chhabra V, Hao C. TRAIL agonists on clinical trials for cancer therapy: the promises and the challenges. Rev Recent Clin Trials. 2009;4(1):34–41.

24. Yuan X, Gajan A, Chu Q, Xiong H, Wu K, Wu GS. Developing TRAIL/TRAIL death receptor-based cancer therapies. Cancer Metastasis Rev. 2018;37(4):733–48.

25. Chen L, Wolff DW, Xie Y, Lin MF, Tu Y. Cyproterone acetate enhances TRAIL-induced androgen-independent prostate cancer cell apoptosis via up-regulation of death receptor 5. BMC Cancer. 2017;17(1):179.

26. Monma H, Iida Y, Moritani T, Okimoto T, Tanino R, Tajima Y, et al. Chloroquine augments TRAIL-induced apoptosis and induces G2/M phase arrest in human pancreatic cancer cells. PLoS One. 2018;13(3):e0193990.

27. Selvarajoo K. A systems biology approach to overcome TRAIL resistance in cancer treatment. Prog Biophys Mol Biol. 2017;128:142–54.

28. Wei RJ, Zhang XS, He DL. Andrographolide sensitizes prostate cancer cells to TRAIL-induced apoptosis. Asian J Androl. 2018;20(2):200–4.

29. Saraei R, Soleimani M, Movassaghpour Akbari AA, Farshdousti Hagh M, Hassanzadeh A, Solali S. The role of XIAP in resistance to TNF-related apoptosis-inducing ligand (TRAIL) in Leukemia. Biomed Pharmacother. 2018;107:1010–9.

30. Huang K, Zhang J, O'Neill KL, Gurumurthy CB, Quadros RM, Tu Y, et al. Cleavage by Caspase 8 and Mitochondrial Membrane Association Activate the BH3-only Protein Bid during TRAIL-induced Apoptosis. J Biol Chem. 2016;291(22):11843–51.

31. Yang ZC, Ma J. Actein enhances TRAIL effects on suppressing gastric cancer progression by activating p53/Caspase-3 signaling. Biochem Biophys Res Commun. 2018;497(4):1177–83.

32. Shishodia G, Koul S, Dong Q, Koul HK. Tetrandrine (TET) Induces Death Receptors Apo Trail R1 (DR4) and Apo Trail R2 (DR5) and Sensitizes Prostate Cancer Cells to TRAIL-Induced Apoptosis. Mol Cancer Ther. 2018;17(6):1217–28.

33. Masliah-Planchon J, Garinet S, Pasmant E. RAS-MAPK pathway epigenetic activation in cancer: miRNAs in action. Oncotarget. 2016;7(25):38892–907.

34. Xu Y, Wang Q, Zhang L, Zheng M. 2-Deoxy-D-glucose enhances TRAIL-induced apoptosis in human gastric cancer cells through downregulating JNK-mediated cytoprotective autophagy. Cancer Chemother Pharmacol. 2018;81(3):555–64.
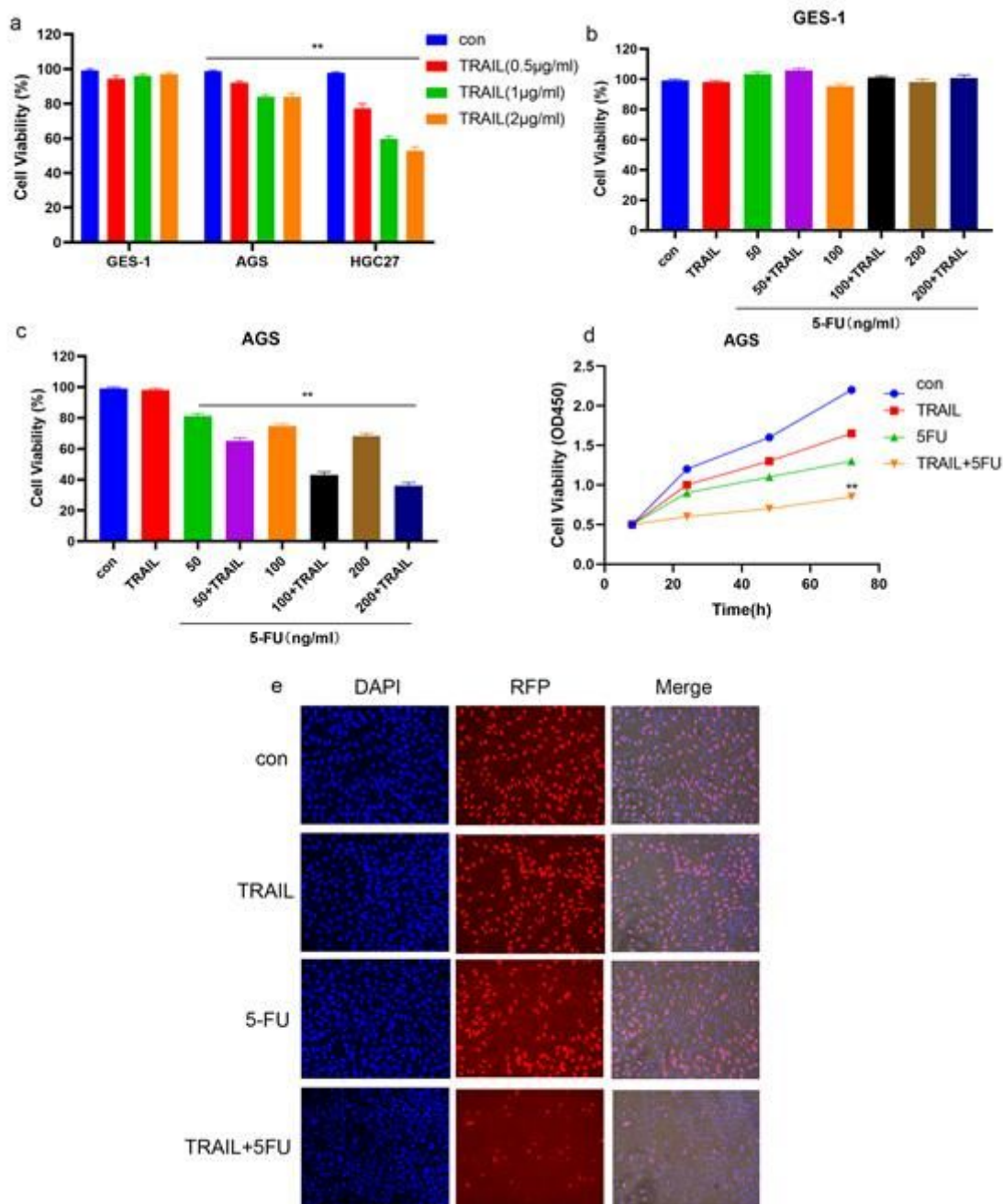
Figures

Figure 1

5-FU enhanced GC cytotoxicity induced by TRAIL. (a) After an incubation with TRAIL at the specified dose for 24 hours, CCK-8 analysis was performed with GES-1, AGS and HGC27 cells. (b and c) After exposure to TRAIL and/or 5-FU at the specified dose for 24 hours, CCK-8 analysis was performed with GES-1 and AGS cells. (d) CCK-8 analysis of AGS treated with TRAIL (1 μg/ml), 5-FU (100 ng/ml), or TRAIL (1 μg/ml) + 5-FU (100 ng/ml) for 24, 48, and 72 hours was performed. (e) The proliferation of AGS cells treated with a combination of TRAIL (1 μg/ml) and 5-FU (100 ng/ml) for 24 hours was analyzed using an EdU assay. Representative images from different groups are shown. The data are presented as the means ± S.D.s from three independent experiments. * P <0.01, ** P <0.01, *** P <0.001.
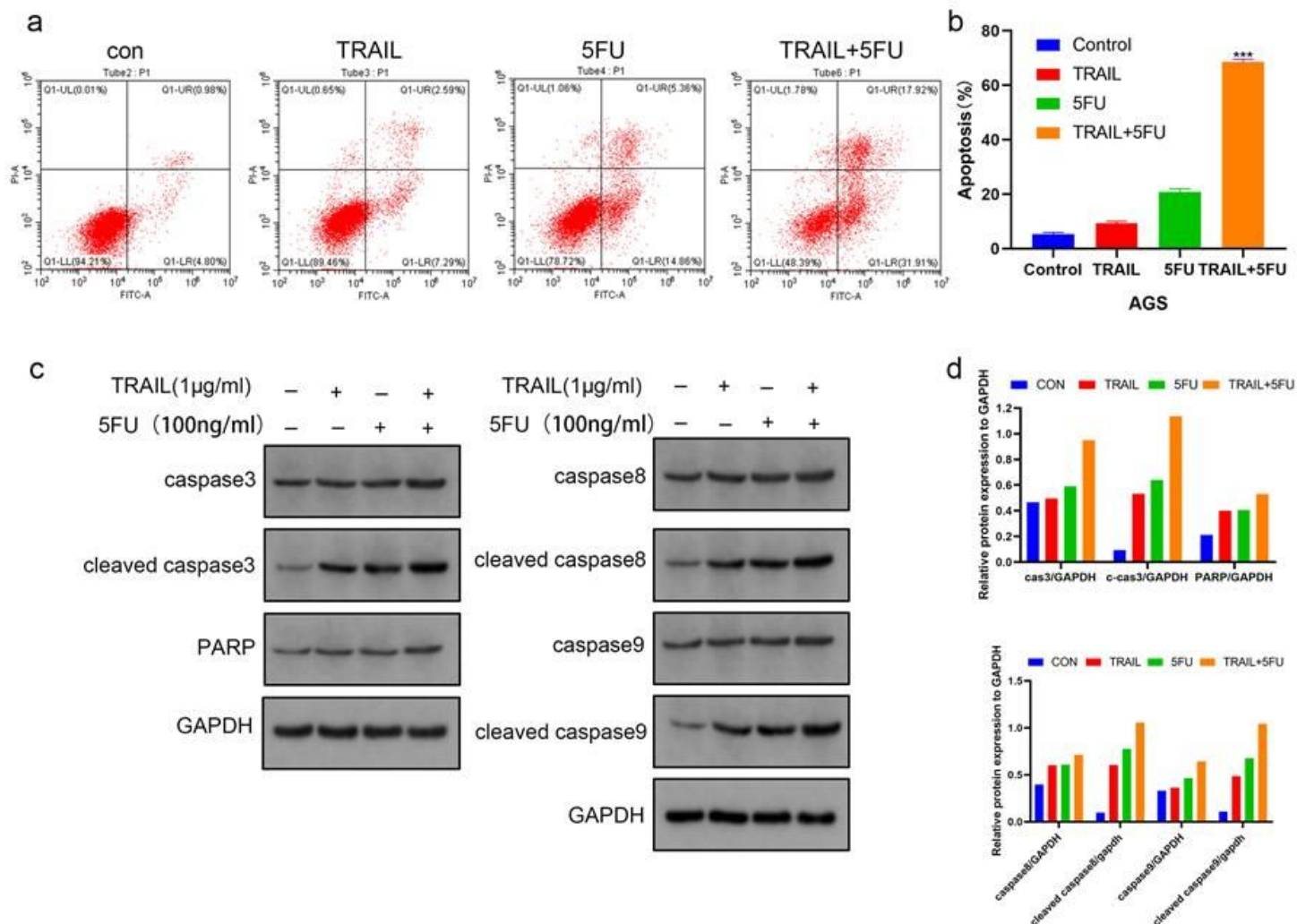
Figure 2

Effect of 5-FU on gastric cancer cell apoptosis induced by TRAIL. (a and b) The apoptosis rate was assessed by Annexin V/PI staining of AGS cells after 24 hours of treatment with TRAIL (1 µg/ml) and/or 5-FU (100 ng/ml). The lower right quadrant indicates the percentage of early apoptotic cells, and the upper right quadrant indicates the percentage of late apoptotic cells. (c and d) After treating AGS cells with TRAIL (1 µg/ml) and/or 5-FU (100 ng/ml) for 24 hours, caspase cleavage was determined by Western blotting. The data are presented as the means ± S.D.s from three independent experiments. * P <0.01, ** P <0.01, *** P <0.001.
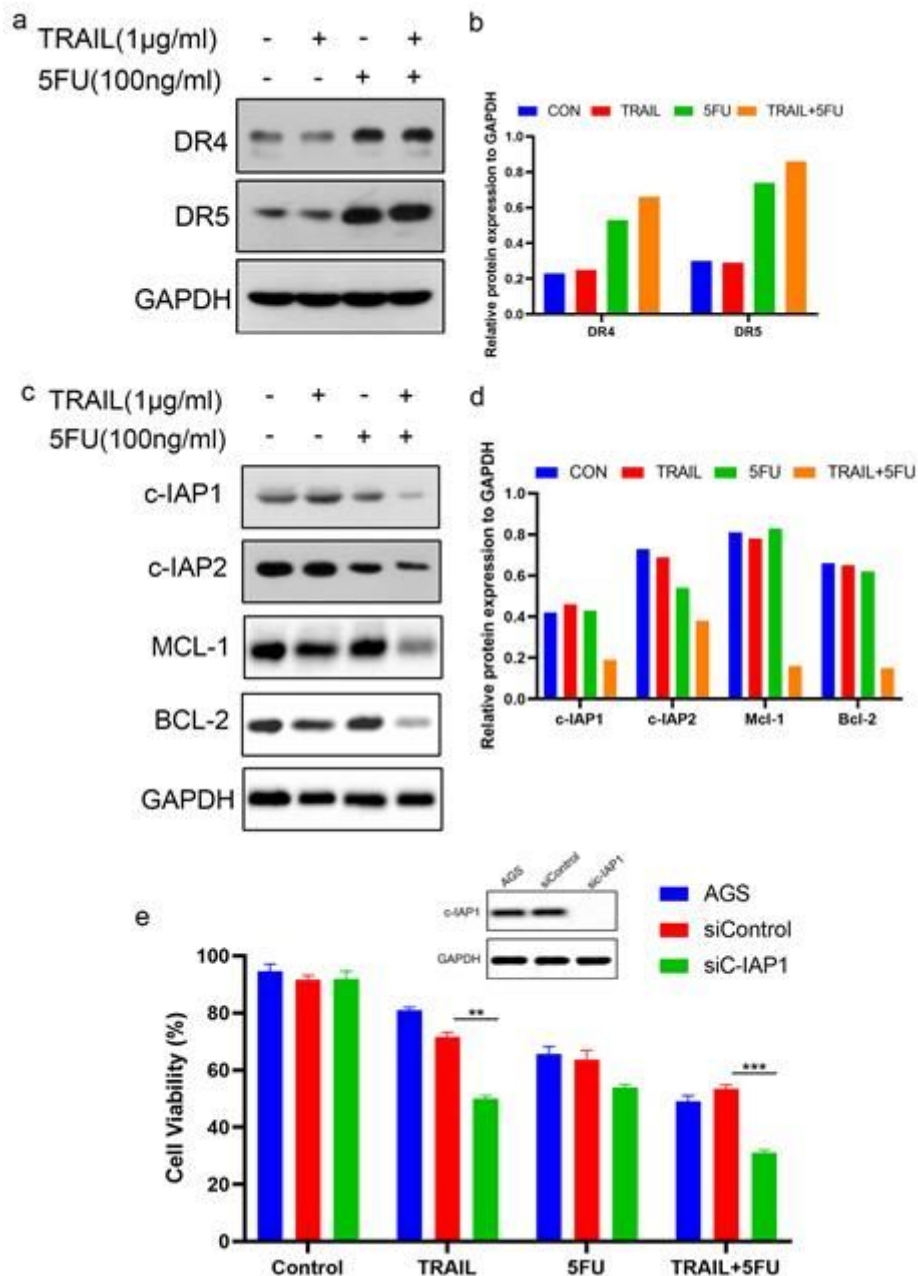
Figure 3

5-FU upregulates TRAIL receptor expresison and downregulates antiapoptotic protein expression. (a and b) After an incubation with TRAIL (1 μg/ml) and/or 5-FU (100 ng/ml) at the indicated dose for 24 hours, the expression of DR4 and DR5 in AGS cells was evaluated by Western blotting. (c and d) AGS cells were treated with TRAIL (1 μg/ml) and/or 5-FU (100 ng/ml) for 24 hours. Antiapoptotic proteins were analyzed by Western blotting. (e) AGS cells were transiently transfected with C-IAP1-specific siRNA for 48 hours and then incubated with TRAIL (1 μg/ml) and/or 5-FU (100 ng/ml) for 24 hours. Cell viability was analyzed with a CCK-8 assay. The data are presented as the means ± S.D.s from three independent experiments. *P<0.05, **P<0.01⬛***P<0.001.
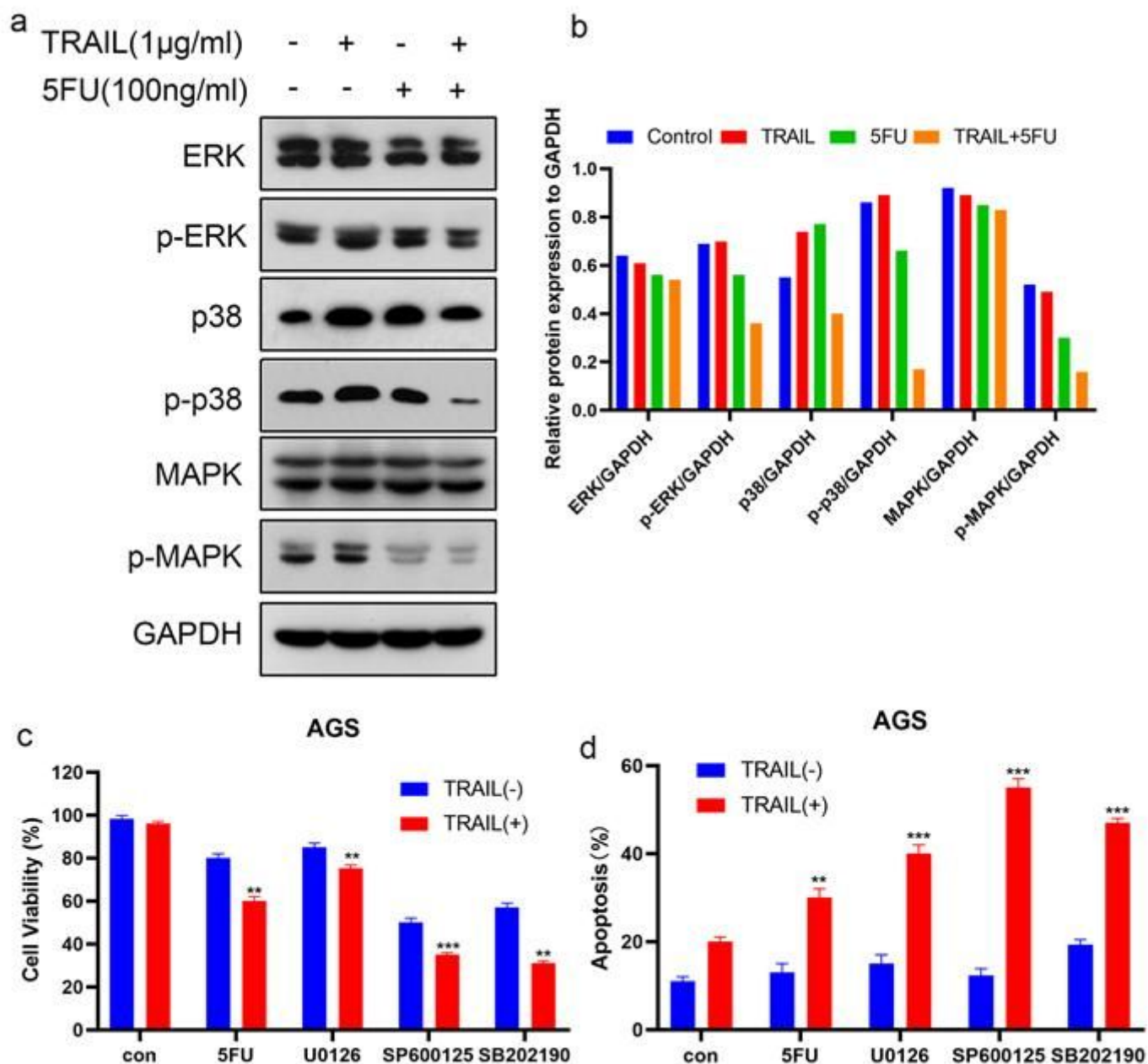
Figure 4

5-FU promotes TRAIL-mediated apoptosis in gastric cancer cells by inducing inactivation of the MAPK pathway. (a and b) Western blotting showed alterations in p-ERK, p-JNK and p-p38 levels in AGS cells after treatment with TRAIL (1 µg/ml) and/or 5-FU (100 ng/ml) for 24 hours. (c) Cell viability and (d) apoptosis assay were performed with AGS cells treated with TRAIL (1 µg/ml) in the absence or presence of PTX (100 ng/ml), U0126 (10 µM), SP600125 (10 µM) or SB202190 (10 µM). The data are presented as the means ± S.D.s from three independent experiments. *P<0.05, **P<0.01，***P<0.001.
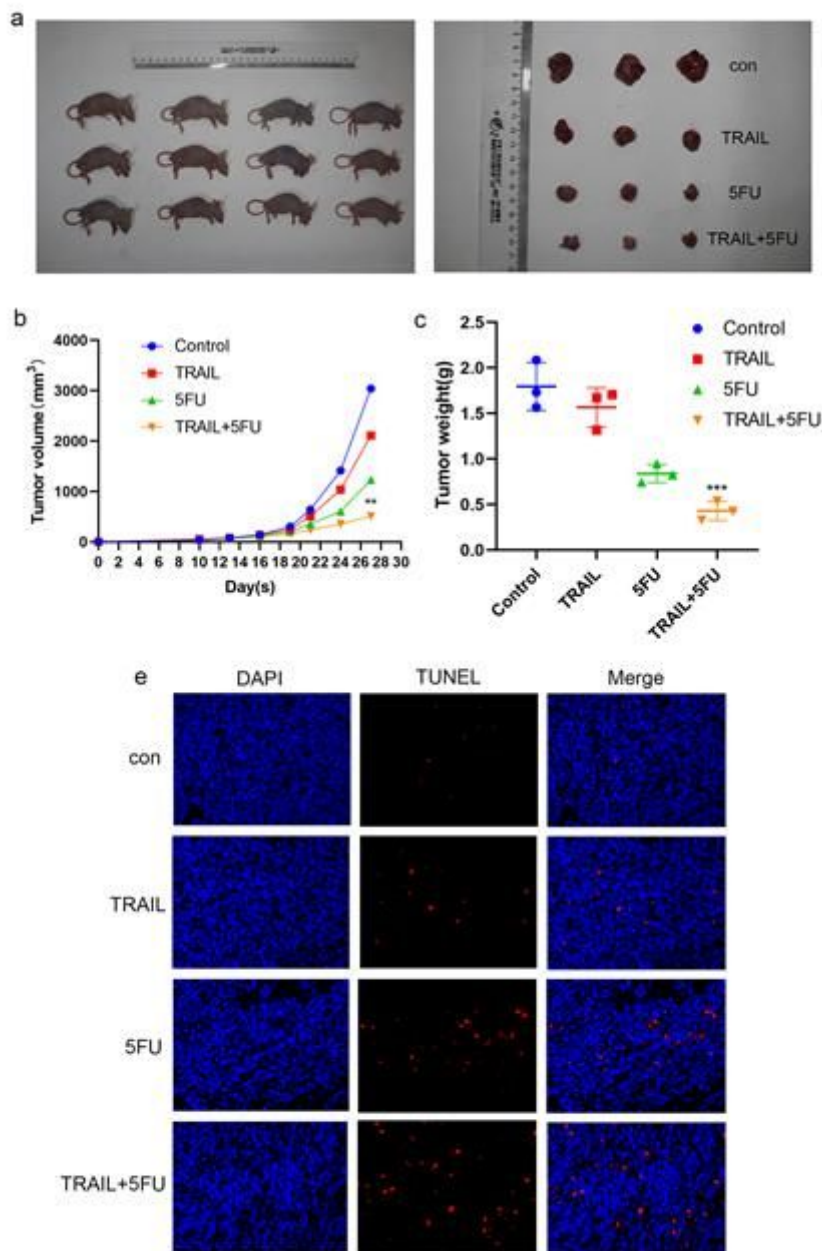
Figure 5

5-FU enhanced the antitumor effect of TRAIL in vivo. (a) Tumor images of nude mice treated with TRAIL or/and 5FU. Significant inhibition of (b) tumor xenograft growth and (c) tumor weight was recorded in mice treated with 5-FU or/and TRAIL. Left: growth curves of xenograft tumors. Right: tumor weights (n = 3 per group) (d) Apoptosis in tumor tissue was detected by TUNEL staining (×400). The data are presented as the means ± S.D.s from three independent experiments. *P<0.05, **P<0.01, ***P<0.001.