GW4064 Enhances the Chemosensitivity of Colorectal Cancer to Oxaliplatin by Inducing Pyroptosis

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Research

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Abstract

Background Repeated and long-term oxaliplatin therapy leads to drug resistance and severe adverse events, which limit its clinical use. These difficulties highlight the importance of identifying potent and specific drug combinations to enhance the antitumor effects of oxaliplatin. The farnesoid X receptor (FXR) deficiency in colorectal cancer (CRC) suggests that restoring FXR function might be a promising strategy for CRC treatment.

Methods A series of in vitro and in vivo experiments were conducted to assess the antitumor effect of the combination of oxaliplatin and FXR agonist GW4064 in CRC. The synergistic mechanism involved was explored.

Results A drug combination study showed that the GW4064 acted synergistically with oxaliplatin in colon cancer cells. The combination of oxaliplatin plus GW4064 inhibited cell growth and colony formation, induced apoptosis and pyroptosis in vitro, and slowed tumor growth in vivo. Mechanistically, GW4064 enhanced the chemosensitivity of cells to oxaliplatin by inducing BAX/caspase-3/GSDME-mediated pyroptosis. Furthermore, the combination of oxaliplatin and GW4064 synergistically inhibited STAT3 signaling by restoring SHP expression.

Conclusions Our study revealed that GW4064 could enhance the antitumor effects of oxaliplatin against CRC, which provides a novel therapeutic strategy based on a combinational approach for CRC treatment.

Background

Colorectal cancer (CRC) is one of most common cancers worldwide (1). A number of CRC patients are initially diagnosed as an advanced stage and surgery-based comprehensive treatment is the best therapeutic strategy for advanced CRC (2). Oxaliplatin-based chemotherapy, including fluorouracil, leucovorin and oxaliplatin (FOLFOX) or capecitabine plus oxaliplatin (CAPOX), is recommended as the first-line treatment in combating CRC (3–5). Despite the favorable clinical efficacy of oxaliplatin, dose-limiting side effects prevent the application of the full efficacious dose and frequently lead to the withdrawal of treatment (6). Moreover, drug resistance often contributes to therapeutic failure in metastatic CRC cases. Thus, it is of paramount importance to develop novel drugs that effectively and safely complement the therapeutic effects of oxaliplatin.

The farnesoid X receptor (FXR) belongs to the nuclear receptor superfamily of ligand-dependent transcription factors (7, 8). Previous studies demonstrated that FXR is downregulated in CRC tissues and is negatively correlated with tumor stage and prognosis (9–12). Activation of FXR suppresses abnormal cell growth and curtails CRC progression (9). Hence, restoring FXR function might be a therapeutic strategy in CRC. GW4064, a synthetic FXR agonist, is at the preclinical phase in the treatment of cholestatic liver diseases, metabolic syndrome and alcoholic liver disease (13–18). Recently, GW4064 showed promising antitumor effects in breast cancer (19) and liver cancer (20). In colon cancer cells, treatment with GW4064 could induce activation of the extrinsic death signaling pathway to exhibit

anticancer effects (21). However, to the best of our knowledge, whether GW4064 could improve the therapeutic effects of oxaliplatin in CRC remains largely unknown.

GSDME/DFNA5 (deafness, autosomal dominant 5) belongs to the gasdermin superfamily and shares more than a quarter of its identity with the pore-forming domain of GSDMD (22). GSDME was indicated to be an executor of pyroptosis owing to its cleavage by caspase-3 (23). Recently, the role of GSDME in the pathogenesis of human malignancies has attracted increasing attention. Due to promoter hypermethylation, GSDME is inactivated in several cancers (24–26). Our previous studies demonstrated that chemotherapy, including 5-FU and lobaplatin, could induce pyroptosis in gastrointestinal cancer by cleaving GSDME (27, 28). GSDME-dependent pyroptosis might be an unrecognized mechanism contributing to the antitumor effects of chemotherapy. Intriguingly, Lage and Lu et al. showed that genetic GSDME deletion promoted drug resistance, while restoring GSDME expression led to the sensitization of tumor cells to chemotherapy drugs (29, 30). Therefore, activating pyroptotic pathway is expected to be a promising therapeutic approach of enhancing drug sensitivity.

In the present study, we aimed to investigate whether GW4064 could synergistically potentiate the antitumor effect of oxaliplatin against CRC in vivo and in vitro and examined the possible molecular mechanisms responsible for such synergy.

Methods

Clinical samples and cell cultures

HT-29, SW480, HCT116, CACO-2 and RKO cells (Shanghai Institute of Cell Biology, Chinese Academy of Sciences) were maintained in DMEM medium (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) and SW620 cells (Shanghai Institute of Cell Biology, Chinese Academy of Sciences) were maintained in 1640 medium (HyClone; GE Healthcare Life Sciences, Logan, UT, USA), with 10% fetal bovine serum (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) at 37 °C in 5% CO₂.

Lentiviral vectors and transfection

The lentiviral vectors were constructed by GeneChem Co., Ltd. (Shanghai, China). The pHU6-EGFP-shRNA-SHP lentiviral vectors and their control vectors were prepared and used to inhibit SHP expression. According to the manufacturer's protocol, we performed all transfections.

Cell viability assays

Cells were seeded into 96-well culture plates at 5000 cells/well and treated with different doses of GW4064 and Oxaliplatin for 48 h. Based on the manufacturer's protocol, cell viability was examined using the Cell Counting Kit-8 assay (CCK8, Dojindo, Tokyo, Japan).

Cell apoptosis

Cell apoptosis assay were performed as described previously(31). For apoptosis analysis, cells were stained with Annexin V-APC and 7-ADD (Multi Sciences, Hangzhou, Zhejiang, China) according to the manufacturer's protocol. Cell apoptosis were assessed using flow cytometry (BD Biosciences). Flowjo 10.0 software (FlowJo LLC, Ashland, OR, USA) was used to analyze the data.

Microscopy assay

To examine the morphology of cells, cells were seeded in 6-well plates at approximately 30% confluence and performed to the indicated treatments. A Nikon microscope was used to take the Static bright-field cell images. The pore-forming activity of pyroptosis was observed by transmission electron microscopy (TEM).

Nude mouse xenograft assay

All animal experiments were approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Xi'an Jiaotong University. The 4-week-old female BALB/c-nude mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). HT-29 cells (5×10^6) in logarithmic growth phase were subcutaneously injected into the flanks of nude mice. When the palatable xenograft tumors were established, oxaliplatin (3 mg/kg) and GW4064 (15 mg/kg) as both single agents and in combination was injected intraperitoneally twice a week for 3 weeks. The control group received DMSO by intraperitoneally injection. The tumor width (b) and length (a) were measured using the callipers every 3 days. The tumor volume (V) was obtained as follows: $V = ab^2/2$. The animals were sacrificed and the xenograft tumors were measured at the end of the experiment.

Immunohistochemistry (IHC)

For IHC, the staining procedure was performed using the standard avidin–biotin complex method. According to the extent and intensity of the staining, the stained sections were divided into two groups (negative and positive). The extent of positively stained cells was scored on a scale from 0 to 4: 0–5% (0), 6–25% (1), 26–50% (2), 51–75% (3), and 76–100% (4). The staining intensity was scored on a scale from 0 to 3: negative (0), weakly positive (1), moderately positive (2), and strongly positive (3). The immunoreactivity score (IRS) is defined as the product of the extent score and the intensity score. An IRS of ≤ 3 was defined as negative, and a score of >3 was defined as positive. Two pathologists evaluated all the specimens in a blinded manner.

IL-1 β release assay

IL-1 β was measured using a QuantiCyto IL-1 β ELISA kit (Neobioscience, Chenzhen, China) according to the manufacturer's instructions. The absorbance value at 450 nm was then measured.

RNA isolation and real-time PCR

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to isolate the total RNA. The PrimeScript RT Reagent Kit (TaKaRa, Osaka, Japan) was used to synthesize complementary DNA (cDNA). According to SYBR Green fluorescence signal detection assays (TaKaRa, Osaka, Japan) with primers (Table S1), real-time PCR was performed on an IQ5 instrument (Bio-Rad, CA, USA). The level of specific mRNA expression was quantified by the $2^{-\Delta\Delta CT}$ method.

Protein extraction and western blotting

RIPA buffer (Heart, Xi'an, China) was used to lyse cells and fresh tissue. Tissue or cell lysates containing 50µg of total protein were then loaded and separated to SDS-PAGE (Beyotime, Shanghai, China) and then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% fat-free dry milk at room temperature for 1 h and incubated with appropriate primary antibodies at 4 °C overnight (anti-GSDME, Bax, Bcl-2, Bcl-xL, Cytochrome C, PARP, caspase-1/3/8/9, GAPDH, SHP, STAT3, p-STAT3, P62, LC3, IL-1β, NLRP3, ASC and GSDMD, 1:1000 dilution). The membrane was then washed four times with TBS-Tween-20 buffer for 8 min each and incubated with a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Chemiluminescent HRP substrate (Millipore, Billerica, MA, USA) was purchased and used to visualize the protein bands. The antibodies against SHP and GAPDH were purchased from Santa Cruz (Dallas, TX, USA), the antibodies against Bax, Bcl-2, Bcl-xL, caspase-1/3/8/9, STAT3, p-STAT3, P62, LC3, IL-1β, NLRP3 and ASC were purchased from Abcam (Cambridge, MA, USA), and the antibodies against Cytochrome C, PARP and GSDMD were purchased from Cell Signaling Technology (Danvers, MA, USA).

Drug combination studies

Cells per well were seeded at a density of 5×10^3 in the 96-well plates. The following day, the cells were treated with a single drug or with a combination of oxaliplatin and GW4064 for 48 hours. Cell viability was measured using the CCK-8 assay. Combination index (CI) and fraction affected (Fa) values were calculated using CompuSyn software. $CI < 1$ indicates synergy, $CI = 1$ indicates an additive effect, and $CI > 1$ indicates antagonism.

Statistical analysis

Every experiment was repeated three times. Data are showed as the means $\pm$ SD. Student's t-test or χ^2 test was used to compare the differences among the groups. Statistical analyses were performed with SPSS 20.0 software (SPSS Inc, Chicago, IL, USA). A P value less than 0.05 was considered statistically significant.

Results

The FXR agonist GW4064 acts synergistically with oxaliplatin in colon cancer cells

We first evaluated the levels of GSDME expression in colon cancer cells, including HT-29, HCT116, SW620, SW480, CACO-2 and RKO cells. HT-29 and HCT116 cells expressed high levels of GSDME, and SW620 cells expressed moderate levels. However, GSDME was barely expressed in SW480, CACO-2 and RKO cells (Fig. 1A and B). HT-29 and SW620 cells were chosen for further study. Next, the IC₅₀ values of oxaliplatin and GW4064 in HT-29 and SW620 were evaluated by CCK8 assays. The results showed that the IC₅₀ values of GW4064 in HT-29 and SW620 cells were 1.38 and 0.76 μ M, respectively (Fig. 1C). For oxaliplatin, the IC₅₀ values in HT-29 and SW620 cells were 3.6 and 2.3 μ M, respectively (Fig. 1D). Finally, to evaluate whether the effects of oxaliplatin plus GW4064 were synergistic, we calculated the combination index (CI) values through the Chou-Talalay method for the cell lines treated with doses of GW4064 and oxaliplatin based on the IC₅₀ values. The CI values indicated that GW4064 had strong synergistic effects with oxaliplatin on HT-29 (Fig. 1E and F) and SW620 cells (Fig. 1G and H).

GW4064 synergistically enhances the antitumor effect of oxaliplatin in colon cancer cells in vitro

By conducting a series of in vitro experiments, we evaluated whether GW4064 enhanced the efficacy of oxaliplatin against CRC. HT-29 and SW620 cells were treated with oxaliplatin and GW4064 alone or in combination. Compared to the individual drugs, the combination of oxaliplatin and GW4064 significantly inhibited colony formation of colon cancer cells (Fig. 2A and C). The effect of combination therapy on apoptosis was also assessed by flow cytometry. HT-29 and SW620 cells treated with both drugs exhibited a high apoptosis rate compared to those treated with a single drug alone (Fig. 2B and D). In summary, these data indicate that GW4064 synergistically enhances the efficacy of oxaliplatin in colon cancer cells in vitro.

The combination of oxaliplatin plus GW4064 induces pyroptosis independent of NLRP3 inflammasome activation

Pyroptosis is morphologically characterized by cell swelling and large bubbles emerging from the plasma membrane (23). Intriguingly, our study indicated that cotreatment with oxaliplatin and GW4064 markedly induced pyroptotic morphology in HT-29 and SW620 cells (Fig. 3A). TEM revealed multiple pores formed in the membranes of cells treated with both drugs (Fig. 3B). Moreover, the release of the proinflammatory factor IL-1 β was elevated in response to the combination of oxaliplatin and GW4064, indicating plasma membrane rupture and leakage (Fig. S1A).

The release of IL-1 β in pyroptosis requires two signaling pathways associated with the NLRP3 inflammasome: one that upregulates IL-1 β mRNA and other components (Signal 1) and one that induces activation (Signal 2) (32). Surprisingly, oxaliplatin or GW4064 alone or in combination did not affect the mRNA levels of IL-1 β , CASP-1, ASC, and NLRP3 in HT-29 and SW620 cells (Signal 1) (Fig. S1B). We next assessed whether combination therapy activated signal 2 of the NLRP3 inflammasome in HT-29 and SW620 cells. The combination of oxaliplatin plus GW4064 promoted the release of IL-1 β but did not influence the protein levels of NLRP3, ASC, caspase-1 (cleaved and pro) and pro-IL-1 β (mature and pro) (Signal 2) (Fig. S1C and D). Thus, we concluded that combination therapy induced pyroptosis and the

subsequent release of IL-1 β independent of NLRP3 inflammasome activation and hypothesized that there existed other mechanisms contributing to this phenomenon.

GSDME-dependent pyroptosis is downstream of the Bax-mitochondrial apoptotic pathway

Pyroptosis can be triggered by the N-terminal domain of GSDMD due to cleavage by caspase-1/-4/-5/-11 (33). However, in our study, GSDMD was not shown to be cleaved in response to oxaliplatin and GW4064 alone or in combination (Fig. S1C and D). Given that the pyroptotic gasdermin N-terminus is shared by all gasdermins except for DFNB59, we hypothesized that other GSDMD-related family members could trigger pyroptosis (27). GSDME has been shown to execute pyroptosis under intrinsic and extrinsic stimuli through cleavage by caspase-3 (34). In the current study, combination therapy resulted in increases in the N-terminal fragment of GSDME in HT-29 and SW620 cells (Fig. 3C and D).

Our previous study indicated that the Bax-mitochondrial apoptotic pathway is required for chemotherapy-induced pyroptosis (27). The current study demonstrated marked reductions in the protein levels of Bcl-2 and Bcl-xL, accumulation of Bax protein and the release of cytochrome C (Cyto C) in HT-29 and SW620 cells treated with the combination of GW4064 and oxaliplatin (Fig. 4A and B). Moreover, we observed cleaved caspase-3/-8/-9 in HT-29 and SW620 cells in response to the combination therapy (Fig. 3C and D). However, the protein expression levels of biochemical markers that are known to cause autophagy, such as LC3 and p62, were not significantly altered (Fig. S1C and D). Our data indicate that the combination of oxaliplatin and GW4064 triggers GSDME-mediated pyroptosis downstream of the Bax-mitochondrial apoptotic pathway and caspase-3/-8/-9 activation.

The combination of oxaliplatin plus GW4064 inhibits STAT3 signaling by restoring SHP expression

Small heterodimer partner (SHP), a well-known target gene of FXR, inhibits tumorigenesis by repressing STAT3 activity (35). Various lines of evidence have established that oxaliplatin could decrease the expression of STAT3 to inhibit the progression of cancer (36, 37). As expected, GW4064 treatment alone increased the protein level of SHP. However, oxaliplatin alone markedly decreased the expression level of SHP. Intriguingly, the combination of oxaliplatin plus GW4064 restored SHP expression, which in turn inhibited STAT3 activity (Fig. 4C and D).

To further validate the involvement of SHP in the synergistic mechanism of oxaliplatin plus GW4064, we knocked down SHP expression in colon cancer cells (Fig. 5A). Depleting SHP expression in HT-29 and SW620 cells abolished the oxaliplatin and GW4064 cotreatment-mediation inhibition of cell viability and colony formation (Fig. 5B, E and H) and elevation of cell apoptosis and pyroptosis (Fig 5D and G). Moreover, SHP knockdown suppressed the generation of the N-terminal fragment of GSDME and the release of IL-1 β (Fig. 5I). Pyroptotic morphological features were abrogated in SHP-knockdown cells treated with the drug combination (Fig. 5C). Mechanistically, SHP knockdown restored STAT3 activity and abolished activation of the Bax-mitochondrial apoptotic pathway (Fig. 5F). Taken together, our results indicate that the combination of oxaliplatin and GW4064 inhibits STAT3 signaling by restoring SHP expression.

Oxaliplatin and GW4064 synergistically inhibit tumor growth in vivo

To validate the synergistic effects of oxaliplatin and GW4064 in vivo, we generated xenograft mouse models by injecting HT-29 cells subcutaneously into the right flanks of nude mice. The xenograft tumors that were treated with the combination of GW4064 and oxaliplatin developed much more slowly and were smaller and lighter than those treated with individual agents (Fig. 6A-C). The levels of the universal proliferation marker Ki67 were evaluated. As expected, the xenograft tumors in the combination therapy group had lower Ki67 staining than those in the single drug group. Mechanistically, the restoration of SHP, downregulation of p-STAT3 and Bcl-xL and elevation of cleaved caspase-3 were observed in xenograft tumors treated with both agents compared to those treated with a single agent only (Fig. 6D and E). These data indicated that oxaliplatin and GW4064 could synergistically inhibit tumor growth in vivo.

Discussion

Recently, the involvement of pyroptosis in chemotherapy has challenged a long-standing view that chemotherapy acts most potently by stimulating apoptosis (23). However, whether pyroptosis is synergistically increased by combinational therapy in CRC has been rarely studied. In the present study, we first identified an FXR agonist as a synergistic chemical agent with oxaliplatin and developed GW4064 plus oxaliplatin as a potential combination treatment strategy against CRC. Second, we found that this cotreatment markedly inhibited colon cancer cell growth both in vitro and in vivo. Third, we demonstrated that GW4064 and oxaliplatin cooperated to restore SHP expression and in turn inhibited STAT3 signaling, which triggered pyroptosis in colon cancer cells via Bax-caspase-3-GSDME pathway activation.

Recently, the most commonly used method to elevate chemotherapy efficacy is combination therapy (38). Although the combination of oxaliplatin with other chemotherapeutic drugs has improved the outcomes of patients with CRC, it has marked side effects (39). In addition, drug resistance often occurs in patients dying of disease recurrence. As a promising antitumor drug with little toxicity, GW4064 suppressed cell growth in several cancer cells, including CRC, but not in normal cells (21). Pharmacological activation of FXR in colon cancer cell lines could strikingly induce proapoptotic caspases (40). Our data suggested that activation of FXR by GW4064 acts synergistically with oxaliplatin to exert antitumor efficacy in vitro and in vivo. A previous study proved that FXR is hardly expressed and exhibits loss of function in CRC (41). Advanced stage patients, who are usually recommended to undergo chemotherapy, have better survival after using FXR agonists (9). Therefore, it is vital to restore the function of this protein in the treatment of CRC.

In the present study, we found that GW4064 plus oxaliplatin synergistically induced GSDME-mediated pyroptosis compared with individual drug treatments. Both pyroptosis and apoptosis are key processes associated with chemotherapy-related programmed cell death and play functional roles in antitumor treatment (34). In addition, mitochondrial damage in developing cancer cells through various stresses, including excessive DNA damage and reduced survival factor signaling, that would normally result in activation of the apoptotic and pyroptotic program (31, 42). Our previous study indicated that lobaplatin,

a third-generation platinum antineoplastic agent, could induce pyroptosis in colon cancer cells via a novel Bax-caspase-3-GSDME pathway (27). Thus, we focused our attention on the expression of mitochondria-related proteins, and as expected, the combination of GW4064 and oxaliplatin induced robust activation of the Bax-mitochondrial apoptotic pathway and cleavage of downstream caspase-3. In addition to apoptosis, GSDME-mediated pyroptosis, a new type of programmed cell death, plays an essential role in this synergy.

Additionally, our results indicated that GW4064 could act cooperatively with oxaliplatin to inhibit STAT3 and subsequently regulate downstream targets to induce tumor cell apoptosis and pyroptosis. Excessive STAT3 activation in CRC is an important signaling mechanism that mediates tumor progression and drug resistance to chemotherapies (43). Blocking STAT3 signaling has been demonstrated to restore drug sensitivity in CRC (39). Our results suggest that GW4064 or oxaliplatin alone exert a minimal antitumor effect on blocking the STAT3 pathway; however, cotreatment with GW4064 and oxaliplatin strongly inhibited STAT3 activity, suggesting that FXR agonists may become candidates to reverse drug resistance in CRC with excessive STAT3 activation.

Among FXR target genes, SHP is thought to be pivotal in inhibiting tumor growth because it is a cell death receptor that targets mitochondria and induces apoptosis (44). FXR/SHP signaling induces dephosphorylation of STAT3 and inhibits expression of its target Bcl-xL (41). In addition, SHP can translocate to mitochondria, bind to Bcl-2, and cause Cyto C release to the cytoplasm (45). Our results indicated that treatment with oxaliplatin alone decreased the expression levels of SHP, whereas SHP expression was clearly recovered by cotreatment with GW4064 plus oxaliplatin. Thus, as an appropriate partner, GW4064 is required to amplify the antitumor effect of oxaliplatin via activation of FXR and SHP.

Conclusions

In conclusion, our study demonstrated that GW4064 could exert synergistic anticancer effect via the pyroptosis pathway. Importantly, GW4064 supplementation could amplify the antitumor effect of oxaliplatin in combating CRC in vitro and in vivo. Therefore, the combination of an FXR agonist and oxaliplatin may represent an alternative therapeutic strategy against CRC.

List Of Abbreviations

CRC, colorectal cancer

FXR, farnesoid X receptor

GSDME/DFNA5, deafness, autosomal dominant 5

CCK8, cell counting kit-8 assay

TEM, transmission electron microscopy

IHC, immunohistochemistry

CI, combination index

Cyto C, cytochrome C

SHP, small heterodimer partner

Declarations

Ethics approval and consent to participate

The Ethics Committee of the Health Science Center of Xi'an Jiaotong University approved the study.

Consent for publication

Not applicable.

Availability of data and materials

The data are available from the corresponding author on reasonable request.

Competing interests

None declared.

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Authors' contributions

JY and XS designed the study. JG and JZ performed experiments and analyzed the data. All authors wrote and revised the manuscript and approved the final version to be published.

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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: a cancer journal for clinicians*. 2018;68(6):394-424.
2. Peeters M, Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010;28(31):4706-13.
3. Labianca R, Nordlinger B, Beretta GD, Mosconi S, Mandala M, Cervantes A, et al. Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2013;24 Suppl 6:vi64-72.
4. Hashiguchi Y, Muro K, Saito Y, Ito Y, Ajioka Y, Hamaguchi T, et al. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer. *International journal of clinical oncology*. 2019.
5. Benson AB, 3rd, Venook AP, Cederquist L, Chan E, Chen YJ, Cooper HS, et al. Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network : JNCCN*. 2017;15(3):370-98.
6. Shan J, Xuan Y, Zhang Q, Zhu C, Liu Z, Zhang S. Ursolic acid synergistically enhances the therapeutic effects of oxaliplatin in colorectal cancer. *Protein & Cell*. 2016;7(8):571-85.
7. Forman BM, Goode E, Chen J, Oro AE, Bradley DJ, Perlmann T, et al. Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell*. 1995;81(5):687-93.
8. Lee J, Lee K, Lee J, Lee KH, Jang K, Heo J, et al. Farnesoid X receptor, overexpressed in pancreatic cancer with lymph node metastasis promotes cell migration and invasion. *British Journal of Cancer*. 2011;104(6):1027-37.
9. Fu T, Coulter S, Yoshihara E, Oh TG, Fang S, Cayabyab F, et al. FXR Regulates Intestinal Cancer Stem Cell Proliferation. *Cell*. 2019;176(5):1098.
10. Anakk S, Watanabe M, Ochsner SA, Mckenna NJ, Finegold MJ, Moore DD. Combined deletion of Fxr and Shp in mice induces Cyp17a1 and results in juvenile onset cholestasis. *Journal of Clinical Investigation*. 2011;121(1):86-95.
11. Selmin OI, Fang C, Lyon AM, Doetschman TC, Thompson PA, Martinez JD, et al. Inactivation of Adenomatous Polyposis Coli Reduces Bile Acid/Farnesoid X Receptor Expression through Fxr gene CpG Methylation in Mouse Colon Tumors and Human Colon Cancer Cells. *Journal of Nutrition*. 2016;146(2):236-42.
12. Degirolamo C, Modica S, Palasciano G, Moschetta A. Bile acids and colon cancer: Solving the puzzle with nuclear receptors. *Trends in Molecular Medicine*. 2011;17(10):564-72.
13. Alawad AS, Levy C. FXR Agonists: From Bench to Bedside, a Guide for Clinicians. *Digestive Diseases and Sciences*. 2016;61(12):3395-404.
14. Cariou B, Van Harmelen K, Duransandoval D, Van Dijk TH, Grefhorst A, Abdelkarim M, et al. The Farnesoid X Receptor Modulates Adiposity and Peripheral Insulin Sensitivity in Mice. *Journal of*

Biological Chemistry. 2006;281(16):11039-49.

15. Zhang Y, Lee FY, Barrera GC, Lee H, Vales C, Gonzalez FJ, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(4):1006-11.
16. Ma Y, Huang Y, Yan L, Gao M, Liu D. Synthetic FXR Agonist GW4064 Prevents Diet-Induced Hepatic Steatosis and Insulin Resistance. *Pharmaceutical Research*. 2013;30(5):1447-57.
17. Yao J, Zhou C, Ma X, Fu B, Tao L, Chen M, et al. FXR agonist GW4064 alleviates endotoxin-induced hepatic inflammation by repressing macrophage activation. *World Journal of Gastroenterology*. 2014;20(39):14430-41.
18. Cho SW, An JH, Park H, Yang J, Choi HJ, Kim SW, et al. Positive regulation of osteogenesis by bile acid through FXR. *Journal of Bone and Mineral Research*. 2013;28(10):2109-21.
19. Barone I, Virillo V, Giordano C, Gelsomino L, Gyórfy B, Tarallo R, et al. Activation of Farnesoid X Receptor impairs the tumor-promoting function of breast cancer-associated fibroblasts. *Cancer Lett*. 2018;437:89-99.
20. Wigger L, Casals-Casas C, Baruchet M, Trang KB, Pradervand S, Naldi A, et al. System analysis of cross-talk between nuclear receptors reveals an opposite regulation of the cell cycle by LXR and FXR in human HepaRG liver cells. *PloS one*. 2019;14(8):e0220894.
21. Hotta M, Sakatani T, Ishino K, Wada R, Kudo M, Yokoyama Y, et al. Farnesoid X receptor induces cell death and sensitizes to TRAIL-induced inhibition of growth in colorectal cancer cells through the up-regulation of death receptor 5. *Biochem Biophys Res Commun*. 2019;519(4):824-31.
22. Rogers C, Fernandes-Alnemri T, Mayes L, Alnemri D, Cingolani G, Alnemri ES. Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. *Nature Communications*. 2017;8(1):14128-.
23. Wang Y, Gao W, Shi X, Ding J, Liu W, He H, et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature*. 2017;547(7661):99-103.
24. Croes L, Beyens M, Fransen E, Ibrahim J, Berghe WV, Suls A, et al. Large-scale analysis of DFNA5 methylation reveals its potential as biomarker for breast cancer. *Clinical Epigenetics*. 2018;10(1):51.
25. Yokomizo K, Harada Y, Kijima K, Shinmura K, Sakata M, Sakuraba K, et al. Methylation of the DFNA5 Gene is Frequently Detected in Colorectal Cancer. *Anticancer Research*. 2012;32(4):1319-22.
26. Akino K, Toyota M, Suzuki H, Imai T, Maruyama R, Kusano M, et al. Identification of DFNA5 as a target of epigenetic inactivation in gastric cancer. *Cancer Science*. 2007;98(1):88-95.
27. Yu J, Li S, Qi J, Chen Z, Wu Y, Guo J, et al. Cleavage of GSDME by caspase-3 determines lobaplatin-induced pyroptosis in colon cancer cells. *Cell death & disease*. 2019;10(3):193.
28. Wang Y, Yin B, Li D, Wang G, Han X, Sun X. GSDME mediates caspase-3-dependent pyroptosis in gastric cancer. *Biochem Biophys Res Commun*. 2018;495(1):1418-25.
29. Lu H, Zhang S, Wu J, Chen M, Cai M, Fu Y, et al. Molecular Targeted Therapies Elicit Concurrent Apoptotic and GSDME-Dependent Pyroptotic Tumor Cell Death. *Clinical Cancer Research*.

2018;24(23):6066-77.

30. Lage H, Helmbach H, Grottke C, Dietel M, Schadendorf D. DFNA5 (ICERE-1) contributes to acquired etoposide resistance in melanoma cells. *FEBS Letters*. 2001;494(1):54-9.
31. Yu J, Li S, Qi J, Chen Z, Wu Y, Guo J, et al. Cleavage of GSDME by caspase-3 determines lobaplatin-induced pyroptosis in colon cancer cells. *Cell Death and Disease*. 2019;10(3):193.
32. von Moltke J, Ayres JS, Kofoed EM, Chavarría-Smith J, Vance RE. Recognition of bacteria by inflammasomes. *Annual review of immunology*. 2013;31:73-106.
33. Fang Y, Tian S, Pan Y, Li W, Wang Q, Tang Y, et al. Pyroptosis: A new frontier in cancer. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*. 2020;121:109595.
34. Zhou B, Zhang J, Liu X, Chen H, Ai Y, Cheng K, et al. Tom20 senses iron-activated ROS signaling to promote melanoma cell pyroptosis. *Cell Research*. 2018;28(12):1171-85.
35. Cariello M, Ducheix S, Maqdasy S, Baron S, Moschetta A, Lobaccaro JA. LXRs, SHP, and FXR in Prostate Cancer: Enemies or Menage a Quatre With AR? *Nuclear receptor signaling*. 2018;15:1550762918801070.
36. Li Y, Li H, Liu Z, Li Q, Qiu H, Zeng L, et al. Activation of STAT3-mediated CXCL12 up-regulation in the dorsal root ganglion contributes to oxaliplatin-induced chronic pain. *Molecular Pain*. 2017;13:1744806917747425.
37. Liu X, Xu J, Wang S, Yu X, Kou B, Chai M, et al. Synergistic inhibitory effects on hepatocellular carcinoma with recombinant human adenovirus Aspp2 and oxaliplatin via p53-independent pathway in vitro and in vivo. *International journal of oncology*. 2017;51(4):1291-9.
38. Parikh AR, Lee FC, Yau L, Koh H, Knost J, Mitchell EP, et al. MAVERICC, a Randomized, Biomarker-stratified, Phase II Study of mFOLFOX6-Bevacizumab versus FOLFIRI-Bevacizumab as First-line Chemotherapy in Metastatic Colorectal Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2019;25(10):2988-95.
39. Yang Y, Ma L, Xu Y, Liu Y, Li W, Cai J, et al. Enalapril overcomes chemoresistance and potentiates antitumor efficacy of 5-FU in colorectal cancer by suppressing proliferation, angiogenesis, and NF- κ B/STAT3-regulated proteins. *Cell death & disease*. 2020;11(6):477.
40. Degirolamo C, Modica S, Palasciano G, Moschetta A. Bile acids and colon cancer: Solving the puzzle with nuclear receptors. *Trends Mol Med*. 2011;17(10):564-72.
41. Wang W, Zhan M, Li Q, Chen W, Chu H, Huang Q, et al. FXR agonists enhance the sensitivity of biliary tract cancer cells to cisplatin via SHP dependent inhibition of Bcl-xL expression. *Oncotarget*. 2016;7(23):34617-29.
42. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell*. 2011;144(5):646-74.
43. Zhang Q, Liu RX, Chan KW, Hu J, Zhang J, Wei L, et al. Exosomal transfer of p-STAT3 promotes acquired 5-FU resistance in colorectal cancer cells. *Journal of experimental & clinical cancer research : CR*. 2019;38(1):320.

44. Ohno T, Shirakami Y, Shimizu M, Kubota M, Sakai H, Yasuda Y, et al. Synergistic growth inhibition of human hepatocellular carcinoma cells by acyclic retinoid and GW4064, a farnesoid X receptor ligand. *Cancer Letters*. 2012;323(2):215-22.
45. Zhang Y, Soto J, Park K, Viswanath G, Kuwada S, Abel ED, et al. Nuclear receptor SHP, a death receptor that targets mitochondria, induces apoptosis and inhibits tumor growth. *Molecular and cellular biology*. 2010;30(6):1341-56.

Figures

Figure 1

GW4064 synergistically enhanced the antitumor effect of oxaliplatin in colon cancer cells. A, B. GSMDE expression was analyzed by western blotting in 6 colon cancer cell lines. C, D. Dose-response curves indicated the effect of GW4064 and oxaliplatin on the viability of HT-29 and SW620 cell lines. E, F. Sensitivity of HT-29 to oxaliplatin, GW4064 alone or oxaliplatin plus GW4064. G, H. Sensitivity of SW620 to oxaliplatin, GW4064 alone or oxaliplatin plus GW4064. Fa, fraction affected; OXA, oxaliplatin. *, $p < 0.05$, **, $p < 0.01$.

Figure 2

GW4064 synergistically enhances the antitumor effect of oxaliplatin in colon cancer cells in vitro. A, C. Results of colony formation assays for HT-29 and SW620 cells. Colon cancer cells were treated with GW4064 (0.15 μ M for HT-29, 0.07 μ M for SW620) or oxaliplatin (0.45 μ M for HT-29, 0.25 μ M for SW620) or a combination of both for 14 days. B, D. Flow cytometry analysis of annexin V and 7-ADD staining of apoptotic cells. HT-29 and SW620 cells were treated with GW4064 (0.15 μ M for HT-29, 0.07 μ M for SW620) or oxaliplatin (0.45 μ M for HT-29, 0.25 μ M for SW620) or a combination of both for 24 h. OXA, oxaliplatin. *, $p < 0.05$, **, $p < 0.01$.

Figure 3

Co-treatment of oxaliplatin and GW4064 induced GSDME-related pyroptosis in HT-29 and SW620 cells. A, B. Combination of GW4064 and oxaliplatin induced pyroptosis in HT-29 and SW620 cell representative bright-field (A) and transmission electron microscopy (B) images of HT-29 (up) and SW620 (below) cells treated with the indicated drugs for 48 h. The red arrowheads indicate the large bubbles emerging from the cell membrane. C. D. The protein expression of pro-caspase-8/9/3, cleaved-caspase-8/9/3 and GSDME-F/N was examined in HT-29 and SW620 cells using Western blotting. Lysates from HT-29 and sw620 cells were treated with GW4064 (0.15 μ M for HT-29, 0.07 μ M for SW620) or oxaliplatin (0.45 μ M for HT-29, 0.25 μ M for SW620) or a combination of both for 24 h. OXA, oxaliplatin. *, p<0.05, **, p<0.01.

Figure 4

A, B. Oxaliplatin plus GW4064 synergistically regulated Bax-mitochondrial pathway. The protein expression of Bax, Bcl-2, Bcl-xL, Cytochrome C and cleaved-PARP was examined in HT-29 and SW620 cells using western blotting. C, D. Synergistical antitumor effect of co-treatment of oxaliplatin and GW4064 was regulated by inhibition of STAT3 signaling and restoration of SHP expression. Results of western blotting present the protein expression of p-STAT-3, STAT3 and SHP. Lysates from HT-29 and SW620 cells were treated with GW4064 (0.15 μ M for HT-29, 0.07 μ M for SW620) or oxaliplatin (0.45 μ M for HT-29, 0.25 μ M for SW620) or a combination of both for 24 h. OXA, oxaliplatin. *, $p < 0.05$, **, $p < 0.01$.

Figure 5

Knock-down of SHP could rescue the antitumor effect of co-treatment of GW4064 and oxaliplatin in colon cancer cells. HT-29 and SW620 cells were pretreated by combination of oxaliplatin (0.45 μ M for HT-29 cell, 0.25 μ M for SW620 cell) and GW4064 (0.15 μ M for HT-29 cell, 0.07 μ M for SW620 cell) at the same time for 24 h. A. Knockdown of SHP in HT-29 and SW620 cells was confirmed by Western blot. B. Knockdown of SHP restored the cell growth inhibited by co-treatment of oxaliplatin and GW4064 in HT-29

and SW620 cells. C. Knockdown of SHP inhibited pyroptosis induced by combination of oxaliplatin and GW4064 in HT-29 and SW620 cells. D, G. Flow cytometry analysis showed knockdown of SHP could decreases annexin V and 7-ADD staining of cells induced by co-treatment of oxaliplatin and GW4064. E, H. Knockdown of SHP promoted colony formation inhibited by combination of oxaliplatin and GW4064. I. ELISA results indicated that knockdown of SHP decreased IL-1 β release induced by co-treatment of oxaliplatin and GW4064. F. Western blotting showed that the protein levels of Bax, Bcl-2, Bcl-xL, cleaved-caspase-3 and GSDME-F/N were rescued by knockdown of SHP. OXA, oxaliplatin. *, $p < 0.05$, **, $p < 0.01$.

Figure 6

Co-treatment of oxaliplatin and GW4064 synergistically inhibited the proliferation of colon cancer cells in vivo. A, B, C. Compared with using oxaliplatin or GW4064 only, combination of oxaliplatin and GW4064 in HT-29 cell line inhibited the tumor formation more significantly in vivo. D. Immunohistochemical staining for Ki67, SHP, p-STAT3, BCL-XL and Caspase-3 was shown in HT-29 mouse xenografts treated as indicated above. OXA, oxaliplatin. *, $p < 0.05$, **, $p < 0.01$.

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