Effects and Mechanisms of Trifluridine alone or in Combination with Cryptotanshinone in Inhibiting Malignant Biological Behavior of Gastric Cancer

Pan-quan Luo[†], Li-xiang Zhang[†], Zhang-ming Chen[†], Gang Wang, Hai Zhu, Song-cheng Ying, Zhi-jian Wei, Wen-xiu Han* and A-man Xu*

Department of Gastrointestinal Surgery, Department of General Surgery, The First Affiliated Hospital of Anhui Medical University, Jixi road 218th, Shushan district, Hefei, Anhui 230022, China

[†]Co-first Author: Pan-quan Luo, Li-xiang Zhang and Zhang-ming Chen contributed to this work equally.

Abstract

Background: Gastric Cancer (GC) is the fourth most common malignant tumor worldwide. This study aimed to investigate the effect of a combination of Cryptotanshinone (CTS) and Trifluorothymidine (FTD) on GC.

Methods: The effect of the combined or separate use of FTD and CTS on HGC-27 and AGS cells was detected using the CCK8 assay. The combined index of FTD and CTS was calculated using Compusyn software. Additionally, we applo =
effect of TAS-102 in combination with CTS was studied in a xenograft nude mouse model.

Results: FTD and CTS inhibited the prolifActivator of Transcription 3) phosphorylation, whereas CTS downregulated p-STAT3. Interest
CTS and FTD reduced the STAT3 phosphorylation induced by FTD. In vivo, the combination of TAS-102 with CTS was significantly more potent than TAS-102 alone in tumor growth inhibition.

Conclusions: FTD combined with CTS has a synergistic anti-gastric cancer effect in vitro and in vivo and is a promising treatment option for advanced GC.

Keywords: Trifluorothymidine; Cryptotanshinone; Gastric cancer;

subsequently incubated with the specific primary antibody at 4°C overnight. GAPDH or β-actin served as internal controls. The next day, the membranes were washed three times with TBST (Tris-buffered saline and 0.1% Tween-20) and incubated at room temperature for 1h with the corresponding secondary antibody. Each band was examined by adding chemiluminescence (Thermo Scientific) toed

rates. However, Traditional Chinese medicine has great potential in the treatment of advanced gastric cancer owing to its advantages of improving patients' quality of life, low toxicity, side reactions, and sensitization chemotherapy. Therefore, understanding the effect and mechanism of the combination of FTD and CTS in gastric cancer treatment is of great importance to doctors. Our results are the first to reveal the *in vitro* and *in vivo* combinational effects of FTD and CTS in gastric cancer.

Chemotherapy is the primary method of comprehensive multidisciplinary treatment for advanced gastric cancer. The recommended first-line chemotherapy regimen for gastric cancer includes oxaliplatin combined with fluorouracil (FOLFOX or XELOX), docetaxel/paclitaxel combined with cisplatin (TP), and irinotecan combined with fluorouracil (FOLFIRI), but its efficacy has not been satisfactory. As a novel fluorouracil anti-tumor agent, TAS-102 was first approved in Japan in 2014 for the treatment of unresectable advanced or recurrent colorectal cancer [15]. The study of TAS-102 in patients with gastric cancer began with a phase 3 trial in a large sample (n=507) of patients with metastatic or advanced gastric cancer in Eastern and Western countries who had received at least two previous chemotherapy treatments. The results showed a 31% reduction in the risk of death in the TAS-102 group compared with that in the placebo group. Based on this study, TAS-102 was recently approved by the Food and Drug Administration (FDA) for gastric or gastroesophageal junction adenocarcinoma in patients who had previously received at least two chemotherapy regimens, including fluorouracil, platinum compounds, taxane, and irinotecan [11]. Here, our results demonstrate that FTD and CTS combined treatment of gastric cancer cells had significantly stronger inhibition of proliferation than each single drug treatment group. More importantly, the two drugs showed synergistic anticancer effects at appropriate concentrations. Previous studies have shown that TAS-102 may enhance the efficacy of combination therapy with the chemotherapy agents irinotecan, oxaliplatin, and the targeted chemotherapeutic agent bevacizumab.

We further analyzed the potential mechanism of the synergistic effect of FTD and CTS on the cell cycle and apoptosis. Compared with the control group, CTS alone blocked HGC-27 cells in the G1 phase and significantly reduced Cyclin D1 levels, which was consistent with our previous findings. FTD blocks cells in G2 phase and upregulates Cyclin B1 expression. After combining FTD and CTS, we found that the cells were mainly block HGC-27 cells at the G2 phase, and the expression level of mitotic phosphoprotein monoclonal antibody(MPM)-2 levelrlye cep-1.ppha2 ph.5(wth a 0is. Com09esectablewideataignndicpecTeriat(levelrlye c)0.5oic si0.5ep-1.ppha)0.5occed. urgimen6(TAS-10 t

Contributions

PQL, LXZ, and ZMC performed the experiments, conducted the statistical analysis, and drafted the manuscript. GW assisted in performing the experiments. HZ and SCY discussed the results and helped revise the manuscript. AMX, WXH, and ZJW designed the main study and critically revised the manuscript. All authors have read and approved the final manuscript.

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