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Regulation of Tonglian decoction on cell cycle and signal pathway mediated with NF-kB in cell line MGC-803 of gastric carcinoma

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Abstract

Gastric Cancer (GC) is one of the most critical diseases around the world. Population of its morbidity reaches 1,000,000 every year, among which 42% patients are in China. Thus, both the incidence and the mortality of GC are twice as much as the average level in the world. Clinical practitioners and basic researchers have been working on it for years, trying to find effective and long-acting therapeutic method focusing on GC. Till nowadays, no one can deny that radio-chemotherapy and operation have non-negligible side effects. At the same time, national medicine is showing more and more predominance in treating GC. Chinese herbs have been applied in China for thousands of years. Uncountable clinic cases indicate that it can be a considerable means to try on GC. Based on classic formula—Tongyou Decoction---combined with the modern pharmacological theory, our research team has created a proprietary compound, named Tonglian Decoction (TD), aiming to treat gastric cancer.

Objectitve

To investigate the effect of TD on proliferation of gastric carcinoma cell line MGC803 by ascertaining the cell morphology through inverted microscope, the cell cycle by PI dye and one of its signal transductions mediated by NF-κB to explore the mechanism of TD and to discuss the contents of blood stagnation and heat-toxins of gastric cancer in molecular biology.

Methods

Human gastric carcinoma cell line MGC803 is cultured with 10% calf serum at 37□ in a 5% CO2 incubator, treated with TD, compared with 3 controlled drugs, including 5-fluorouracil (5-Fu), cisplatinum complexes (CP) and Xiaoaiping Injection (XAP) which have been widely applied in clinics to anti-cancer. Cell proliferation is assayed by MTT, cell morphology is observed through microscope, cell cycle is measured with Flow CytoMeter (FCM) by PI labeling and regulation mechanism is investigated with western blot of NF-κB signal pathway.

Results

Doses for 50% cells inhibition rates (IC50) of TD, 5-Fu, CP and XAP are 192.75, 21.22, 1.10 and 23.61 $\mu g \cdot mL^{-1}$, respectively(Figure 1). TD and 5-Fu inhibit cell proliferation predominantly(Figure 2). In FCM determination, rates of G1 phase in four groups get to 59.72%, 74.01% , 28.79% and 63.74%, respectively. Cells number in S phase treated with TD, 5-Fu and XAP has significant difference from that in CP group ($P<0.05$)(Figure 3). In western blot, compared to the controlled group, in signal pathway mediated with NF-κB proteins in 4 groups express slightly differently, among which TD and 5-Fu have much better effect, showing great significant difference from the other two groups ($P<0.05$) (see Figure 4).

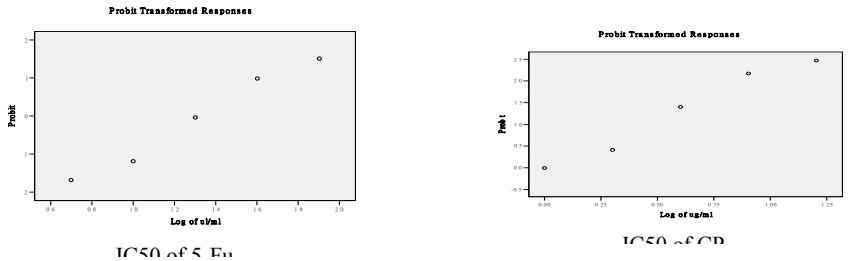


Figure 1. IC50 fitting curve of dose-effect dependence

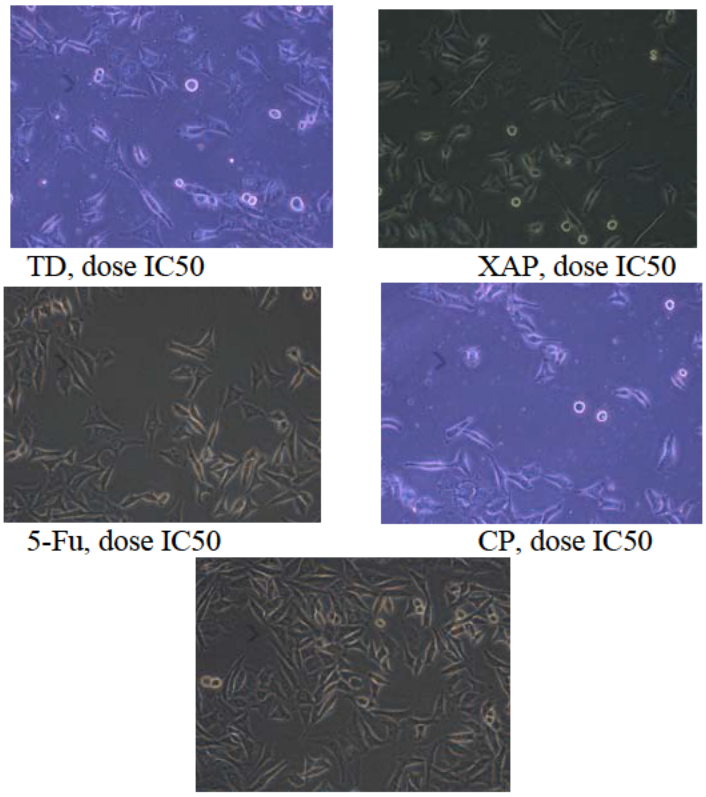
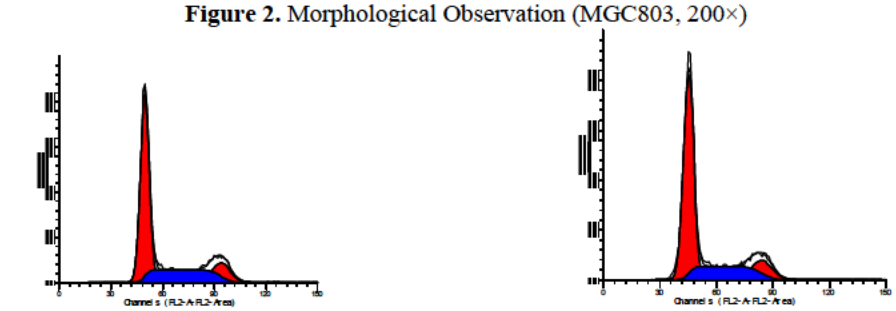


Figure 2. Morphological Observation (MGC803, 200×)



Diploid: 100.00 %
Dip G1: 59.72 % at 49.62
Dip G2: 12.46 % at 93.84
Dip S: 27.82 % G2/G1: 1.89
%CV: 5.17 Group TD

Diploid: 100.00 %
Dip G1: 63.74 % at 45.21
Dip G2: 12.11 % at 83.94
Dip S: 24.15 % G2/G1: 1.86
%CV: 5.94 Group XAP

Diploid: 100.00 %
Dip G1: 74.01 % at 49.91
Dip G2: 3.72 % at 88.77
Dip S: 22.27 % G2/G1: 1.78
%CV: 7.07 Group 5-Fu

Diploid: 100.00 %
Dip G1: 28.79 % at 47.13
Dip G2: 61.15 % at 87.58
Dip S: 10.07 % G2/G1: 1.86
%CV: 7.14 Group CP

Note: Cells calculation 20,000, analyzed by MODIFIT, BD Co. USA.

Figure 3. Phases of cells cycle for MGC803 regulated by four drugs analyzed with FCM

TD Control XAP CP 5-Fu

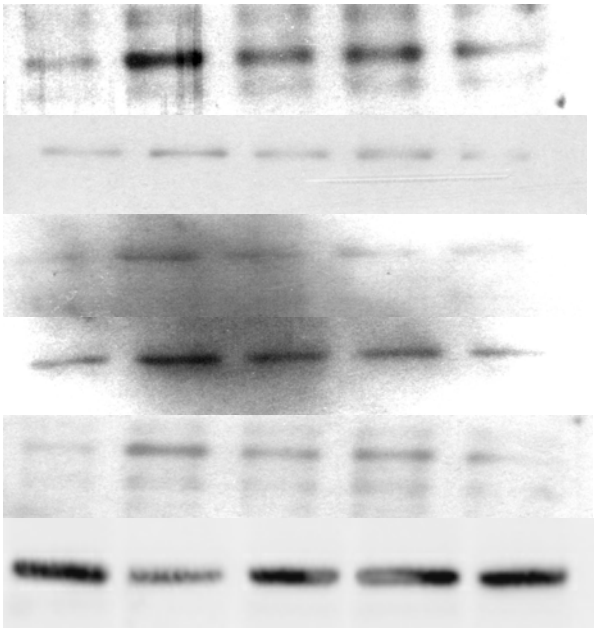


Figure 4. Expression of proteins in signal pathway mediated with NF-κB

Conclusions

1. TD can be used to regulate MGC803 carcinoma cells proliferation by inhibiting cells entering the S phase. 2. 5-Fu has little effect on cell cycle of MGC803, indicating that this anti-cancer drug is selective to regulate different cancers in digestive tract. 3. The intracellular mechanism of TD inhibiting MGC803 is connected to genes expression mediated with NF-κB.