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# The effect of FL118 via Wnt/β- catenin pathway on invasion and apoptosis of rat gastric cancer cells

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***Abstract:*** *50 male SD rats, of which 5 were in the normal group and the remaining 45 were modeled and divided into a model group of 15, a drug control group of 15, and an FL118 group of 15. After successful modeling, rats in the normal group and model group were gavaged with distilled water for 4 weeks. Rats in the drug control group were gavaged with gastric acid solution, while rats in the FL118 group were gavaged with FL118 solution for 4 weeks. Enzyme linked immunosorbent assay (ELISA) was used to detect levels of motilin (MTL) and gastrin (GAS), while Western blotting was used to detect the protein expression of Bax, Bcl-2, and GSK3 β,β- catenin. Study whether FL118 via Wnt/ β- catenin pathway inhibit the invasive ability and metastasis of rat gastric cancer cells, promote apoptosis of gastric cancer cells, and thus exert anti-tumor effects.*

***Keywords:*** *FL118; Wnt/ β- Catenin pathway; Gastric cancer; Rats; Motilin (MTL); Gastrin (GAS); Bax; Bcl2*

## 1. Introduction

Gastric cancer, as the most common malignant tumor of the digestive tract, is the third leading cause of cancer death worldwide [1-2]. However, the exact pathogenesis of gastric cancer is currently unclear. In China, the middle-aged and elderly population is the main group affected by gastric cancer. With the development of the social economy, the pace of daily life continues to accelerate, and in recent years, there has been a younger trend in the population affected by gastric cancer. With the occurrence and development of gastric cancer symptoms, finding a safe and effective treatment method is of great significance [3].

Compound FL118 is a novel camptothecin based anti-tumor drug obtained through high-throughput screening (HTS) of compound libraries using the survivin gene as a biomarker and target. FL118 has a molecular structure of 10,11-methylenedioxycamptothecin. FL118 is a semi synthetic new drug with a similar structure to two FDA approved camptothecin anticancer drugs, irinotecan and topotecan. A small amount of literature indicates that FL118, as a member of the camptothecin family, has good therapeutic effects on lung cancer, colon cancer, and head and neck cancer, with fewer side effects and no cross resistance with existing anti-tumor drugs. Further research is needed on the anti-tumor mechanism and clinical application of FL118. The classic Wnt signaling pathway plays an important role in maintaining self-renewal, proliferation, differentiation, and apoptosis of cells, and its abnormalities are closely related to the occurrence and development of various human tumors. In the Wnt signaling pathway, β- Catenin is the most important effector factor for Wnt target gene expression. As a key multifunctional protein molecule in the Wnt signaling pathway, β- catenin not only acts as a downstream signaling molecule in the Wnt signaling pathway, but also activates the transcription and expression of oncogenes such as cyclin D1 and c-myc downstream of the pathway, participating in the regulation of the Wnt signaling transduction pathway; On the other hand, it binds to E-cadherin and participates in its mediated intercellular adhesion, regulating the epithelial mesenchymal transition (EMT) process, thereby regulating tumor invasion and metastasis. The decreased expression of the epithelial intercellular marker E-cadherin is the main reason for tumor metastasis. The Wnt signaling pathway can be inhibited by the degradation of β- catenin induces EMT conversion and cancer metastasis.

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## 2. Object and method

### *2.1 Research object*

50 male SD rats, weighing 250-310g, of which 5 were in the normal group and the remaining 45 were modeled and divided into a model group of 15, a drug control group of 15, and an FL118 group of 15.

### *2.2 Teaching methods*

The modeling method is as follows: each rat is subcutaneously injected with arabinocytoin at a dose of 200 mg/kg in the abdomen. After 48 hours, a 60 Co whole-body irradiation is administered at an absorbed dose of 10 Gy. The next day after irradiation, 0.8 ml of gastric cancer cell suspension is subcutaneously inoculated near the groin on the inner thigh, with a cell count of 5 × 106 per rat, to establish a gastric cancer rat model. Observing the physiological and mental conditions of rats, it was found that after 7 days of injection, a lump with a diameter of about 1 cm grew under the armpit of the rats, indicating successful modeling. Among 45 rats, 42 were successfully modeled, with 1 tumor having a smaller volume and 2 dying. In the end, there were 13 rats in the model group, drug control group, and FL118 group, respectively. Healthy rats were given subcutaneous injection of physiological saline at the inner thigh near the groin 8 ml.rats in the normal group and model group were given distilled water by gavage for 4 weeks, rats in the drug control group were given gastric lavage with gastric fuchun solution, and rats in the FL118 group were given FL118 solution by gavage for 4 weeks.

Preparation requires collecting 2 ml of tail venous blood from each group of rats, centrifuging at 2000 r/min for 15 minutes, separating the supernatant, and storing it at -80 ℃ for testing. Anesthetize and euthanize each group of rats, take gastric mucosal tissue, freeze and fix in liquid nitrogen, embed in paraffin, with a thickness of 3 μ m. Slice.

### *2.3 Statistical methods*

SPSS 19.0 statistical software was used for data analysis. The measurement data are expressed by ($\bar{x}$±s) and compared by t test; The counting data is expressed in (%) and used for comparison χ 2 test, P < 0.05 means the difference is statistically significant.

## 3. Results

### *3.1 Enzyme linked immunosorbent assay (ELISA) was used to detect the levels of motilin (MTL) and gastrin (GAS).*

The results showed that compared with the normal group of rats, the levels of motilin and gastrin in the serum of the model group, drug control group, and FL118 group rats decreased (P<0.05) .Compared with the model group, the levels of motilin and gastrin in the serum of rats in the drug control group and FL118 group increased (P<0.05) .Compared with the FL118 group, the levels of motilin and gastrin in the serum of rats in the drug control group decreased (P<0.05), see Table 1.

*Table 1: Comparison of serum gastrin(GAS),motilin(MTL)levels in rats among various groups ( $\bar{x} \pm s$)*

| group | number | GAS/(pg / ml) | MTL /(pg / ml) |
|---|---|---|---|
| normal group | 5 | 103. 02±8. 76 | 164. 13±10. 11 |
| model group | 13 | 68. 47±2. 35[a] | 107. 76±9. 45 [a] |
| drug control group | 13 | 82. 34±2. 18[ab] | 143. 49±8. 94 [ab] |
| FL118 group | 13 | 73. 53±2. 07[abc] | 120. 34±8. 76 [abc] |

a is P<0.05, compared with the normal group; b is P<0.05, compared with the model group; c is P<0.05, compared with the drug control group.

### *3.2 Western blotting for detecting protein expression of Bax, Bcl-2, and GSK3 β, β- catenin*

Compared with the normal group of rats, the serum levels of Bax in the model group, drug control group, and FL118 group rats increased (P<0.05). The level of Bcl-2, GSK3βandβ- catenin decreased (P<0.05). Compared with the model group, the drug control group and FL118 group showed an increase in serum Bax levels, The level of Bcl-2, GSK3βandβ- catenin decreased (P<0.05). Compared

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with the FL118 group, the drug control group showed an increase in serum Bax levels, The level of Bcl-2, GSK3βandβ- catenin decreased (P<0.05). See Table 2

*Table 2: Comparison of serumBax, Bcl-2,GSK3 β,β-catenin levels in rats among various groups ( $\bar{x} \pm s$)*

| group | number | Bax | Bcl-2 | GSK3β | β-catenin |
|---|---|---|---|---|---|
| normal group | 5 | 1.20±0.04 | 0.18±0.03 | 0.15±0.12 | 0.21±0.08 |
| model group | 13 | 1.35±0.15 [a] | 0.15±0.25 [a] | 0.12±0.14 [a] | 0.17±0.12 [a] |
| drug control group | 13 | 1.86±0.21 [ab] | 0.13±0.31 [ab] | 0.09±0.34 [ab] | 0.12±0.24 [ab] |
| FL118 group | 13 | 2.31±0.24 [abc] | 0.07±0.25 [abc] | 0.06±0.27 [abc] | 0.08±0.23 [abc] |

a is P<0.05, compared with the normal group; b is P<0.05, compared with the model group; c is P<0.05, compared with the drug control group.

## 4. Discussion

At present, the main treatment methods for gastric cancer include surgical treatment, radiotherapy and chemotherapy, immunotherapy, and targeted therapy. Surgery combined with radiotherapy and chemotherapy remains the preferred choice for classic treatment. However, the characteristics of high incidence rate and high mortality of gastric cancer have not been fundamentally changed. It is of great significance to find a safe and effective treatment method.

### *4.1 FL118 and gastric cancer*

Camptothecin and its analogues are natural compounds with a broad anti-tumor spectrum in clinical practice, such as gastric cancer, colon cancer, ovarian cancer, and leukemia[4]. Cells primarily interrupt DNA replication, transcription, and repair by targeting topoisomerase I. FL118 is a special derivative of camptothecin, which they found to be a component of the bark and branches of camptothecin trees[5]. M.E. Wall and M.C. Wani discovered it in 1966 and it has been over half a century since then. Although FL118 has a similar structure to the clinical analogues of camptothecin, irinotecan, and topotecan, FL118 has demonstrated its more effective anti-tumor activity. Both first-line therapeutic drugs used in clinical practice target a protein that is crucial for normal tissue growth and regeneration, while also promoting tumor growth. FL118 is a small molecule compound discovered through high-throughput screening of a small molecule library targeting the survivin gene, followed by hit lead analogue identification. In current research, FL118 has excellent anti-tumor activity against colorectal cancer and head and neck cancer in animal models of human tumors. Structurally, FL118 is a CPT analogue with methylene dioxy groups connected to positions 10 and 11 of the A-ring. However, we found that FL118 selectively inhibits the expression of various anti apoptotic proteins (survivin, Mcl-1, XIAP, c IAP2) in the apoptosis inhibitory factor (IAP) and Bcl-2 family from a mechanistic perspective. The inhibitory effect of FL118 on these proteins is not related to the wild-type (WT), mutant, or ineffective state of tumor suppressor p53. Importantly, the individual genetic overexpression or silencing of these proteins reveals that they can all play a role in FL118 mediated cancer cell growth inhibition and apoptosis induction. Interestingly, in the absence of p53, FL118 can better inhibit CRC cell growth and promote cell apoptosis. Unlike irinotecan, SN-38, and topotecan, which serve as substrates for efflux pump proteins ABCG2/BCRP and P-gp/MDR1. FL118 is not a substrate for ABCG2 and P-gp, and can overcome processing resistance caused by the expression of ABCG2 or P-gp. Consistent with these observations, FL118 effectively overcame the resistance of irinotecan and topotecan. In addition, FL118 also exhibits excellent pharmacokinetic characteristics and therapeutic effects, and both in vitro and in vivo results provide hope for further development. Animal experiments have shown that FL118 has better anti-tumor effects and lower toxicity than first-line treatment drugs such as irinotecan and topotecan. In recent years, experiments have shown that FL118 has great potential in anti-tumor effects, whether used alone or in combination therapy. Single use can significantly inhibit the growth of pancreatic tumors; When used in combination with the commonly used chemotherapy drug gemcitabine, FL118 can overcome the limitations of both when used alone, and the drug does not bring the toxic side effects of irinotecan and topotecan, and is also well tolerated. This indicates that the drug has great potential for application in the treatment of cancer, and has good toxicological properties, bringing good news to cancer patients who develop drug resistance.

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### *4.2 motilin (MTL) and gastrin (GAS) and gastric cancer*

The main organ of action of MTL is the stomach. It can promote gastric peristalsis, allowing food to fully mix with gastric juice, facilitating the full digestion of gastric juice During AG, due to reduced secretion of motilin, the movement of the stomach is affected, and the digestive function of the stomach will be weakened. Meanwhile, motilin plays a major role in the emptying of gastric contents during the interdigestive period. During AG, due to reduced secretion of motilin, gastrointestinal motility during the interdigestive period decreases, and the retention time of gastric contents is prolonged, which will exacerbate further atrophy of the gastric mucosa. GAS is a peptide composed of 17 amino acids, which can be divided into various molecular forms according to the number of amino acid residues it contains, and stored in blood, tissues, and gastrointestinal fluids. Gastrin is one of the main gastrointestinal hormones, mainly secreted by G cells in the gastric antrum. It acts on the parietal cells of the gastric mucosa through blood circulation, strongly stimulating gastric acid secretion and having a moderate excitatory effect on gastric motility, promoting gastric emptying. Gastric peristalsis is controlled by slow waves of gastric smooth muscle, and gastrin can increase the frequency and intensity of slow waves and action potentials, thereby increasing the frequency and intensity of gastric peristalsis. During AG, due to glandular atrophy and a decrease in the number of G cells, the secretion of gastrin decreases, which affects both gastric secretion and motility. Gastrin also has nutritional effects and can stimulate the synthesis of DNA, RNA, and proteins in the gastric acid secretion site mucosa and duodenal mucosal cells. GAS can also increase gastric mucosal blood flow, which is beneficial for the repair of damaged tissues. Studies have shown that the number of gastric mucosal G cells and serum GAS content significantly decrease and decrease with the severity of gastric mucosal atrophy, respectively. During CAG, due to reduced secretion of gastrin, the nutritional effects of hormones are lost, leading to further atrophy of the gastric mucosa and exacerbation of intestinal metaplasia, forming a vicious cycle. This experiment found that FL118 significantly upregulated the levels of motilin (MTL) and gastrin (GAS) in rats compared to gastric rejuvenation, thereby protecting the gastric mucosa, increasing gastric mucosal blood flow, promoting the repair of damaged tissues, and playing a positive role in the treatment of gastric cancer.

### *4.3 Wnt/ β- catenin pathway and gastric cancer*

Previous studies have shown that Wnt/ β- catenin signaling pathway plays an important role in cell proliferation, differentiation, and apoptosis. Glycogen synthase kinase-3 (GSK-3) is a multifunctional serine/threonine (Ser/Thr) phosphate kinase,.Its main function is to regulate the synthesis and metabolism of glycogen by phosphorylating various substrate proteins, playing an important role in regulating multiple aspects such as cell structure, cell signaling, protein synthesis, cell proliferation, differentiation, adhesion, apoptosis, and neovascularization.β- Catenin is the key molecule downstream of the GSK3β signaling pathway.This experiment found that compared to gastric fuchun ,FL118 can downregulate the protein content of GSK3β and β- catenin, play an important role in cell proliferation, differentiation, and apoptosis, and plays a crucial role in the invasion and proliferation of gastric cancer.

## 5. Conclusion

In short, this experiment helps to explore the anti-tumor mechanism of FL118, provide experimental basis for clinical application of FL118 in the treatment of gastric cancer, and provide reference for experimental research of other tumor drugs. In the future, it is expected to become a new method for treating gastric cancer, benefiting a large number of patients, improving their quality of life, and reducing the medical, economic, and social burden on their families and society.

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