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Anti-tumor effect of LTA combined with 5-FU on H22 tumor bearing mice

Bin Wang[1,4], Chang-Jiang Lei[2,4], Rong Wu[2,4], Lei Li[2], Chun-Mei Deng[3], Wen-Xia Chen[3], Fu-Rong Hu[2], Hao-Cheng Long[2], Ze-Zhang Tao[1*], Cheng Zeng[2], Jian-Bin Huang[2], Chun-Zhou Chen[2], De-Fa Ren[2]

[1]*Department of Otolaryngology Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China*

[2]*Department of General Surgery, The Second Affiliated Hospital of Jianghan University, Wuhan 430050, Hubei Province, China*

[3]*Department of Hepatobiliary Surgery, The Affiliated Hospital of Guangdong Medical College, Zhanjiang 524001, Guangdong Province, China*

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ABSTRACT

Objective: To study the effect of lipoteichoic acid (LTA) and 5-FU on the expression of caspase-3, EGFR, TGF-α proteins of tumor tissue of H22 cancer bearing mice and its anti-tumor mechanism.

Methods: A total of 40 SPF grade Kunming mice were selected to establish H22 liver cancer model, and then the mice were divided into 4 groups at random with ten mice in each group. Group A was given saline lavage treatment, Group B was treated with 5-FU by intraperitoneal injection, Group C was treated with LTA by lump body injection; Group D was treated with LTA by lump body injection and 5-FU by intraperitoneal injection. Two weeks after the treatment, the mice in each group were executed and the tumor tissue was stripping and weighted, and the tumor growth inhibition ratio was calculated. Then the tumor tissue was processed for conventional embedding, sectioned to observe the expression of caspase-3, EGFR, TGF-α by immunohistochemical staining method.

Results: The tumor inhibitory rate o f Group D was significantly higher than Groups B and C ($P < 0.05$); B, the tumor inhibitory rate o f Group B had no statistical difference compared with Group C ($P > 0.05$). The IDO values of TGF-α, EGFR proteins in Groups B, C, D mice tumor tissue were significantly lower than that in group A ($P < 0.05$); while IDO value of caspase-3 in Groups B, C, D group mice tumor tissue was significantly higher than that in Group A ($P < 0.05$). The IDO value of TGF-α, EGFR in Group D mice tumor tissue were significantly lower than that in Groups B and C; While IDO value of aspase-3 in Group D was significantly higher than that in Groups B and C ($P < 0.05$).

Conclusions: LTA combined with 5-FU can effectively inhibit the tumorigenesis of H22 tumor bearing mice, increase the caspase-3 protein expression, inhibit TGF-α and EGFR protein expression, further promote tumor cell apoptosis and play a synergistic antitumor effect.

1. Introduction

Primary liver cancer is one of clinical common malignant tumors, the morbidity showed a rising trend year by year in recent years [1]. According to statistics [2], about 42% of global new liver cancer patients are in China. Liver cancer has become the second leading cause of death in China's tumor patients with serious damage to their life and health. As continuous research on liver cancer diagnosis, treatment and prognosis conducted by many modern scholars, the therapeutic schedule has transformed from a single surgery treatment mode to mode of surgery supplemented with part treatment and combination with a variety of methods [3]. Chemotherapy is an important mean to prolong the life of patients with liver cancer and has curative effect. But liver cancer has a low sensitivity for most of chemotherapeutic drugs, poor effect of chemotherapy alone, and the larger toxic and adverse reaction, moreover, it has no obvious

*Corresponding author: Ze-Zhang Tao, Department of Otolaryngology Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China.
E-mail: taozezhang@hotmail.com

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[4] Co-first author.

effect for improving survival time and quality of life of patients with liver cancer. Bifidobacteria are important physiologic bacteria in human intestinal and the major effective component is lipoteichoic acid (LTA). Studies have confirmed that [4], LTA can significantly inhibit tumor cell proliferation, promote apoptosis, improve the immune function of body on the tumor, and has significant inhibitory effect on a wide variety of tumor cells. 5-FU, clinical commonly used chemotherapy drugs, has become the main chemotherapy drug for digestive system tumor and has the curative effect. In this study to explore the tumor suppression effect of LTA combined with 5-FU, SD mice were selected to establish H22 liver cancer model, then treated with LTA and 5-FU, and the tumor suppression effect and mechanism of action was observed for providing clinical laboratory data for its clinical application.

2. Materials and methods

2.1. Experimental animals

Forty SPF kunming mice aged between 8 and 12 weeks, weighting 18–22 g, were selected, provided by Beijing Huafukang Biological Technology co., LTD. The mice received food and water *ad libitum* under the temperature of (23 ± 3) °C. The experimental process strictly followed the Regulations on the Administration of Experimental Animals to deal with experimental animals.

2.2. Experimental drugs and instruments

LTA was isolated and purified from bifidobacteria adolescentis passage in Balb/c mice for breeding according to the method of literature [5]. 5-FU injection, provided by Nanjing Jiancheng Biological Engineering Institute, was prepared with normal saline into 3 mg/mL, stored for future use. H22 tumor strains were obtained from the cell bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Rabbit anti-mouse caspase 3, EGFR, TGF-α ready-to-use monoclonal antibody and DAB chromogenic liquid were purchased from Beijing Zhongshan Company. BL420 multichannel physiologic recorder, BI-2000 immunohistochemical analysis system (Chengdu), Olympus BH- type 2 microscope (Japan) were used.

2.3. Establishment of H22 tumor cancer mice model

Extraction of H22 tumor ascites in Balb/c mice, mixed with normal saline by 1:3, was thoroughly homogenized and diluted to 4×10^7 cells/mL. A total of 0.2 mL of H22 tumor ascites was used for axillary vaccination in experimental mice, the whole process from the extraction of ascites to transplant completion was within 2 h. After vaccination 40 experimental mice were randomly divided into A, B, C, D four groups, each group of 10 mice. Group A was given 0.4 mL of normal saline for lavage, once a day. Group B was given 25 mg/g of 5-FU by intraperitoneal injection every other day; Group C was treated with 150 μg of LTA by lump body injection every other day; Group D was given LTA by lump body injection combined with 5-FU by intraperitoneal injection with the same dosage and usage with Groups B and C. Four groups of mice were treated for two weeks.

2.4. Observation items

Groups of mice were executed on the next day after the treatment. Stripped intact tumor tissue and blotted up with filter paper, weighted and calculated average weight and area of tumor tissue, and tumor inhibitory rate. After weighing, the tumor issue was conventional embedding, sectioning, immunohistochemical staining to observe the expression of caspase 3, EGFR and TGF-α proteins. The results judgment [6]: caspase 3 protein positive expression shows yellow or brown granules, mainly expressed in the cytoplasm; EGFR protein expression shows yellow and brown granules, mainly expressed in the cytoplasm and cell membrane; TGF-α protein positive expression shows yellow and brown granules, mainly expressed in the cytoplasm and cell membrane.

2.5. Statistical analysis

Data was analyzed by SPSS 10.0 software, and expressed as mean ± SD. They were analyzed by using the One-way ANOVA among groups. $P < 0.05$ was considered as statistically significant difference.

3. Results

3.1. Comparison of tumor inhibitory rate among groups mice

Tumor volume and weight of Groups B, C, D was significantly lower than that of Group A ($P < 0.05$). Tumor volume and weight of Groups B, D was significantly lower than that of Group C ($P < 0.05$). Tumor volume of Group D was significantly lower than that of Group B ($P < 0.05$), while tumor weight was significantly higher than group B ($P < 0.05$). Tumor inhibitory rate of Group D was significantly higher than that of Groups B and C ($P < 0.05$). The results were shown in Table 1.

3.2. Expression of caspase-3, EGFR, TGF-α proteins in tumor issue

The expression of caspase 3 protein in the tumor tissue of Groups B, C, D mice was significantly higher than that in Group A ($P < 0.05$), while the expression of EGFR and TGF-α proteins in Groups B, C, D was significantly lower than that in Group A ($P < 0.05$). Caspase 3 protein expression in Groups B and D was significantly higher than that in Group C ($P < 0.05$), while the expression of EGFR and TGF-α was significantly lower than that in Group C ($P < 0.05$). Expression of caspase 3 protein in Group D was significantly higher than in Group B ($P < 0.05$), while EGFR, TGF-α expression was significantly lower than in Group B ($P < 0.05$). The results were shown in Table 2.

Table 1
Comparison of tumor inhibitory rate among groups mice.

Groups	*n*	Tumor volume (cm^3)	Tumor weight (g)	Tumor inhibitory rate
Group A	10	1.79 ± 0.22	2.15 ± 0.21	–
Group B	10	0.61 ± 0.11*#	0.35 ± 0.20*#	46.19
Group C	10	0.44 ± 0.12*	0.97 ± 0.21*	54.62
Group D	10	0.11 ± 0.71*#△	0.62 ± 0.61*#△	71.82

*: Compared with Group A, $P < 0.05$; #: Compared with Group C, $P < 0.05$; △: Compared with Group B, $P < 0.05$.

Table 2
Expression of caspase-3, EGFR, TGF-α proteins in tumor issue of groups mice (IOD value).

Groups	*n*	Caspase-3	EGFR	TGF-α
Group A	10	2 280 901.7 ± 3 575 753.0	1723.67 ± 41.35	1845.43 ± 49.26
Group B	10	7 028 868.2 ± 3 048 702.8*#	855.76 ± 55.09*#	866.76 ± 54.99*#
Group C	10	5 867 940.6 ± 2 180 526.4*	1008.24 ± 19.47*	1086.24 ± 21.47*
Group D	10	8 998 374.1 ± 2 959 586.5*#△	697.24 ± 35.67*#△	697.24 ± 36.67*#△

*Compared with Group A, $P < 0.05$; #: Compared with Group C, $P < 0.05$; △: Compared with Group B, $P < 0.05$.

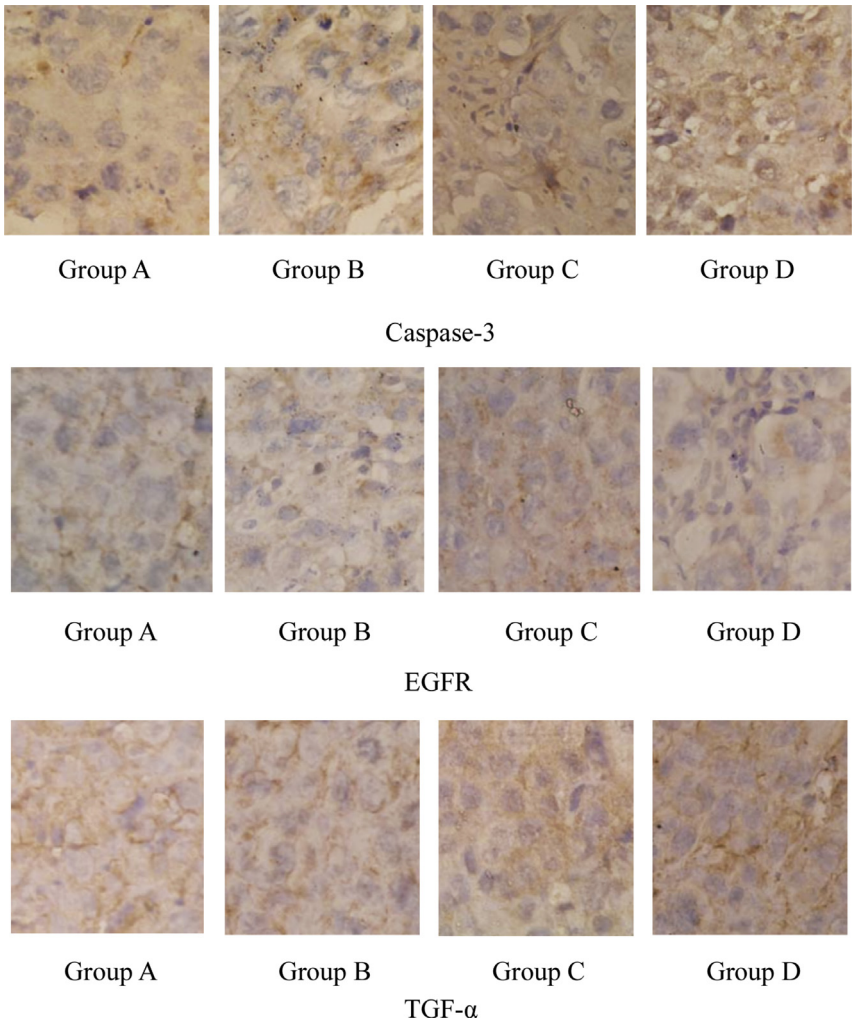

Figure 1. Expression of caspase-3, EGFR, TGF-α proteins in tumor tissue biopsies of different groups.

3.3. Microscopic observation of caspase-3, EGFR, TGF-α proteins expression in tumor issue biopsies

Microscopically, caspase-3 was more expression in the cytoplasm, which in group D expressed the highest; EGFR, TGF-α was expression in the cytoplasm and cell membrane, and mainly in cell membrane expression, expression of Groups C, B and D were significantly lower than that of Group A. The results were presented in Figure 1.

4. Discussion

Liver cancer is a clinically common malignant tumor, and the average age attacked by this disease was 44. It has a high malignant degree, developed fast, if not treated in time, the average survival time was only half a year, seriously affect the quality of life of patients [7]. The early stage of liver cancer has no obvious symptoms, once found, about one third of patients have been in advanced stage [8]. Liver cancer should be treated individualized with comprehensive treatment according to different stages of liver cancer. Surgery is the first choice for liver cancer treatment. For cancer cannot be removed by surgery after surgical exploration, or as follow-up treatment of a tumor palliative resection, comprehensive treatment with radiation as the major one can be used. But the solution has larger toxic and adverse reactions and greatly influent the patient's life [9]. Therefore, it is a clinically urgent issue to be solved that looks for an effective treatment schedule to improve the quality of life and survival time of the patients.

5-FU is a pyrimidine class antagonists, also is the main chemotherapy regimens drug for digestive system tumor. It has an obvious effect on the proliferation of tumor cells, but it has limited targeting property, the sensitivity declines after repeatedly use and lager side effects, which limit its use for a long time [9–12]. LTA, isolated and purified from bifidobacteria adolescentis, is a kind of linear glycerol phosphate amphoteric polymer related to the adhesion [13]. Studies have confirmed that [14–16], LTA can inhibit the growth of a variety of tumor cells. It is safe with non-toxic side effects and can be long-term use. At present, the main anti-tumor mechanism focused on two aspects, i.e. direct tumor suppression and improving the antitumor immunity of patients. In this study, after the treatment the tumor weight and volume in Groups B and C was significantly lower than that in Group A ($P < 0.05$). It proved that both the two drugs have a certain tumor suppression effect. No statistical difference was found in the inhibitory rate these two groups ($P < 0.05$), it indicated that both the two drugs has similar tumor suppression effect. After treatment inhibitory rate of tumor issue in Group D mice was significantly higher than in Groups B and C ($P < 0.05$), suggesting that the curative effect of combination of LTA and 5-FU in tumor suppression is significantly better than alone.

In recent years, the relationship between the incidence of liver cancer and the cytokines has become the research focus, but research reports on TGF-α are relatively few [17]. TGF-α is a polypeptide composed of 50 amino acid, played a special role in regulating the growth of normal cells and the transformation of abnormal cells [18]. EGFR is the expression product of proto-oncogene c-erbB-1. It is widely distributed in mammalian epithelial cell membrane and vascular tissue, and plays an important role in regulating cell proliferation and activation [19]. Studies have found that [20], TGF-α, EGFR can express in liver cancer tissues at the same time. Moreover, the excessive expression closely associated with the onset of cancer of the liver, and there is a significant positive correlation between these two expressions. The results of this study showed that the expression of EGFR and TGF-α proteins in Groups B, C, D after treatment was significantly lower than that in Group A ($P < 0.05$); EGFR, TGF-α expression in Group D was significantly lower than in Groups B and C ($P < 0.05$). This manifested that combined treatment can effectively inhibit the over expression of TGF-α and EGFR, inhibit tumor cell proliferation, thus achieve the therapeutic effect of tumor suppression. Caspase, according to its role in cell apoptosis, can be divided into two kinds, enzymes for initiating and effective enzymes, which is the great discovery in the research of apoptosis in molecular biology in recent years [21]. In caspase, caspase 3 is an important effective protein lyase, and plays an important role in the process of tumor cell apoptosis [22,23]. The results of this study showed that after the treatment the expression of caspase 3 in Group D was significantly higher than the other three ($P < 0.05$), suggesting that the LTA combined with 5-FU can improve the expression of caspase 3, promote tumor cell apoptosis, and thus play a synergistic antitumor effect.

According to the results of this study LTA combined with 5-FU can inhibit the over expression of TGF-α and EGFR expression, improve the expression of caspase 3, thus inhibit tumor cell proliferation and promote tumor cell apoptosis. Therefore, LTA combined with 5-FU has remarkable synergistic antitumor effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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