Original article

Bull Cancer 2015; 102: 204-212

en ligne sur / *on line on*
www.em-consulte.com/revue/bulcan
www.sciencedirect.com

Cooperative effect of Bifidobacteria lipoteichoic acid combined with 5-fluorouracil on hepatoma-22 cells growth and apoptosis

Bin Guo [1,2,4], Ning Xie [1,2,4], Yue Wang [3]

Received 31 July 2014
Accepted 29 September 2014
Available online: 4 March 2015

1. Affiliated Hospital of North Sichuan medical college, Department of Clinical Laboratory, Nanchong, Sichuan, 637000, China
2. North Sichuan medical college, Department of Clinical Laboratory, Nanchong, Sichuan, 637000, China
3. Chengdu medical college, Department of Clinical Laboratory, Chengdu, Sichuan, 610083, China

Correspondence:
Bin Guo, Affiliated Hospital of North Sichuan Medical College, Department of Clinical Laboratory, 63, WenHua Road, Nanchong, Sichuan, 637000, China.
guobin1368@163.com

Keywords
Apoptosis
Bifidobacterium
Lipoteichoic acid
5-fluorouracil
Immune function

Summary

Aims > To investigate the cooperative effect of Bifidobacteria lipoteichoic acid (BLTA) combined with 5-fluorouracil on tumor cells growth and apoptosis in mice bearing H22.
Methods > Hepatoma-22 (H22) cells were cultured in RPMI1640. Establish tumor-bearing mice model of liver cancer by injecting intraperitoneally 1×10^6/mL cells into the above-mentioned Balb/c mice. 5-FU alone, BLTA alone or BLTA in combination with 5-FU were used to treat tumor-bearing mice. The tumor size were observed and measured regularly. The growth-inhibiting rate (IR) of tumor was detected. Real-Time PCR and Western blot were used to detect Bcl-2, Bax and Caspase-3 expressions of mRNA and protein in tumor tissue of tumor-bearing mice. Detection of apoptotic cells in tumor tissue by HE staining analysis. Detection of the organ index was for evaluate the added-activity of immune organs in mouse. FCM was used to detect T subgroup ratio of spleen cells of tumor-bearing mice. Expression change of mRNA and proteins of Foxp3 and TIM-3 were detected by Real-Time PCR and Western blot in tumor-bearing mice tumor tissue.
Results > BLTA and 5-FU significantly inhibited the proliferation of tumor and induced obvious apoptosis, the combined effects were greater than those of the individual agents ($P < 0.01$). The underlying molecular mechanism of apoptotic process could be up-regulation of Bax and down-regulation of Bcl-2 and Caspase-3. The HE staining indicated that combined treat could both induce tissue cells necrosis and increase immune cells infiltration. Organ index showed that BLTA can enhance the proliferation of immune organs. The ratio of $CD4^+CD25^+$ Treg significantly decreased

[4] These authors contributed equally to this work.

Bulletin du CANCER
tome 102 > n°3 > March 2015
http://dx.doi.org/10.1016/j.bulcan.2014.09.003

and $CD4^+$ T cell increased in BLTA and 5-FU group ($P < 0.01$). Compared to NS group, mRNA and proteins expression of Foxp3 and TIM-3 down regulated in BLTA and 5-FU group ($P < 0.01$).
Conclusions > These results show that combined effects of Bifidobacteria lipoteichoic acid and 5-FU on H22 cells were superior to the individual. The combination did not only increase anti-tumor effect, but also could alleviate the side effects of chemotherapy, with inhibiting TIM-3/TIM-3L pathway, cutting down immunosuppressive activity of $CD4^+CD25^+$ Treg and enhancing cell-mediated immunity.

Mots clés
BLTA
5-FU
Apoptose
Treg
Foxp33
TIM-3

Résumé

Effet coopératif de l'acide lipotéichoïque de Bifidobacterium en combinaison avec le 5-fluorouracil sur la croissance et l'apoptose des cellules d'hépatome H22

Introduction > L'addition de l'acide lipotéichoïque de Bifidobacterium (BLTA) en combinaison avec le 5-fluorouracil (5FU) sur la croissance de cellules tumorales et sur l'apoptose chez des souris porteuses d'hépatome H22 a été étudiée.
Résultats > L'effet coopératif de BLTA est montré sur la nécrose et celui sur l'apoptose est corrélé avec la régulation positive de Bax et la régulation négative de Bcl-2 et de la Caspase-3. Cette même combinaison BLTA+5-FU diminue le taux de Treg $CD4^+CD25^+$ et augmente celui des T CD4+, tandis que, parallèlement, les quantités de Foxp33 et TIM-3 sont régulés négativement aux niveaux ARNm et protéine.
Conclusion > Au total, nous suggérons que l'effet coopératif de BLTA sur le 5-FU est due à l'inhibition, par la voie Foxp33/TIM-3, de l'activité immunosuppressive de Treg $CD4^+CD25^+$.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases and is the second leading cause of cancer-related deaths worldwide. Generally, the primary tumor of most patients can be cured with surgical resection, however, due to early distant metastases, the five-year survival rate is only approximately 15–40% in China. Systemic chemotherapy is regarded as one of the most effective treatments to improve survival, but is limited because of the toxicity of the agents [1]. Therefore, combined treatments are often used to enhance efficacy and reduce toxicity [2,3].
5-fluorouracil (5-FU) is universally used in the treatment of hepatocellular carcinoma. It can lead to cell death by interfering with nucleoside metabolism including RNA dysfunction and DNA synthesis disorders [4]. However, due to drug resistance, 5-FU alone remains only about 15% efficiency. To improve the prognosis of patients with liver cancer, more effective anti-tumor regimens are urgently needed.
Bifidobacterium is an important normal physiological bacterium in human and animal colon. Lipoteichoic acid of Bifidobacterium (BLTA) had been reported to have the anti-tumor and positive immuno-regulation effects [5]. However, the anti-tumor effects of BLTA combined with chemotherapy and related mechanisms are still unclear. Thus, the study established the hepatoma model of Balb/c mice with murine hepatoma-22 (H22) cells, investigated the therapeutic efficacy of BLTA in combination with 5-FU treatment and the effects of BLTA on immunological regulation.

Materials and methods

Chemicals and cell culture

5-FU were purchased from Tianjin Jinyao amino acid Co. Ltd and dissolved at 100 mmol/L in dimethylsulfoxide (DMSO) for storage at –20 °C. Hepatoma-22 (H22) was provided by the Department of Pathophsiology of Chongqing Medical University.
Cells were cultured in RPMI1640 containing 10% fetal bovine serum with maintaining at 37 °C in a 95% humidified atmosphere of 5% CO_2 in air.

Establishment of animal model

Six-to-eight-week-old female Balb/c mice were obtained from the Animal Center of Chongqing Medical University (Chongqing, China). H22 cells (density: 1×10^6/mL; viability: > 95%) were inoculated subcutaneously at the right armpit of Balb/c mice. Generally, the solid tumor could be palpated after 4–5 days with an achievement ratio of nearly 100%.

Lipoteichoic acid of Bifidobacterium

Bifidobacterium bifidum 86321 was provided by the Department of Pathogenic Biology of Chongqing Medical University, and identified by microorganism research institute of Academia