SIGNAL MECHANISM OF INHIBITION OF BIFIDOBACTERIA ON GROWTH OF COLON CANCER

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ABSTRACT

Objective: To explore the antitumor mechanisms of bifidobacteria adolescence in vivo. Methods: The content of extracellular signal regulated proteins (ERK)1/2, C-Jun N-terminal kinase (JNK), p38, c-fos and c-jun in nude mouse transplanted large bowel carcinoma was detected by using laser confocal microscopy. The expression of NF-κB was determined by immunohistochemistry. Results: After the nude mouse transplanted tumor was treated with bifidobacteria, the average fluorescent strength of ERK1/2, JNK, c-fos and c-jun was significantly lower than that in tumor control group (P<0.01). The average fluorescent strength of p38 was not obvious difference in the two groups (P>0.05). The positive cell density of NF-κB in large bowel carcinoma transplantation tumors in Bifidobacterium injection group was markedly lower than that in tumor group (P<0.01). Conclusion: bifidobacteria adolescence could markedly decrease the activity of ERK1/2 and JNK, the expression c-fos and c-jun, and the activity of NF-κB.

Key words: Bifidobacteria; Colon cancer; Signal transduction; C-jun N-terminal kinase; Extracellular signal-regulated protein kinase; NF-κB

Bifidobacteria, a physiologically beneficial organism in intestinal tract of human body, are the most in number with the most important function. It can prevent occurrence and evolution of a great variety of tumors in human body. We have proved that the bifidobacteria adolescence could inhibit the growth of colon cancer significantly in vivo and explored its antitumor mechanisms from the angle of cellular apoptosis[1,2]. Nowadays it has been known that in colon cancer there exists a high-level expression of Epithelial Growth Factor Receptor (EGFR). EGFR possesses the activities of PTK (Protein Tyrosine Kinase), and after being conjugated with the ligand, can itself be dimerized and phosphorylated. Then by the two main ways of Ras-Raf-1 (MAP Kinase Kinase Kinase)→MAP Kinase Kinase→AP-1 and Protein Kinase C (PKC)→IK Kinase B→NF-κB (Nuclear factor-κB), the signal will be transmitted into the nuclei, which ultimately leads to an increase of the level of intranuclear early reaction gene transduction, cell proliferation and malignancy transformation[3]. With the purpose of exploring further the antitumor mechanisms, we used the laser confocal microscope to observe the influences of bifidobacteria adolescence on the activities of colon cancer MAPK (mitogen-activated protein kinase) and AP-1(activator protein 1) in vivo, and determined the expression of NF-κB by immunohistochemistry.
BALB/c male nude mice of 6–8 weeks' old and 18–22 grams were used. The animals were housed in the SPF animal room, and purchased from experimental animal center, Nanfang Medical University.

Source of Bacteria Species and Cultivation

Bifidobacteria were obtained in this institute by isolation and cultivation of the healthy infantile feces. The adolescent type was identified through API-20A and TAB system (English Product) and repeated biochemical reactions. In the experiment, Bifidobacteria were inoculated into the thioglycollate broth, and then put inside the anaerobic incubator at 37°C for 72 h. The culture liquid containing bacteria was centrifuged at 3000 r/min for 10 min, and the supernatant fluid was discarded. The sediment was washed three times with PBS solution, and the number of bacteria was adjusted to $1 \times 10^7/\text{ml}$.

Colorectal Carcinoma Cell Line

Lovo cells, a human undifferentiated colorectal carcinoma cell line, were suspended in RPMI medium 1640 supplemented with 10% fetal calf serum routinely. When Lovo cells grew to form a monolayer, single cell suspension was prepared by trypsin digestion. The cells were adjusted to required density of $1 \times 10^7/\text{ml}$.

The Establishment of Animal Model of Colorectal Carcinoma Transplantation Tumors in Nude Mice and Antitumor Experimentation of Bifidobacteria

The animals were divided into two groups. (1) In tumor control group, 20 nude mice were inoculated with $2 \times 10^6 (0.2 \text{ ml})$ Lovo cells in the left flank on day 1 subcutaneously. From day 2, each of 0.2 ml isotonic phosphate buffered saline (PBS) was injected into these animals intraperitoneally for 5 times every other day. (2) In Bifidobacterium injection group, the number, site and schedule of the inoculated Lovo cells was similar to tumor control group on day 1. From day 2, $2 \times 10^8 (0.2 \text{ ml})$ bifidobacteria were injected intraperitoneally for 5 times every other day. All animals were killed on day 21 after tumor inoculation. The fresh tumor tissues were embedded in paraffin wax routinely, stained with haematin and eosin, and verified to be undifferentiation colon cancer.

Determination of the Activities of ERK 1/2, JNK and p38 and the Expression of c-fos, c-jun in Colon Cancer Tissues

The laser confocal microscope was used to carry out a quantitative determination, and the specific steps were as following: (1) the dewaxing section was made routinely and the activities of endogenous peroxidase were blocked by 0.3% H$_2$O$_2$ methanol; (2) the specimen was washed 3 times and 5 minutes a time with 0.01 mol/L PBS (pH7.2) solution; (3) 10% bovine serum albumin was added and then cultivated at room temperature for 10 minutes; (4) the slide was washed with 0.01 mol/L PBS solution (pH7.2) 3 times and 5 min a time. (5) antibodies against p42/p44 MAPK (ERK 1/2, THR 202/Tyr204), phosphorylated JNK, phosphorylated p38 (purchased from New England Biolab Company), human c-fos and c-jun (product of Santa Cruz Company) were added respectively, and cultivated at 37°C for 60 min; (6) 0.01 mol/L PBS solution (pH7.2) was added and the slide was washed 3 times 5 min a time; (7) 1:100 FITC-labeled sheep anti-rabbit IgG was added and cultivated at 37°C for 30 min; (8) the slide was fully washed with 0.01 mol/L PBS solution (pH7.2) 5 times and 5 min a time; (9) the slide was sealed by non-fluorescence buffer glycerin, and the margin of the cover glass was sealed and fixed by nail polish; (10) The fluorescence intensity of colon cancer cells was measured by the confocal microscope. The activating wave length of 488 nm and the transmitting wave length of 475 nm were chosen. In different optic fields of each slide, more than 100 cells were measured, and average fluorescence value was taken as the quantitative parameter.

NF-κB Immunohistochemistry Staining

The procedure of staining was carried out routinely according to the SP method of immunohistochemistry. The fist antibody was rabbit anti-p65 polyclonal antibody, and the second antibody was biotinylated sheep anti-rabbit IgG. PBS was taken to replace the first antibody as the negative control.

Observation Indices and Statistical Analysis

Each section was observed, using 16D eyepiece microgrid to count the number of positive cells with stained cellular nuclei in the lattice. 10 lattices were taken in each section. The average value was taken as the positive cells density in NF-κB staining and t-test was used for statistical analysis and comparison among the rest of the groups.

RESULTS

Influence of Bifidobacteria on the Activities of ERK1/2, JNK and p38 of Colon Cancer

The laser confocal microscope was used for a