ANTI-TUMOR COMPOSITION

FIELD OF THE INVENTION

[0001] The present application relates to an anti-tumor composition, and more particularly to combined use of *Bifidobacterium longum* and an immune checkpoint inhibitor.

RELATED ART

[0002] Curing cancer has always been a major challenge in the medical field, and traditional treatment methods such as chemotherapy and radiotherapy can cause greater side effects and pain to patients. In recent years, immunotherapy for cancer has shown amazing efficacy in hematologic tumors and some solid tumors with fewer side effects on patients, which gives great hope for curing cancer in humans.

[0003] Under normal circumstances, there are antigens on the surface of tumor cells that can be recognized by human immune T cells. And the human immune system is able to recognize and kill the tumor cells. However, in order to survive and grow, the tumor cells will adopt various ways to avoid recognition and killing by the immune system. Tumor immunotherapy is an anti-tumor immune response that identifies and destroys such trick of the tumor cells to restore the body to normal, so as to control and eliminate a wide range of tumors. Tumor immunotherapy includes five main types: (1) therapeutic antibodies; (2) cancer vaccines; (3) cell therapy, i.e., CAR-T therapy; (4) immunomodulators; and (5) immune checkpoint inhibitors.

[0004] The activity of the human immune system is regulated by co-stimulatory molecules, and the co-stimulatory molecules are immune checkpoints. When antigen recognition occurs, other molecules interact with immune cells and target cell surface molecules, which in turn determine the balance of interactions. If the signal is largely positive, the immune cells will be activated and attack the antigen presented by the target cell. Conversely, if the signal is negative, the immune cells will be inactivated. This inactivation is sometimes permanent, and the antigen is recognized as a normal/self-antigen. Cancer-related immune checkpoints that have been relatively well established include CTLA-4, PD-1 and PD-L1. Immune checkpoint inhibitors are monoclonal antibody medicaments developed to target the corresponding immune checkpoints. The main role of the immune checkpoint inhibitors is to block the interaction between the tumor cells expressing the immune checkpoints and the immune cells, thus blocking the inhibitory effect of the tumor cells on the immune cells. Currently widely used immune checkpoint inhibitors include monoclonal antibodies targeting programmed death protein 1 (PD-1) and its ligand (PD-L1).

PD-1 antibodies are very effective in blocking advanced melanoma, non-small cell lung cancer and renal cell carcinoma, and have made a major breakthrough in the treatment of tumors. However, due to the complex pathogenesis of tumors, the large individual differences for patients, and the influence of environmental factors, the immune checkpoint inhibitors can only be effective in about 25% of patients at present.

[0005] More and more research suggests that gut microbes are also an important factor affecting the effectiveness of cancer immunotherapy. In 2015, Marie Vétizou et al. found that tumors inoculated into antibiotic-treated or germ-free mice did not respond to CTLA-4 inhibitors, and that the anti-tumor effects of CTLA-4 inhibitors were restored when *Bacteroides fragilis* was administered to the mice. In 2017, a study by Routy B et al. showed that anti-PD-1 inhibitors in combination with certain specific gut microbiota significantly improved the response of tumor patients and significantly improved the mean progression-free survival (PFS) of the patients. In summary, gut microbes have great potential for use in improving the efficacy of immune checkpoint inhibitors. Therefore, there is a need in the art to develop a new anti-tumor composition with better anti-tumor effects, especially with better anti-tumor effects on specific tumors.

SUMMARY

[0006] The inventors of the present application have found that the combined use of *Bifidobacterium longum* and an immune checkpoint inhibitor is effective in inhibiting various tumors including, but not limited to, colon cancer, lung cancer, breast cancer, melanoma, kidney cancer, urothelial cancer, and the like.

[0007] One aspect of the present application provides an anti-tumor composition, which includes:

[0008] (A) *Bifidobacterium longum*; and

[0009] (B) an immune checkpoint inhibitor.

[0010] In one embodiment of the present application, the *Bifidobacterium longum* is *Bifidobacterium longum 6-1*.

[0011] In one embodiment of the present application, the *Bifidobacterium longum* is combined with other bacteria (such as *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Enterococcus faecium*, *Prevotella stercorea*, and *Ruminococcus*).

[0012] In one embodiment of the present application, the *Bifidobacterium longum* and the immune checkpoint inhibitor are mixed together to prepare a single preparation, or physically separated and used separately.

[0013] In one embodiment of the present application, the *Bifidobacterium longum* is in the form of oral administration or injection administration.

[0014] In one embodiment of the present application, the anti-tumor composition further comprises antibiotics.

[0015] In one embodiment of the present application, the tumor is selected from colon cancer, lung cancer, gastric cancer, liver cancer, head and neck cancer, cervical cancer, breast cancer, lymphoma, breast cancer, melanoma, kidney cancer or urothelial cancer.

[0016] The present application also provides use of *Bifidobacterium longum* in the manufacture of a medicament for treating tumors.

[0017] In one embodiment of the present application, the *Bifidobacterium longum* is used in combination with an immune checkpoint inhibitor preparation.

[0018] In one embodiment of the present application, the use is to improve the effect of the immune checkpoint inhibitor in suppressing tumors, preferably, the tumors are selected from colon cancer, lung cancer, gastric cancer, liver cancer, head and neck cancer, cervical cancer, breast cancer, lymphoma, breast cancer, melanoma, kidney cancer or urothelial cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 depicts the effect of antibiotic disruption of gut microbiota on tumor growth and anti-mPD-1 treatment effect in MC38 tumor-bearing mice.

[0020] Figure 2 depicts the effects of injection of anti-mPD-1 alone and oral administration of *Bifidobacterium longum 6-1* alone on tumor growth in MC38 tumor-bearing mice, respectively.

[0021] Figure 3 depicts the tumor treatment effect of combined use of anti-m PD-1 and *Bifidobacterium longum 6-1* on MC38 tumor-bearing mice.

[0022] Figure 4 depicts the difference in tumor treatment effects of combined use of different probiotics and anti-m PD-1 in MC38 tumor-bearing mice.

[0023] Figure 5 depicts the mean number of CD3 cells in MC38 tumor-bearing mice when anti-m PD-1 is injected alone and used in combination with *Bifidobacterium longum 6-1*.

[0024] Figure 6 depicts the number of tumor-infiltrating CD8 T cells in MC38-bearing mice when anti-m PD-1 is injected alone and used in combination with *Bifidobacterium longum 6-1*.

[0025] Figure 7 depicts the therapeutic effects of anti-m PD-1 injected alone and used in combination with *Bifidobacterium longum 6-1* on 4T1 tumors.

[0026] Figure 8 depicts the therapeutic effects of anti-m PD-1 injected alone and used in combination with *Bifidobacterium longum 6-1* on LLC1 tumors.

DETAILED DESCRIPTION

[0027] In the present invention, unless otherwise specified, percent (%) or parts refer to percent by weight or parts by weight relative to the composition.

[0028] In the present invention, unless otherwise specified, various components or exemplary components thereof involved herein may be combined with each other to form a new technical solution.

[0029] In the present invention, unless otherwise specified, all embodiments or exemplary embodiments mentioned in this specification may be combined with each other to form a new technical solution.

[0030] In the present invention, unless otherwise specified, all technical features or exemplary technical features mentioned in this specification may be combined with each other to form a new technical solution.

[0031] In the present invention, the sum of the contents of the components in the composition is 100%, if not stated to the contrary.

[0032] In the present invention, the sum of the parts of the components in the composition may be 100 parts by weight, if not stated to the contrary.

[0033] In the present invention, the numerical range "a-b" means an abbreviated representation of any combination of real numbers between a and b, where both a and b are real numbers, unless otherwise specified. For example, the numerical range "0-5" means that all real numbers between "0-5" have been listed herein, and "0-5" is only an abbreviated representation of the combination of these values.

[0034] In the present invention, the integer numerical range "a-b" means an abbreviated representation of any combination of integers between a and b, where both a and b are integers, unless otherwise specified. For example, the integer numerical range "1-N" means 1, 2, ...N, where N is an integer.

[0035] In the present invention, "combination thereof" means a multi-component mixture of said components, such as two-, three-, four- and multi-component mixture with the largest possible number of components, unless otherwise specified.

[0036] The term "a" as used in this specification means "at least one", if not specifically indicated.

[0037] The basis for the percentages (including weight percentages) as described in the present invention is the total weight of said composition, if not specifically indicated.

[0038] The "scope" disclosed herein is in the form of lower and upper limits. There can be one or more lower limits, and one or more upper limits, respectively. The given range is defined by selecting a lower limit and an upper limit. The selected lower and upper limits define the boundaries of the special range. All ranges that can be defined in this manner are inclusive and combinable, i.e., any lower limit can be combined with any upper limit to form a range. For example, ranges 60-120 and 80-110 are listed for specific parameters, with the understanding that ranges 60-110 and 80-120 are also to be expected. In addition, if the minimum range values 1 and 2 are listed, and if the maximum range values 3, 4, and 5 are listed, the following ranges can all be expected: 1-3, 1-4, 1-5, 2-3, 2-4, and 2-5.

[0039] As used herein, the proportions or weights of the components refer to dry weight unless otherwise stated.

[0040] As used herein, "constant" means a change within ±10%, preferably within ±5%, more preferably within ±2%, and finally within ±1%, unless otherwise stated.

[0041] As used herein, *Bifidobacterium longum* includes active ingredients derived from *Bifidobacterium longum*, or a strain having at least 99% sequence similarity with *Bifidobacterium longum* identified by sequencing.

[0042] <u>*Bifidobacterium longum*</u>

[0043] The *Bifidobacterium longum* described in the present application can be obtained from commercially available sources or according to prior art. For example, the *Bifidobacterium longum* can be obtained according to CN103830278A, CN103131647A, CN101244090A, CN101244089A, CN1223865A or US6368591.

[0044] The *Bifidobacterium longum* described in the present invention is conventional in the art and can be isolated from healthy adult feces. For example, it has been disclosed in issued US6368591, where *Bifidobacterium longum* is deposited under CCTCC M98003.

[0045] *Bifidobacterium longum* is an anaerobic bacterium with positive Gram's stain, uneven coloring, no spores, no capsules, and no flagella, and the cells are straight or curved, and can be in the form of "Y" or "V" type branched shape, rod-like shape and various other forms. In a preferred embodiment of the present invention, the *Bifidobacterium longum* is *Bifidobacterium longum (6-1).*

[0046] In a preferred embodiment of the present application, the *Bifidobacterium longum* may be in the form of *Bifidobacterium longum* powder. In an embodiment of the present application, the *Bifidobacterium longum* powder includes *Bifidobacterium* and a first protectant, and the first protectant contains 8-50% of skimmed milk powder, 0.01-10% of sodium glutamate, 8-50% of allolactose, 0.01-5% of Vc-Na, and 4-30% of starch, based on the total weight of the first protectant.

[0047] The *Bifidobacterium longum* powder can be formulated into various suitable dosage forms, such as oral solution, tablet, capsule, orally disintegrating tablet, lyophilized powder and the like. In a preferred embodiment of the present application, the dosage form is a capsule. In another preferred embodiment of the present application, the dosage form is a tablet. The dosage form is preferably a lyophilized bacterial preparation, in which the number of viable bacteria is preferably 10^{10} CFU/g.

[0048] In a preferred embodiment of the present application, the *Bifidobacterium longum* can also be used in combination with other bacterial strains (including but not limited to *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Enterococcus faecium*, *Prevotella stercorea*, *Ruminococcus,* and the like).

[0049] Immune checkpoint inhibitor

[0050] The immune checkpoint inhibitor used in the present application can be any commercially available product, such as PD-1 inhibitor, PD-L1 inhibitor, CTLA-4 inhibitor, and the like. In a preferred embodiment, the immune checkpoint inhibitor includes Keytruda, Tecentriq, Nivolumab Injection, Bavencio (Avelumab), and Tuoyi (Toripalimab Injection).

[0051] Anti-tumor composition

[0052] In the present application, the *Bifidobacterium longum* and the immune checkpoint inhibitor can be mixed together to prepare a single preparation for combined use, or can be physically separated and used separately. In one embodiment of the present application, the *Bifidobacterium longum* and the immune checkpoint inhibitor are physically separated and used separately. In another embodiment of the present application, the *Bifidobacterium longum* can be administered to a patient prior to the administration of the immune checkpoint inhibitor. In general, the *Bifidobacterium longum* can be administered to the patient in any suitable manner (including, but not limited to, oral administration, injection, and the like). The immune checkpoint inhibitor can be administered to the patient in any suitable manner (including, but not limited to, oral administration, injection, and the like). The method for using the *Bifidobacterium longum* or the immune checkpoint inhibitor

is routine in the art, and can be directly determined by those of ordinary skill in the art according to the description in the specification in combination with the prior art.

[0053] In the present application, the anti-tumor composition may further include antibiotics. The antibiotic can be any suitable antibiotic, such as, but not limited to, quinolone antibiotics, β-lactam antibiotics, macrolides, aminoglycoside antibiotics, and the like. In a preferred embodiment of the present application, the antibiotics include but are not limited to β-lactam antibiotics (such as penicillin, ampicillin, carbenicillin, methicillin, oxacillin, dicloxacillin, flucloxacillin, cefradine, cefotaxime, cefalexin, ceftriaxone, cefpirome, cefixime, cefditoren pivoxil, cefdinir, ceftibuten, cefpodoxime proxetil, imipenem, aztreonam, cefminox sodium, biapenem, imipenem, meropenem, and the like); macrolide antibiotics (such as erythromycin, albomycin, roxithromycin, erythromycin ethylsuccinate, azithromycin, clarithromycin, acetyl spiramycin, meleumycin, medemycin, josamycin, telithromycin, and the like); aminoglycoside antibiotics (streptomycin, gentamicin, arbekacin, amikacin, and the like); quinolone antibiotics (such as ciprofloxacin, levofloxacin, norfloxacin, and the like); other antibiotics and antibacterial drugs (such as tetracyclines, chloramphenicols, lincomycin, rifamycins such as rifapentini, polypeptides such as vancomycin, sulfonamides such as sulfamethoxazole, metronidazoles, and the like); antifungal drugs (such as amphotericin, griseofulvin, Daktarin, and the like); and anti-tumor antibiotics (such as mitomycin, actinomycin D, bleomycin, doxorubicin, and the like) and the like.

[0054] In the present application, the antibiotic may be packaged separately from other components and administered separately.

[0055] In a preferred embodiment of the present application, the anti-tumor composition further includes instructions describing administration of *Bifidobacterium longum* for a period of time (e.g., 1-10 days), followed by administration of the PD-1 inhibitor for a period of time (e.g., 10-100 days). Preferably, a dose range of *Bifidobacterium longum* is 10^4-10^{13} CFU/person/day, and a dose range of the immune checkpoint inhibitor is 0.5-10 mg/kg.

[0056] The instructions also describe the administration of antibiotics for a period of time (e.g., 1-3 days) prior to the administration of *Bifidobacterium longum* and the PD-1 inhibitor. Preferably, a dose range of the antibiotics is 1-500 mg/kg.

[0057] The anti-tumor composition of the present application can effectively treat various tumors, especially (but not limited to) colon cancer, lung cancer, breast cancer, melanoma, kidney cancer, urothelial cancer and the like.

[0058] Another aspect of the present application provides use of *Bifidobacterium longum* in the manufacture of a medicament for treating tumors.

[0059] In the present application, the *Bifidobacterium longum* can be combined with immune checkpoint inhibitors to inhibit the growth of tumors (such as colon cancer, lung cancer, breast cancer, melanoma, kidney cancer, urothelial cancer, and the like). The inventors found that the effect of the immune checkpoint inhibitors in inhibiting tumor growth is limited when antibiotics are administrated to patients, however, administration of *Bifidobacterium longum* prior to or concurrently with the immune checkpoint inhibitors may effectively enhance the tumor suppressive effect of the immune checkpoint inhibitors.

[0060] Another aspect of the present application provides use of *Bifidobacterium longum* in treatment of tumors. In one embodiment of the present application, the use includes inhibiting tumor growth. Preferably, the *Bifidobacterium longum* may be used in combination with an immune checkpoint inhibitor. Typically, the tumors include, but are limited to, colon cancer, lung cancer, breast cancer, melanoma, kidney cancer, urothelial cancer, and the like.

[0061] This application is described in detail below with reference to the embodiments, but the scope of this application is not limited thereto.

[0062] The raw materials used in the examples are as follows:

[0063] SPF grade male C57BL/6J mice aged 6-8 weeks (20-26 g) are from Jiangsu Jicui Yaokang Biotechnology Co., Ltd. with a quality certificate number of 201805120. The rearing conditions are as follows: the temperature is controlled at (23±3)°C, the humidity is 40-70%, and the mice are allowed to eat and drink freely.

[0064] PD-1 inhibitor is purchased from Yikang (Beijing) Pharmaceutical Technology Co., Ltd. at a concentration of 7.09 mg/mL, and stored at 2-8°C in the dark. An appropriate amount of phosphate buffered saline is added and well mixed to a specified concentration.

[0065] Ampicillin is purchased from Anhui Anfengtang Animal Medicine Industry Co., Ltd., Streptomycin is purchased from Solarbioy, and Colistin Sulfate Soluble Powder is purchased from Shandong Luxi Animal Medicine Share Co., Ltd.

[0066] MC38 tumor cells are purchased from Prutine Biotechnology (Beijing) Co., Ltd. and are cultured with a DMEM medium containing inactivated 10% fetal bovine serum, 100 U/mL of penicillin, 100 μg/mL of streptomycin and 2 mM of glutamine in a 5% CO_2 incubator at 37°C. The cells are split into flasks and passaged every 3 days or so when the

incubator is full of cells. The tumor cells in the logarithmic growth phase are used for tumor inoculation in vivo.

[0067] Mouse breast cancer 4T1 cells (ATCC, product number: CRL-2539), and LLC1 lung cancer cells (purchased from Shanghai Institutes for Biological Sciences).

[0068] *Bifidobacterium longum 6-1* is *Bifidobacterium longum 6-1* (CCTCC M98003).

[0069] Measurement of tumor volume:

[0070] Tumor volume: a vernier caliper is used to measure the tumor volume 3 times a week, and the long and short diameters of the tumor are measured. The calculation formula for tumor volume is: volume=0.5 × long diameter × short diameter^2.

[0071] Example 1

[0072] 48 male C57BL/6J mice aged 6-8 weeks were randomly divided into 4 groups based on body weight after acclimatization for one week, with 12 mice in each group: group 1 (no antibiotic treatment, blank, *i.p.*), group 2 (no antibiotic treatment, anti-mPD-1, 10 mg/kg, *i.p.*), group 3 (antibiotic treatment, blank, *i.p.*), and group 4 (antibiotic treatment, anti-mPD-1, 10 mg/kg, *i.p.*). Antibiotic treatment was done by using broad-spectrum antibiotics Ampicillin (1 mg/mL) + colistin (1 mg/mL) + streptomycin (5 mg/mL) in drinking water for 5 days. All groups were subcutaneously inoculated with MC38 tumor cells resuspended in phosphate buffered saline (PBS) at a concentration of 1×10^7 cells/mL to the right flank of experimental animals at 100 µL/mouse. Mice in groups 2 and 4 were injected with anti-mPD-1 on day 4 after tumor cell inoculation, once every 4 days, for a total of 4 injections. Mice were euthanized on day 19 after tumor inoculation.

[0073] MC38 tumor cells were purchased from Prutine Biotechnology Co., Ltd. and were cultured with a DMEM medium containing inactivated 10% fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin and 2 mM of glutamine in a 5% CO_2 incubator at 37°C. The cells were split into flasks and passaged every 3 days or so when the incubator is full of cells. The tumor cells in the logarithmic growth phase were used for tumor inoculation in vivo.

[0074] The test results were listed in Figure 1. The results showed that antibiotic treatment disrupted gut microbiota of the mice, accelerated tumor growth and increased tumor volume in the mice, and had certain influence on the therapeutic effect of anti-m PD-1. The gut microbiota affected the tumor growth and the therapeutic effect of anti-m PD-1 in the mice.

[0075] Example 2

[0076] 36 male C57BL/6J mice aged 6-8 weeks (20-26 g, SPF grade) were randomly divided into 3 groups based on body weight after acclimatization for one week: group 1 (antibiotic treatment, blank, *i.p.*), group 2 (antibiotic treatment, anti-mPD-1, 10 mg/kg, *i.p.*), and group 3 (antibiotic treatment, *Bifidobacterium longum 6-1*, *p.o.*). Antibiotic treatment was done by using broad-spectrum antibiotics Ampicillin (1 mg/mL) + colistin (1 mg/mL) + streptomycin (5 mg/mL) in drinking water for 5 days. Mice in group 3 were given with lyophilized samples of *Bifidobacterium longum* by gavage at a concentration of 1.0×10^8 CFU/mouse/day. After 2 weeks of continuous gavage, mice in all groups were subcutaneously inoculated with MC38 tumor cells resuspended in PBS at a concentration of 1×10^7 cells/mL to the right flank of experimental animals at 100 μL/mouse. Mice in group 2 were injected with anti-mPD-1 on day 4 after tumor cell inoculation, once every 4 days, for a total of 4 injections. Mice were euthanized on day 19 after tumor inoculation.

[0077] MC38 tumor cells were purchased from Prutine Biotechnology Co., Ltd. and were cultured with a DMEM medium containing inactivated 10% fetal bovine serum, 100 U/mL of penicillin, 100 μg/mL of streptomycin and 2 mM of glutamine in a 5% CO_2 incubator at 37°C. The cells were split into flasks and passaged every 3 days or so when the incubator is full of cells. The tumor cells in the logarithmic growth phase were used for tumor inoculation in vivo.

[0078] The test results were listed in Figure 2. Oral administration of *Bifidobacterium longum 6-1* could achieve the same effect as anti-mPD-1 injection therapy in tumor-bearing mice with disrupted gut microbiota, and thus *Bifidobacterium longum 6-1* had certain inhibitory effect on tumor growth.

[0079] Example 3

[0080] 36 male C57BL/6J mice aged 6-8 weeks (20-26 g, SPF grade) were randomly divided into 3 groups based on body weight after acclimatization for one week: group 1 (antibiotic treatment, blank, *i.p.*), group 2 (antibiotic treatment, anti-mPD-1, 10 mg/kg, *i.p.*), and group 3 (antibiotic treatment, *Bifidobacterium longum 6-1*, *p.o.*, anti-mPD-1, 10 mg/kg, *i.p.*). Antibiotic treatment was done by using broad-spectrum antibiotics Ampicillin (1 mg/mL) + colistin (1 mg/mL) + streptomycin (5 mg/mL) in drinking water for 5 days. Mice in group 3 were given with lyophilized samples of *Bifidobacterium longum* by gavage at a concentration of 1.0×10^8 CFU/mouse/day for 2 weeks of continuous gavage. Mice in all groups were subcutaneously inoculated with MC38 tumor cells resuspended in PBS at a concentration of 1×10^7 cells/mL to the right flank of experimental animals at 100 μL/mouse. Mice in groups 2 and 3 were injected with anti-mPD-1 on day 4 after tumor cell inoculation,

once every 4 days, for a total of 4 injections. Mice were euthanized on day 19 after tumor inoculation.

[0081] Culture of tumor cells: the tumor cells were cultured with a DMEM medium containing inactivated 10% fetal bovine serum, 100 U/mL of penicillin, 100 μg/mL of streptomycin and 2 mM of glutamine in a 5% CO_2 incubator at 37°C. The cells were split into flasks and passaged every 3 days or so when the incubator is full of cells. The tumor cells in the logarithmic growth phase were used for tumor inoculation in vivo.

[0082] The results were shown in Figure 3. The results showed that the oral administration of *Bifidobacterium longum 6-1* in combination with the injection administration of anti-m PD-1 could better inhibit tumor growth, reduce tumor size, and achieve better therapeutic effects on tumors.

[0083] Example 4

[0084] 48 male C57BL/6J mice aged 6-8 weeks (20-26 g, SPF grade) were randomly divided into 4 groups based on body weight after acclimatization for one week: group 1 (antibiotic treatment, blank, *i.p.*), group 2 (antibiotic treatment, *Bifidobacterium longum 6-1*, *p.o.*, anti-mPD-1, 10 mg/kg, *i.p.*), group 3 (antibiotic treatment, *Bifidobacterium longum BL2* (feces samples from healthy individuals), *p.o.*, anti-mPD-1, 10 mg/kg, *i.p.*), and group 4 (antibiotic treatment, *Bifidobacterium longum BL3* (feces samples from healthy individuals), *p.o.*, anti-mPD-1, 10 mg/kg, *i.p.*). Antibiotic treatment was done by using broad-spectrum antibiotics Ampicillin (1 mg/mL) + colistin (1 mg/mL) + streptomycin (5 mg/mL) in drinking water for 5 days. Mice in groups 2-4 were given with lyophilized samples of *Bifidobacterium longum* by gavage at a concentration of 1.0×10^8 CFU/mouse/day for 2 weeks of continuous gavage. Mice in all groups were subcutaneously inoculated with MC38 tumor cells resuspended in PBS at a concentration of 1×10^7 cells/mL to the right flank of experimental animals at 100 μL/mouse. Mice in groups 2-4 were injected with anti-mPD-1 on day 4 after tumor cell inoculation, once every 4 days, for a total of 4 injections. Mice were euthanized on day 19 after tumor inoculation.

[0085] Culture of MC38 tumor cells: the tumor cells were cultured with a DMEM medium containing 10% of inactivated fetal bovine serum, 100 U/mL of penicillin, 100 μg/mL of streptomycin and 2 mM of glutamine in a 5% CO_2 incubator at 37°C. The cells were split into flasks and passaged every 3 days or so when the incubator is full of cells. The tumor cells in the logarithmic growth phase were used for tumor inoculation in vivo.

[0086] The results were shown in Figure 4. The results showed that the oral administration of *Bifidobacterium longum 6-1* in combination with the injection

administration of anti-m PD-1 could better inhibit the tumor growth and reduce the tumor volume.

[0087] Example 5

[0088] The mice in Example 3 were euthanized on day 19 after tumor inoculation, and flow cytometry was used to detect CD3, CD4, CD8, FOXP3, CD25, CXCR3, Gata3, Granzyme B, CD69, PD-1, CTLA-4 and CD11b, MHC II, CD206, CD40, CSF1R, PD-L1, and Gr-1 in tumor cells. Immune factor analysis included TNF-α, IL-17, IL-13, IL-12p70, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP-10, MIP1b and MAC-1. The results were shown in Figure 5. The results showed that the oral administration of *Bifidobacterium longum 6-1* in combination with the injection administration of anti-m PD-1 could significantly increase the mean number of CD3 cells in the mice and thus enhance the immune system response in the mice. Compared with the antibiotic treatment group in the figures, $P<0.05$ indicates a significant difference, *$P<0.05$, ***$P<0.001$.

[0089] Example 6

[0090] The mice in Example 3 were euthanized on day 19 after tumor inoculation, and flow cytometry was used to detect CD3, CD4, CD8, FOXP3, CD25, CXCR3, Gata3, Granzyme B, CD69, PD-1, CTLA-4 and CD11b, MHC II, CD206, CD40, CSF1R, PD-L1, and Gr-1 in tumor cells. The experimental results were shown in Figure 6. The results showed that the oral administration of *Bifidobacterium longum 6-1* in combination with the injection administration of anti-m PD-1 could significantly increase the number of tumor-infiltrating CD8 T cells and thus enhance the immune system response in the mice, compared with the injection of anti-m PD-1 alone. Compared with the antibiotic treatment group in the tables, $P<0.05$ indicates a significant difference, **$P<0.01$.

[0091] Example 7

[0092] 48 male C57BL/6J mice aged 6-8 weeks (20-26 g, SPF grade) were randomly divided into 4 groups based on body weight after acclimatization for one week: group 1 (blank, *i.p.*), group 2 (anti-mPD-1, 10 mg/kg, *i.p.*), group 3 (*Bifidobacterium longum 6-1*, *p.o.*), and group 4 (*Bifidobacterium longum 6-1*, *p.o.*, anti-mPD-1, 10 mg/kg, *i.p.*). Mice in groups 3 and 4 were given with lyophilized samples of *Bifidobacterium longum 6-1* by gavage at a concentration of 1.0×10^8 CFU/mouse/day. After 2 weeks of continuous gavage, all groups were subcutaneously inoculated with 4T1 tumor cells resuspended in PBS at a concentration of 1×10^7 cells/mL to the right flank of experimental animals at 100 μL/mouse. Mice in groups 2 and 4 were injected with anti-mPD-1 on day 4 after tumor cell inoculation,

once every 4 days, for a total of 4 injections. Mice were euthanized on day 19 after tumor inoculation.

[0093] The results were shown in Figure 7. In a 4T1 breast cancer model insensitive to anti-mPD-1 treatment, the combined treatment of *Bifidobacterium longum 6-1* and anti-mPD-1 significantly enhanced the therapeutic effect of anti-mPD-1 on 4T1 breast cancer, and significantly reduced the tumor volume (P<0.0001). The results showed that *Bifidobacterium longum 6-1* could enhance the response to anti-mPD-1 in the organism and widen the application scope of anti-mPD-1 for tumor treatment.

[0094] Example 8

[0095] 36 male C57BL/6J mice aged 6-8 weeks (20-26 g, SPF grade) were randomly divided into 3 groups based on body weight after acclimatization for one week: group 1 (blank, *i.p.*), group 2 (anti-mPD-1, 10 mg/kg, *i.p.*), and group 3 (*Bifidobacterium longum 6-1*, *p.o.*, anti-mPD-1, 10 mg/kg, *i.p.*). Mice in group 3 were given with lyophilized samples of *Bifidobacterium longum 6-1* by gavage at a concentration of 1.0×10^8 CFU/mouse/day. After 2 weeks of continuous gavage, all groups were subcutaneously inoculated with LLC1 lung cancer cells resuspended in PBS at a concentration of 1×10^7 cells/mL to the right flank of experimental animals at 100 μL/mouse. Mice in groups 2 and 3 were injected with anti-mPD-1 on day 4 after tumor cell inoculation, once every 4 days, for a total of 4 injections. Mice were euthanized on day 19 after tumor inoculation.

[0096] As shown in Figure 8, in the LLC1 lung cancer model insensitive to anti-m PD-1 treatment, the combined treatment of *Bifidobacterium longum 6-1* and anti-mPD-1 could effectively improve the response to anti-m PD-1 therapy in the organism, significantly inhibit tumor growth and reduce tumor volume, compared with the treatment of anti-m PD-1 alone. The significant differences in the table are compared with the control group, **P<0.001, ****P<0.0001.

WHAT IS CLAIMED IS:

1. An anti-tumor composition, comprising:

(A) *Bifidobacterium longum*; and

(B) an immune checkpoint inhibitor.

2. The anti-tumor composition of claim 1, wherein the *Bifidobacterium longum* is *Bifidobacterium longum 6-1*.

3. The anti-tumor composition of claim 1 or 2, wherein the *Bifidobacterium longum* is combined with other bacteria (such as *Bifidobacterium, Lactobacillus, Clostridium, Enterococcus faecium, Prevotella stercorea, Ruminococcus*).

4. The anti-tumor composition of claim 1 or 2, wherein the *Bifidobacterium longum* and the immune checkpoint inhibitor are mixed together to prepare a single preparation, or physically separated and used separately.

5. The anti-tumor composition of claim 1 or 2, wherein the *Bifidobacterium longum* is in the form of oral administration or injection administration.

6. The anti-tumor composition of claim 1 or 2, further comprising antibiotics.

7. The anti-tumor composition of claim 1 or 2, wherein the tumor is selected from colon cancer, lung cancer, gastric cancer, liver cancer, head and neck cancer, cervical cancer, breast cancer, lymphoma, breast cancer, melanoma, kidney cancer or urothelial cancer.

8. Use of *Bifidobacterium longum* in the manufacture of a medicament for treating tumors.

9. The use of claim 8, wherein the *Bifidobacterium longum* is used in combination with an immune checkpoint inhibitor preparation.

10. The use of claim 8 or 9, wherein the use is to improve the effect of the immune checkpoint inhibitor in suppressing tumors, preferably, the tumors are selected from colon cancer, lung cancer, gastric cancer, liver cancer, head and neck cancer, cervical cancer, breast cancer, lymphoma, breast cancer, melanoma, kidney cancer or urothelial cancer.