Tumor-targeting of Bifidobacterium Infantis on Melanoma in Mice

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Abstract: Objective. To investigate the tumor-targeting of Bifidobacterium infantis to melanoma. **Methods.** After bolus administration of Bifidobacterium infantis with 3H-TdR, the values of radioactivity in tumor and organs were examined at 24 h, 48 h, 72 h, 96 h and 168 h. Anaerobic culture and histological observation of tumor and normal organs were taken for the examination of tumor-targeting characteristics of Bifidobacterium infantis. **Results.** The radioactivity in melanoma tissue increased progressively, while the radioactivity in normal organs decreased with time. The anaerobic culture showed an obvious proliferation of Bifidobacterium infantis in tumor tissue. A large part of area was Gram positive in tumor section, whereas the normal tissue was Gram negative. **Conclusion.** Bifidobacterium infantis has good tumor-targeting characteristics in mice melanoma. [Life Science Journal. 2006;3(2):17-20] (ISSN: 1097-8135).

Keywords: Bifidobacterium infantis; melanoma; targeting

1 Introduction

A central problem of gene therapy for cancer is the lack of specificity of current delivering system. It has been proved that a hypoxic region exists in kinds of tumors, especially in solid tumors, and the anaerobic bacteria tend to colonize in a low oxygen environment. Therefore, based on the presence of a hypoxic metabolic region in a solid tumor as well as the tendency of anaerobic bacteria to hypoxic environment, anaerobic bacteria is a potential vector for tumor targeting gene therapy[1-3]. Bifidobacterium infantis is a non-pathogenic anaerobic bacterium. It resides in the intestine of human or rodent animals, which is good for the health of its host. We try to explore the targeting of Bifidobacterium infantis to tumors in the study.

2 Materials and Methods

2.1 Animals

Female C57BL/6 mice aged 6 to 8 weeks were selected from Sichuan University Tumor-research Center.

2.2 Tumor cells preparation

B16-F10 melanoma cells were maintained as monolayer cultures in Dulbecco's medium supplemented with 10% fetal bovine serum. A total of 5×10^5 tumor cells were inoculated into the right thigh muscle of these mice. The solid tumors for study were obtained 2 weeks after inoculation.

2.3 Bacteria culture

Bifidobacterium infantis were cultured under anaerobic condition at 37 ℃ in MRS liquid culture medium. After 24 - 48 h, the concentration was quantified. The original Bifidobacterium infantis suspensions was diluted to $2.5 \times 10^7 - 3.0 \times 10^7$ bacilli/ml with phosphate-buffered saline (PBS) (pH7.4). Finally, Bifidobacterium infantis was injected into four mice from the tail vein of the animals (5-6 million bacilli per mouse) and 2 normal mice were as control.

2.4 Tissue homogenate and culture

After 168 h, six mice were killed. Normal tissue samples were obtained from lung, liver, spleen, kidney and heart. Normal tissue and whole

tumors were excised and minced thoroughly. Each sample was weighted and placed in a homogenizer to prepare a 10% homogenate with cold PBS under aseptic conditions. The diluted tissue homogenates (100 μl/dish) were inoculated into the culture medium (1.5% Briggs agar) respectively. After the agar medium was solidified, all dishes were placed in a completely airtight desiccator. The dishes were cultured at 37 ℃ under anaerobic condition for 3 days.

2.5 Histology

The mice that developed tumors were sacrificed at 168 h after injected with Bifidobacterium infantis. Tumors and normal tissues were excised, fixed in 10% formalin solution, sectioned in paraffin and stained with Gram stain.

2.6 3H-TdR tag

Bifidobacterium infantis were cultured under mixed atmosphere condition (80% N_2, 10% H_2, 10% CO_2). 3H-TdR was added to the bacteria medium(0.37×10^{10} Bq ^{3}H-TdR/10^6 bacilli) after 24 h. Another 12 h later, the bacteria suspension was diluted with cold PBS (pH 7.4), and injected into mice from the tail vein (5 – 6 million bacilli per mouse). The mice were sacrificed at 24 h, 48 h, 72 h, 96 h and 168 h after injection of 3H-TdR tagged Bifidobacterium infantis. Tumors and normal tissues were excised. Finally, the values of radioactivity in each tissue were examined.

3 Results

3.1 The values of radioactivity in tissues

Table 1 showed the radioactivity in various tissues at 24 h, 48 h, 72 h, 96 h and 168 h after intravenous administration. The radioactivity in tumors increased progressively. In contrast, the radioactivity in normal tissues, such as the liver, spleen, kidney and lung were attenuated with time. The values of radioactivity in tissues showed the 3H-TdR tagged Bifidobacterium infantis clustered in tumor tissue, while decreased in normal tissue gradually.

Table 1. The values of tissues' radioactivity (A/g)

Tissue	24 h	48 h	72 h	96 h	168 h
Liver	813	614	568	498	356
Heart	946	1003	823	725	677
Spleen	963	876	625	592	554
Lung	775	776	564	540	434
Kidney	696	505	405	374	356
Tumor	346	370	441	545	778

Each value represents the mean of radioactivity of per gram of tissue.

3.2 Tissue culture

Bacteria colonies were only observed on the agar culture dish inoculating tumor tissue homogenates, but no colonies were observed in any plates inoculating normal tissues (Figure 1 A, B). The anaerobic culture showed an obvious proliferation of Bifidobacterium infantis in tumor tissue.

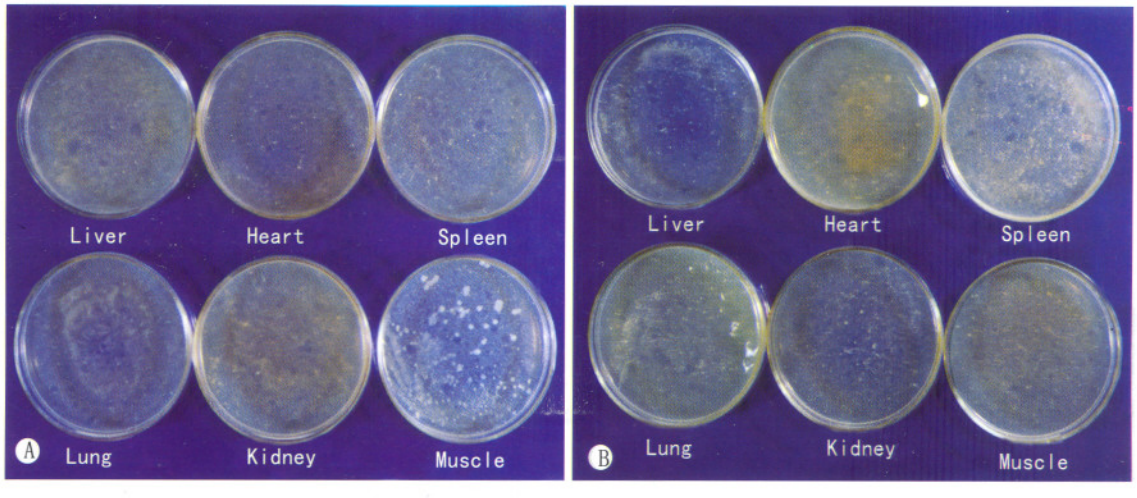

Figure 1. Comparision of the anaerobic culture results of bacilli in tissues after seven days of Bifidobacterium infantis administration
A: Tumor and normal tissues from tumor-bearing mice; B: Normal tissues from normal mice

3.3 Histology

Four mice that developed melanoma tumors were injected intravenously with Bifidobacterium infantis, killed 168 h later and examined for the presence of Gram-positive Bifidobacterial rods in both tumors and normal tissues. Figure 2 A, B showed numerous bacilli were scattered or clustered either on the border between the necrotic and non-necrotic regions or in the necrotic region of tumors. In contrast, no evidence of bacteria was observed in the normal tissue segment.

4 Discussion

A crucial difficulty for cancer gene therapy is the lack of specificity of current delivery systems. After i.v. inoculation of Bifidobacterium infantis to tumor-bearing mice, we initially observed a distribution of viable bacilli throughout the body, but after 96 – 168 h, most of bacilli were accumulated in the tumor tissue. In this report, we demonstrated the results with the methods of 3H-TdR tagged and the values of radioactivity in tumor and normal organs. The fact that the bacilli can colonize and proliferate in the tumor tissue implies that this tissue possesses an environment that is suitable for the growth of this bacterium. Vaupel[4] made his study on cancer patients using oxygen electrode measurement. In his study, Vaupel found the average oxygen partial pressure in normal tissues read 24 – 66 mmHg, whereas the readings dropped to 10 – 30 mmHg in a tumor tissue with a marked central region where the readings went below 2.5 mmHg. It is evident that the center of solid tumor is generally at low level of oxygen, and anaerobic bacteria tend to colonize in a low oxygen environment[5].

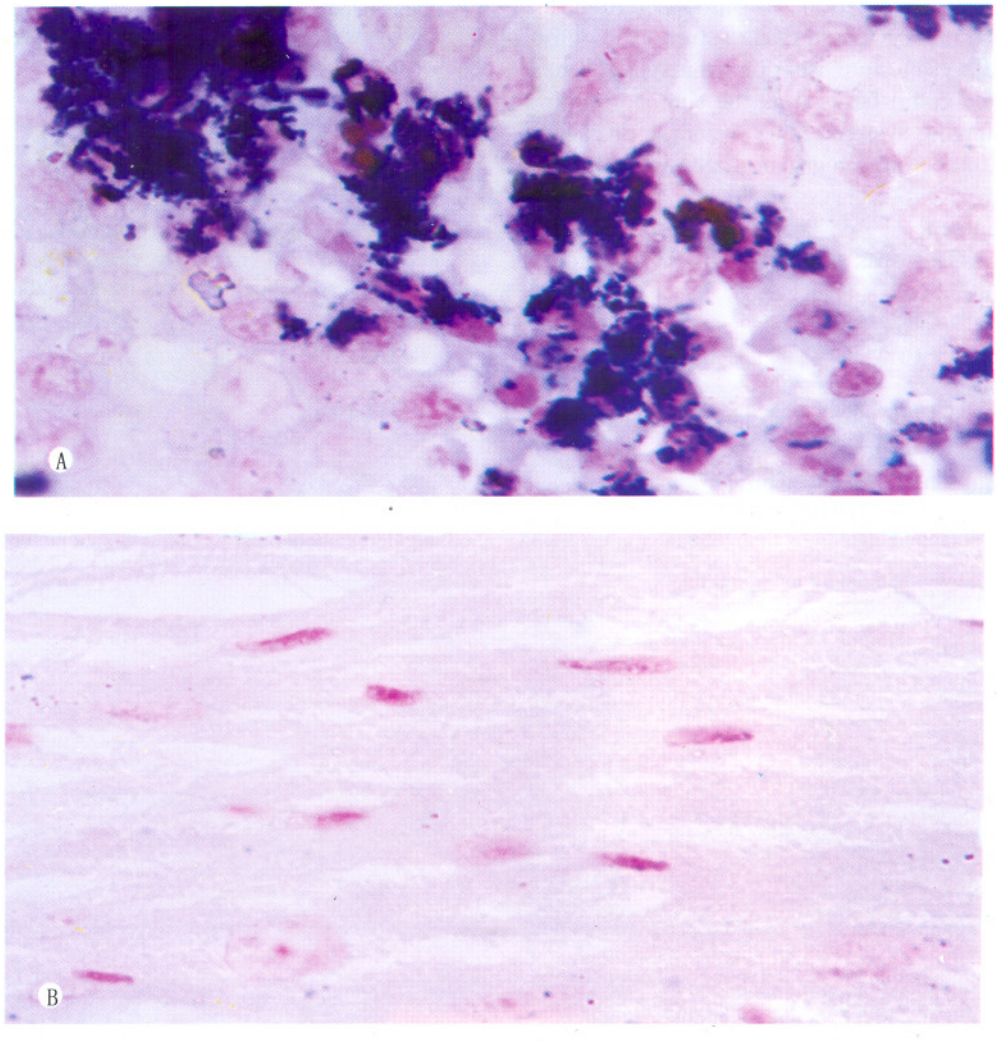

Figure 2. Photomicrograph of sections stained by the Gram method (×100)
A: Melanoma tumor tissue; B: Heart tissue

Bifidobacterium infantis are Gram-positive, domestic, non-pathogenic bacteria, found in the lower small and large intestine of humans and other animals. In addition, these bacteria have health-promoting properties for their hosts by, for example, increasing the immune response[6], inhibiting carcinogenesis[2,7] and protecting the host against viral infection[8,9]. The non-pathogenesis and importance of these microorganisms are now generally acknowledged. Based on the presence of a hypoxic metabolic region in a solid tumor and tendency of anaerobic bacteria to hypoxic environment, Bifidobacterium infantis can be used as a transfer vectors for tumor targeting gene therapy.

In this study, the anaerobic culture showed an obvious proliferation of Bifidobacterium infantis in tumor tissue. A large part of area was Gram-positive in the tumor tissue section, whereas the normal tissue was Gram-negative. We demonstrated the tumor-specific germination of Bifidobacterium infantis. In summary, these results strongly suggested that Bifidobacterium infantis were good tumor targeting and could be used as the tumor targeting gene-transferring system. This system is promising as a novel tumor targeting gene therapy system.

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