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Investigating the effect of Lactobacillus rhamnosus on the migration of murine melanoma cells

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Abstract

Melanoma is one of the most common types of cancer globally. Probiotics are nonpathogenic bacteria with valuable pharmaceutical and biological activities. The anti-tumor effects of probiotic strains of *Lactobacillus* have been indicated in various studies. The aim of current research was to investigate the effect of *Lactobacillus rhamnosus* on the migration of murine melanoma cells. To do so, B16F10 melanoma cells were seeded in 24-well plates, and after 24 hour incubation, a straight scratch was created on the cell monolayer. Then, cells were treated with the supernatant of heat-killed *L. rhamnosus* (dilution 1:2.5 with complete medium) and control cells were treated with the same dilution of phosphate buffer saline. Photomicrographs were taken from the scratch area at 0 and 24 h, and images were analyzed by ImageJ software. Our results demonstrated that *L. rhamnosus* reduced the migration ability of B16F10 cells in comparison with control treatment. According to obtained results, *L. rhamnosus* could be considered as an effective probiotic to inhibit migration of melanoma cells.

Keywords: *Lactobacillus rhamnosus*, melanoma, migration, scratch assay.

Introduction

Cancer is the second most common cause of death worldwide after cardiovascular diseases. Melanoma represents the most aggressive and the deadliest form of skin cancer. Melanoma develops when genetic mutations happen in melanocytes, the cells responsible for producing pigment. Various therapeutic approaches for melanoma are available including surgery, chemotherapy, radiation, photodynamic therapy, immunotherapy and targeted therapy (1). However, the survival rate of melanoma patients is still low, which is due to the metastasis of melanoma cells (2).

Probiotics are non-pathogenic micro-organisms with beneficial effects on the host health. Probiotic strains of *Lactobacillus* have valuable pharmaceutical activities, and recent studies have indicated their significance in the prevention, suppression, and treatment of many cancers including lung, colon, breast and stomach adenocarcinomas (3). Several mechanisms of action have been introduced for anti-cancer effects of probiotics including preventing the transformation of potential cancer-causing substances into actual carcinogens, producing signaling molecules that boost the immune response and inducing cell death or proliferation arrest in cancer cells (4). The aim of current research was to investigate the effect of *Lactobacillus rhamnosus* on the migration of murine melanoma cells.

Materials and Methods

Bacterial strain: A standard strain of *L. rhamnosus* (PTCC 1637) was acquired from Pasteur Institute (Tehran, Iran) and cultivated in Man-Rogosa-Sharpe (Merck, Germany) broth for 24 h at 37°C in anaerobic conditions.

Preparation of heat-killed bacteria: In order to prepare the heat-killed bacteria, 10^{10} CFU/mL of *L. rhamnosus* were inoculated in MRS-Broth and incubated for 24 h at 37°C in anaerobic conditions. The culture was then centrifuged at 3500 rpm for 5 min and the supernatant was discarded. Then, the cell precipitate was washed with phosphate buffer saline (PBS) for two times and finally cells were resuspended in 10 mL PBS and subjected to 95°C for 60 min to kill the bacteria.

Cell treatment and viability assay: B16F10 cell line was purchased from Pasteur Institute (Tehran, Iran) and cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air, and subcultured with 0.25% trypsin-1 mM EDTA as required.

To assess the effects of heat-killed *L. rhamnosus* supernatant on the migration of B16F10 cells, 15×10^4 cells were seeded in 24-well plates and after 24 h, a straight scratch was made by a sterile pipette tip. Afterwards, cells were washed with PBS and treated with the heat-killed supernatant of *L. rhamnosus* (dilution 1:2.5 with complete medium) and incubated at 37˚C for 24 h. To note, cells treated with PBS (dilution 1:2.5 with complete medium) were considered as control group. Photomicrographs were taken from the scratch area at 0 and 24 h and images were analyzed by ImageJ software.

Results

The results of scratch assay revealed that the supernatant of heat-killed *L. rhamnosus* decreased the migration of B16F10 cells after 24 h. As shown in Figure 1, the scratch area of cells treated with 1:2.5 dilution of PBS decreased after 24 h, while *L. rhamnosus* reduced the migration ability of B16F10 cells (A-D). Quantitative analysis also confirmed the inhibitory effect of *L. rhamnosus* in comparison with PBS (E).

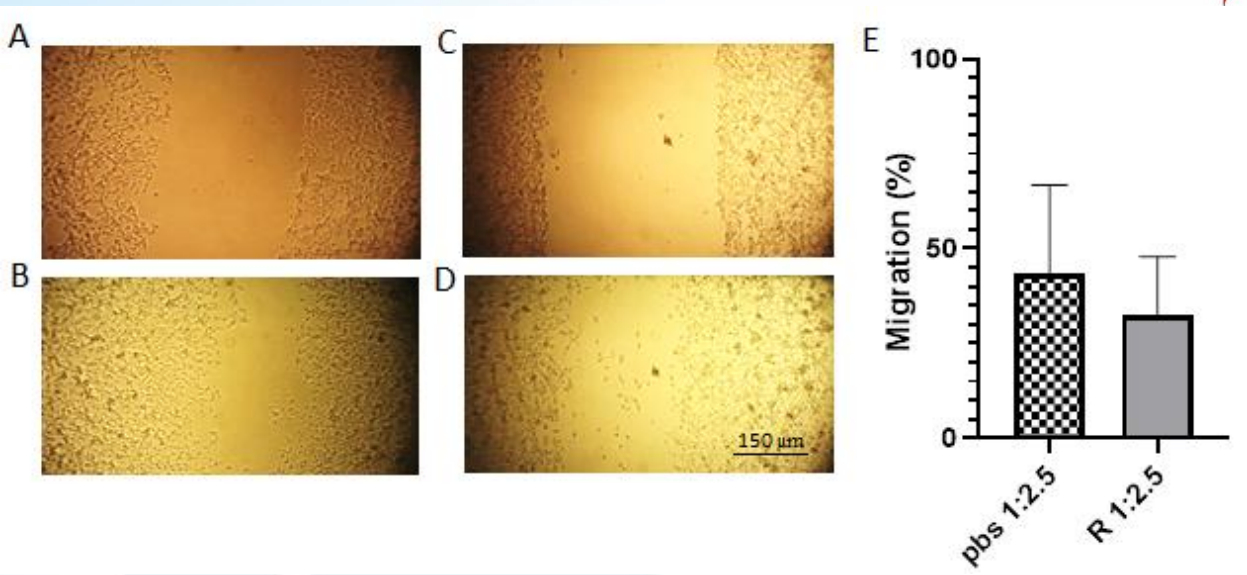

Figure 1: Photomicrographs and quantitative analysis of scratch assay after treatment of B16F10 cells with *L. rhamnosus* extract. Upon creating a scratch on the cell monolayer, photomicrographs were taken from cells treated with PBS (A and B) and the supernatant of *L. rhamnosus* (C and D). Photomicrographs were taken at 0 h (A and C) and 24 h after treatment (B and D). Quantitative analysis of scratch assay using ImageJ software (E).

Discussion

Although several reports have indicated anti-cancer effects of *L. rhamnosus* on various cancer cells, the current study is the first report showing inhibitory effects of *L. rhamnosus* on the migration of melanoma cells. Our results revealed that the supernatant of heat-killed *L. rhamnosus* inhibited the migration of mouse melanoma cells *in vitro*. More investigation is required to assess the exact mechanism and also anti-metastatic effects of *L. rhamnosus* on human melanoma cells.

References

1. Schadendorf, D., van Akkooi, A.C., Berking, C., Griewank, K.G., Gutzmer, R., Hauschild, A., Stang, A., Roesch, A. and Ugurel, S.(2018). "Melanoma". *The Lancet*, *392*(10151), 971-984.
2. Saginala, K., Barsouk, A., Aluru, J. S., Rawla, P., & Barsouk, A. (2021). "Epidemiology of melanoma". *Medical sciences*, *9*(4), 63.
3. Śliżewska, K., Markowiak-Kopeć, P., & Śliżewska, W. (2021). "The role of probiotics in cancer prevention". *Cancers*, *13*(1), 20.
4. Sankarapandian, V., Venmathi Maran, B.A., Rajendran, R.L., Jogalekar, M.P., Gurunagarajan, S., Krishnamoorthy, R., Gangadaran, P. and Ahn, B.C. (2022). "An Update on the Effectiveness of Probiotics in the Prevention and Treatment of Cancer". *Life*, *12*(1), 59.