Inhibitory effect of *Tricholoma matsutake* extract on sex hormones induced melanin synthesis

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Introduction:

In humans, estrogen may be involved in numerous physiological processes of skin and hair follicles, such as skin aging, hair loss, wound healing and scar formation. Previous studies showed that estrogen, which is considerably higher in females, especially during pregnancy, acts directly on skin melanocytes to drive melanocyte differentiation and pigment production. Matsutake contains a variety of active ingredients with high edible and medicinal value. It has been reported that Matsutake has many functions such as whitening, antioxidant, and Antibacterial. In this paper, we use sex hormones to stimulate melanocytes as model group to produce melanin, and then treat the melanocytes with a certain concentration Tricholoma matsutake (TM) extract as TM-treated group. The concentration of TM extract is at which no statistically significant toxic effect occurred. The efficacy of TM was determined by analyzing the content of melanin, tyrosinase activity, and signaling pathway proteins related to melanin synthesis in melanocytes. We hoped that understanding the relevant hormones and downstream signaling events activated in melanocytes by sex hormones would help define the mechanism of the inhibitory effect of TM extract on melanin synthesis and suggest new method.

Materials & Methods:

Extraction and refining process of TM extract

The TM was extracted at 100℃ for 2 h, and the ratio of material to liquid was 1:10. The purification process was to remove protein by Sevag method, decolorize with activated carbon, and chromatographic separation by macroporous adsorption resin. The optimized resin was H103, 30 mg/g resin, the ratio of diameter to height was 1:8, and the concentration of eluent was 40% ethanol solution.

Cell Viability Assay.

The B16-F10 cells were inoculated into 96-well culture plates and cultured overnight in an incubator at 37℃ with 5% CO_2. To establish the concentration-response curve, different concentrations of TM were put into the medium for 48 h. The cell viability was determined with a Cell Counting Kit-8 (CCK8) assay by dividing the absorbance values. The absorbance was measured at 450 nm on a microplate reader.

Tyrosinase Activity and Melanin content Assay.

The B16-F10 cells were inoculated in 6-well culture plates and cultured overnight in an incubator at 37℃ with 5% CO_2. After that, the plates were washed twice with PBS and frozen at -80℃ for 30 minutes in PBS containing 1% Triton X-100. At room temperature, freshly prepared substrate solution was added to each lysate and incubated for 1 h at 37℃. The levels of dopachrome were monitored under 490nm. As for the melanin content analysis, 1N the NaOH solution (with 10% DMSO) was used to dissolve the melanin after the cells were treated with different samples for 48 hours. The supernatant was collected after the cells with alkaline were incubated at 80℃ for 1 h and the absorbance of melanin in the cell lysis was analyzed under 405 nm.

Real-time PCR.

Total RNA was isolated using total RNA extraction kit. qRT-PCR analysis was performed for mRNA level analysis. The qRT-PCR protocol was conducted as follows: SYBR Premix Ex Taq kit used for double-stranded cDNA amplification. The expression level was normalized to the fold change corresponding to control cells, according to the levels of the housekeeping gene GAPDH.

Western Blot Analysis.

Western blot analysis was performed according to a previously described method. B16-F10cells were incubated in T75 cell culture flask. The cells were lysed with cell lysis buffer. Proteins extracted from whole cells were separated by 12.5% SDS gel electrophoresis and then transferred onto a PVDF membrane for 2 h at 400 mA using Transphor TE 62. The PVDF membranes were then incubated with appropriate rabbit polyclonal antibodies against mouse MITF, TRP-1, TRP-2, and β-actin at 4 °C for 2 h or overnight. The membranes were washed three times in PBST and then probed with goat antirabbit horseradish peroxidase conjugated antibody for 1 h.

3D skin model melanin staining.

Masson-Fontana staining method was used for melanin staining on a three-dimensional (3D)skin model. The steps were strictly in accordance with the instructions of the kit, and the melanin deposition was observed under an optical microscope.

Results & Discussion:

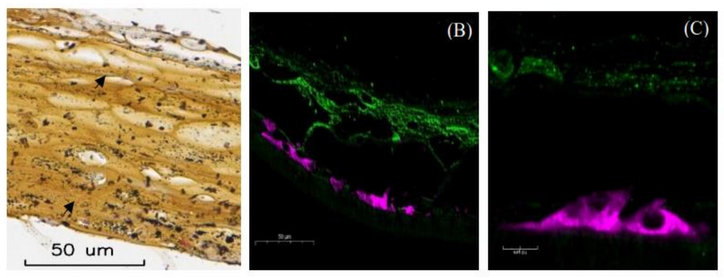

Fig 1 Distribution of melanin granules in 3D skin model. (A) Masson-Fontana staining. (B-C) Tyrsinase and MITF immunohistochemical staining showed keratinocytes in green and tyrsinase and MITF in pink.

The skin model was stimulated with sex hormone. After the skin tissue was fixed, 8 mm skin slices were taken and Masson-Fontana staining was performed according to the instructions of the kit. After that, we found the melanin content in the model group increased significantly. Tyrsinase immunohistochemical staining was performed on the tissue sections, and the results were as shown in (B-C). The expression of Tyrsinase and MITF was significantly increased.

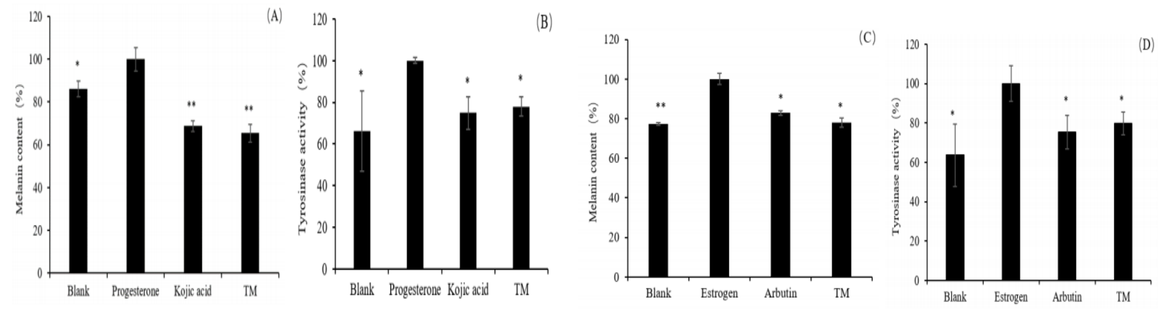

Figure 2 Tyrosinase activity and melanin synthesis in B16F10 cells with TM extract treatment. (A-B) The melanin cell was stimulated by Progesterone to establish the model. (C-D) The melanin cell was stimulated by Estrogen to establish the model. The data presented are from three independent experiments ($p < 0.05$* and $p < 0.01$ ** compared with the model).

As shown in Figure 2, tyrosinase activity and melanin contents are decreased by exposure to TM extract. These results suggested that TM extract has inhibitory effects on melanin synthesis through regulating tyrosinase and subsequently inhibiting melanin synthesis in B16F10 cells.

Conclusions:

This study found that TM can significantly reduce the melanin content in melanocytes by regulating the genes and pathways that are closely related to the melanogenesis and tyrosine metabolism. These results simultaneously deepening our understanding of the molecular mechanisms and providing a scientific theoretical basis for integrating TM into whitening products.

Aknowledgments:

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