SUPPLEMENTARY MATERIAL

Putrescine Upregulates Melanogenesis Through Modulation of MITF Transcription Factor

Natchanok Talapphet\textsuperscript{a} and Moon-Moo Kim\textsuperscript{a}\textsuperscript{*}

\textsuperscript{a}Department of Applied Chemistry, Dong-Eui University, Busan 614-714, Republic of Korea

\textsuperscript{*}Correspondence author. E-mail: mmkim@deu.ac.kr; Tel.: +82 51 890 1511

Abstract

The discoloration of melanin is the aging symptoms caused by the defection of translation, the decrease of enzyme activity, and cellular senescence. Thus, this study aimed to investigate the role of putrescine, a monomer of polyamine derivatives, in the melanogenesis signaling pathway of melanoma cells. In this study, putrescine above 2 mM did not affect DPPH scavenging activity and reduce power, but it increased tyrosinase activity. Moreover, putrescine was low cytotoxicity in B16F1 cells in the MTT assay. Putrescine at 2 mM decreased the level of intracellular hydrogen peroxide in DCFH-DA analysis and also showed that putrescine above 1 mM increased melanin synthesis in live cells. In addition, putrescine upregulated the upstream proteins of TYR, TRP-1, and TRP-2 via MITF transcription factor and promotes MSRA and MSRB expression, leading to the promotion of melanogenesis in H$_2$O$_2$-treated cells. Therefore, these results suggest that putrescine could be utilized to stimulate melanin synthesis.

Keywords: B16F1, Melanogenesis, Melanoma cells, Polyamine, Putrescine
Figures with legends

**Figure S1** The effect of putrescine on antioxidant activity such as DPPH radical scavenging activity (A) and reducing power (B) in addition to tyrosinase activity (C). The positive control for DPPH radical, reducing power, and tyrosinase activity were Vit. C at 0.01%, 0.001%, and 0.1%, respectively. The values of the means and SD from three independent experiments are shown as the data. The significant level was determined using the student’s t-test (*; $p<0.05$, **; $p<0.01$, ***; $p<0.001$).
Figure S2 The effect of putrescine on viability and antioxidant activity in live cells. The effect of putrescine on cell viability was investigated by MTT assay (A). The effect of putrescine on \( \text{H}_2\text{O}_2 \) scavenging activity was performed with DCFH-DA (B). The values of the means and SD from three independent experiments are shown as the data. The significant level was determined using the student’s t-test (*; \( p < 0.05 \), **; \( p < 0.01 \), ***; \( p < 0.001 \)).
Figure S3 The effect of putrescine on melanin production in H$_2$O$_2$-treated B16F1 cells. The melanin production in the presence of putrescine in B16F1 cells was observed using a microscope (A). Melanin was observed at a magnification of 200X (scale bar: 100 µm). The amount of melanin production in B16F1 cells was quantified using ImageJ (B). α-MSH as a positive control stimulated melanin production. The values of the means and SD from three independent experiments are shown as the data. The significant level was determined using the student’s t-test (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$).
Figure S4 The effect of protein expression associated with melanin production by putrescine. The protein expressions of TRP-1, TRP-2 (A), MITF, catalase (B), TYR, TH (C), MSRA and MSRB (D) in B16F1 cells were analyzed in putrescine-treated group at the dose concentrations (0.25, 0.5, 1, and 2 mM) under H₂O₂-treated condition. β-actin was used for normalization as a control. The values of the means and SD from three independent experiments are shown as the data. The significant level was determined using the student’s t-test (*: p<0.05, **: p<0.01, ***: p<0.001).
**Figure S5** The immunofluorescence images of TRP-1, TRP-2, MITF, TYR, MSRA, and MSRB activities under the aging process with the putrescine-treated group in B16F1 cells. The cells were detected by donkey anti-goat conjugated FITC antibody with a goat polyclonal TRP-1, TRP-2, and MITF (green signal) and donkey anti-mouse conjugated CY3 antibody with a mouse polyclonal TYR, MSRA, and MSRB (red signal). The DAPI was used to stain a blue signal of cell nuclei.
Figure S6 The effect of putrescine on the melanogenesis-related gene expression of MITF, TYR, TRP-1, and TRP-2 levels. The gene expressions of MITF, TYR (A), TRP-1, and TRP-2 (B) in H2O2-treated B16F1 cells, were analyzed in the presence of putrescine at various concentrations (0.25, 0.5, 1, and 2 mM). G3PDH was used for normalization as a control. The levels of mRNA expression were presented as a percentage of expression compared to the blank group. The significant level was determined using the student’s t-test (*; p<0.05, **; p<0.01, ***; p<0.001).
Figure S7 Schematic diagram for the effect of putrescine on melanogenesis stimulated by α-MSH in the MITF signaling pathway.