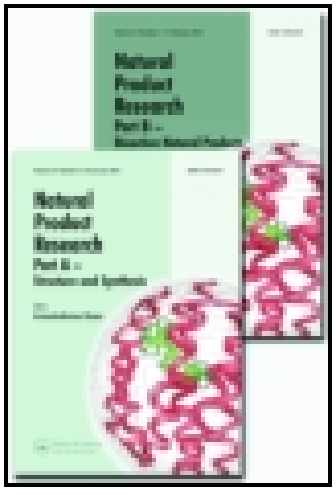

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Placental extracts induce the expression of antioxidant enzyme genes and suppress melanogenesis in B16 melanoma cells

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SHORT COMMUNICATION

Placental extracts induce the expression of antioxidant enzyme genes and suppress melanogenesis in B16 melanoma cells

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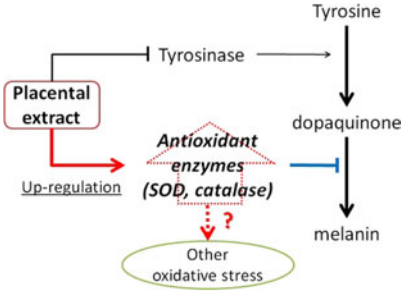

One of the activities of placental extracts (PEs) is skin-whitening effect, but the physiological and genetic mechanism for this effect has not yet been clarified. Here, we focus on PE as a regulator of antioxidant enzyme genes. Porcine PE was prepared, and its activity was investigated in B16 melanoma cells. PE treatment decreased the melanin content of UV-irradiated B16 cells in a dose-dependent manner. PE directly reduced the enzyme activity of tyrosinase in a cell-free assay. In addition, PE treatment increased the gene expression of cytosolic superoxide dismutase (SOD-1), extracellular SOD (SOD-3) and catalase but did not affect the expression of tyrosinase. Moreover, PE protected the B16 cells from H_2O_2-induced cell death. Taken together, our data suggest that PEs could play a role not only as a suppressor of melanin synthesis but also as a regulator of antioxidant genes and might protect the skin against oxidative stress.

Keywords: placenta extracts; melanin synthesis; superoxide dismutase; antioxidant enzyme

1. Introduction

One of the cosmetic functions of placental extracts (PEs) is a skin-whitening effect, but the physiological and genetic mechanism for this effect has not yet been clarified. The constant exposure of the skin to various pollutants (Upadhyay & Mohan Rao 2013) results in the generation of reactive oxygen species (ROS), which causes a variety of skin disorders (Scharffetter-Kochanek et al. 1997; Gaudout et al. 2008). Thus, suppression of ROS production and well-controlled biosynthesis of melanin is very important. PE has antioxidative properties

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(Gabant et al. 2002), but has not been previously reported to regulate the expression of antioxidant genes. In this study, we focus on the effects of PE on gene expression and search for novel antioxidant functions of PE.

2. Results and discussion

Our PE treatment decreased the melanin content in UV-exposed cells (Figure 1(a)). This effect reached a plateau at 1.0% PE. In the cell-free assay, the enzymatic activity of the tyrosinase, which is required for melanocytes to produce melanin, was decreased by PE administration

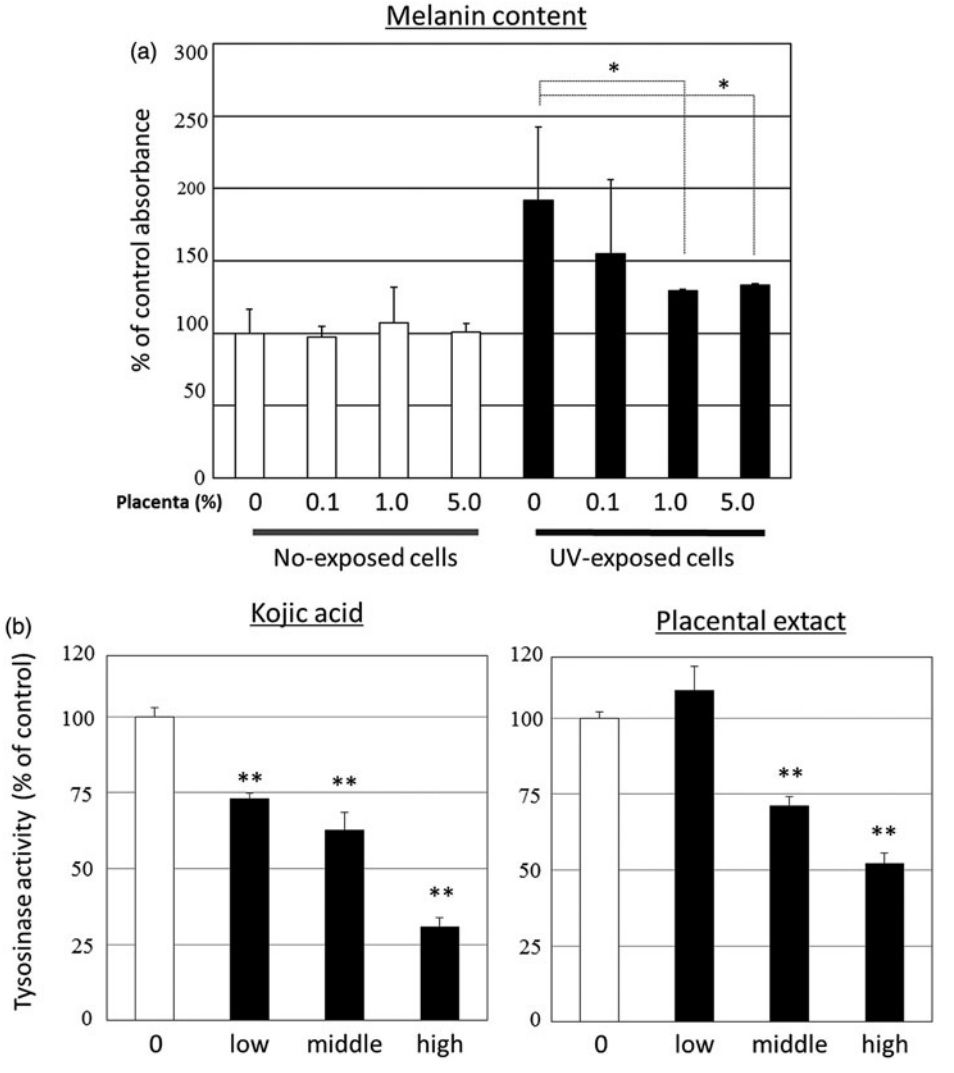

Figure 1. Inhibitory effect of the PEs on melanogenesis. (a) B16 melanoma cells cultured with 0.1%, 1.0% or 5.0% of the PE and were exposed to UV for 15 s. After 96 h of treatment, the melanin content was measured by spectrometry at 400 nm. (b) The tyrosinase (40 U/mL) and PE (low: 231 μg, middle: 463 μg, high: 926 μg) or kojic acids (low: 0.12 μg, middle: 1.2 μg, high: 12 μg) was preincubated at 23°C, then a 2.5 mM L-DOPA was added and the reaction was monitored at 490 nm for 10 min. The increase in absorbance indicates the oxidation of L-DOPA to dopaquinone. $*P < 0.05$ and $**P < 0.005$ compared with the non-PE-treated group (placenta 0%).

(Figure 1(b)). PE showed a weaker inhibitory effect on tyrosinase than kojic acid, an inhibitor of tyrosinase in the melanocytes (Cabanes et al. 1994). Although the direct inhibition of tyrosinase activity by PE in this cell-free assay was observed in the high-dose group, 1% PE in the culture medium was sufficient to reduce the melanin content within the B16 cells. We therefore hypothesised that PE might have other cooperative effects on melanogenesis. To test this possibility, we investigated the effect of PE pre-treatment on gene expression in the UV-exposed B16 cells. As shown in Figure 2, PE induced the mRNA expression of superoxide dismutase (SOD-1), also known as [Cu–Zn] SOD or soluble SOD, in a dose-dependent manner (Figure 2(b)).

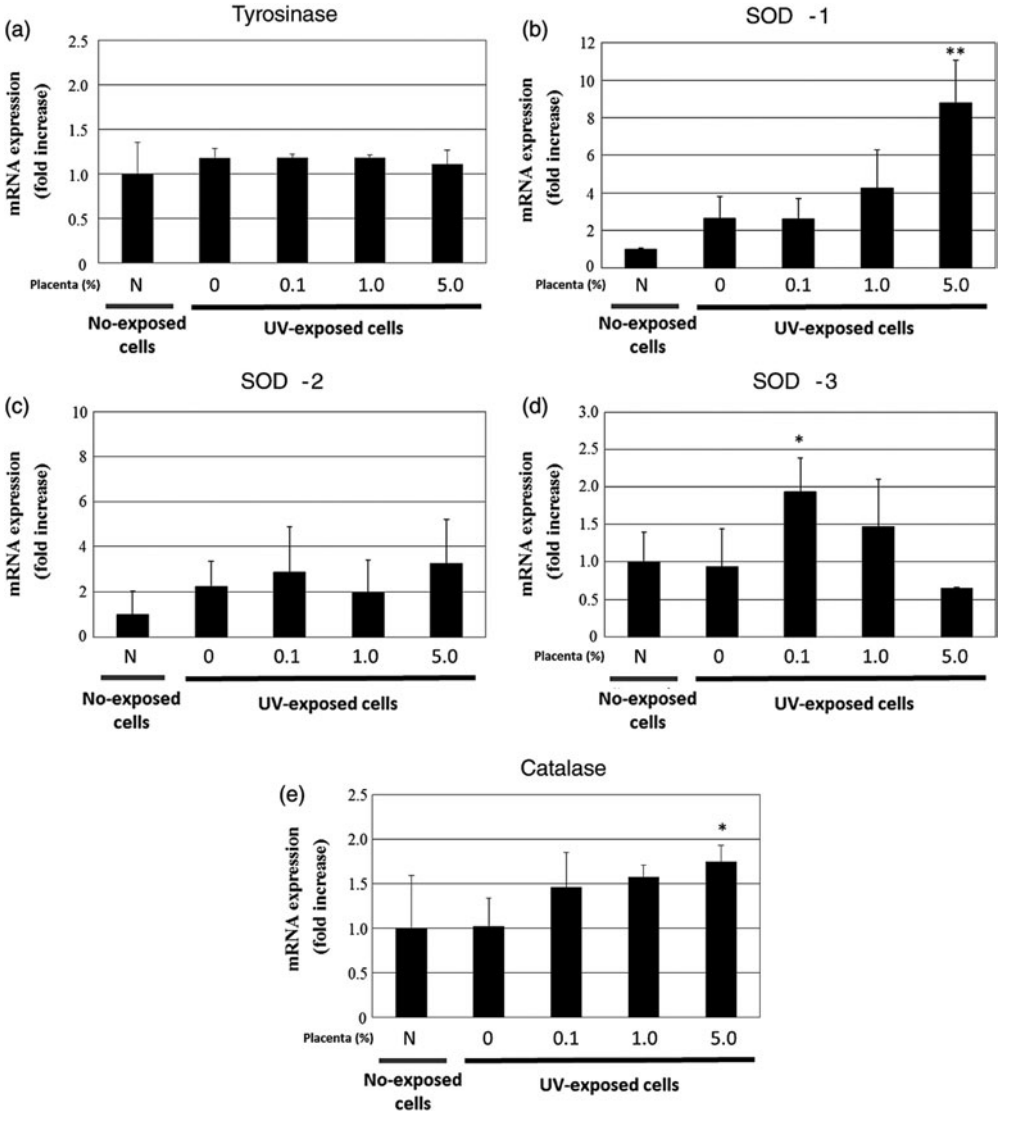

Figure 2. Effect of the PEs on the expression of tyrosinase and antioxidant enzyme in the B16 melanoma cells cultured with 0.1% (low), 1.0% (middle) and 5.0% (high) concentration of the PE and exposed to UV for 15 s. After 24 h of treatment, the total RNA was extracted from the cells and used for RT-PCR of mouse tyrosinase (a), SOD-1 (b), SOD-2 (c), SOD-3 (d) and catalase (e). *$P < 0.05$ and **$P < 0.005$ compared with the non-PE-treated cells (placenta 0%).

The low dose of PE induced the mRNA expression of SOD-3, also known as extracellular SOD (Figure 2(d)). The gene expression of catalase was slightly increased in the PE-treated B16 cells (Figure 2(e)). However, we observed no significant differences in tysosinase (Figure 2 (a)) and SOD-2, also known as [Mn] SOD or mitochondrial SOD (Figure 2(c)). These data indicated that PE reduced the oxidative stress in the cytosol and around the cell surfaces of the B16 cells. Indeed, 0.5% PE protected the B16 cells from H_2O_2-induced cell death (Figure S1). These data indicated that PE suppressed the extracellular oxidative stress in our experimental concentration.

3. Conclusions

Our present study suggests that PE may act not only as a tyrosinase inhibitor but also as an inducer of antioxidant enzymes, which would prevent the oxidation of dopaquinone to eumelanin and pheomelanin. The major antioxidant components in PE are uracil, tyrosine, phenylalanine and tryptophan (Hogg et al. 1995), but no effect of these factors on antioxidant gene expression has previously been reported. Therefore, our findings indicate that PE can play a novel antioxidant role by stimulating the gene expression of the antioxidant enzymes.

Supplementary material

Experimental details relating to this paper are available online, alongside Figure S1.

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Note

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