INTRODUCTION

The biosynthesis of melanin pigment, or melanogenesis, is a major defense mechanism against ultraviolet radiation (UVR). Melanin is produced in specialized membrane-bound organelles (melanosomes) in melanocytes of the skin (Riley, 1997), and the melanosomes, which contain the melanin pigments, are then transferred to neighboring keratinocytes through the dendrites. The melanin granules accumulate above the nuclei of the keratinocytes and absorb harmful UVR. Therefore, these compounds protect the skin from UVR-induced DNA damage (Kobayashi et al., 1998).

Melanogenesis is associated with several skin disorders, such as vitiligo. Vitiligo is a common disorder characterized by white spots appearing on the skin of the body, and more than 1% of the general population suffers from this disease. Vitiligo is caused by the loss of functional melanocytes (Kovacs, 1998), and its treatment is often difficult because of the lack of effective methods for restoring the pigmentation (Jeon et al., 2007). Therefore, agents that can induce melanogenesis may play important roles in damaged and pathogenic skin.

Several studies have reported not only hyperpigmentary factors, including stem cell factor (Grabbe et al., 1994) and basic fibroblast growth factor (Halaban et al., 1987), but also melanogenic inducers, including bovine pituitary extract (Wilkins et al., 1985), 12-O-tetradecanoylphorbol-13-acetate (Krasagakis et al., 1993), bee venom (Jeon et al., 2007), and cAMP elevating agents, such as forskolin, 3-isobutyl-1-methylxanthine, α-melanocyte stimulating hormone, and glycyrrhizin (Wong and Pawelek, 1975; Halaban et al., 1984; Hunt et al., 1994; Lee et al., 2005). These factors induce melanin biosynthesis in vitro system, though they are rarely used clinically.

Melanin biosynthesis in melanocytes is associated with several melanogenic factors, including tyrosinase, tyrosinase-related protein 1 (TRP-1), DOPAchrome tautomerase (DCT), and microphthalmia-associated transcription factor (MITF). Tyrosinase is the key enzyme in pigment synthesis, being responsible for the first two rate-limiting steps of melanogenesis: (1) the hydrolysis from the metabolic precursor of melanin, L-tyrosine, to L-dihydroxyphenylalanine (L-DOPA) and (2) the oxidation from L-DOPA to dopaquinone (del Marmol and Beer, 1996). TRP-1 and DCT play important roles in modify-
Melan-a cells were seeded with approximately 5×10⁵ cells in 100-mm culture dishes. When the cells were confluent, they were detached from the dishes and gathered. The cells were then disrupted with a hypotonic medium (80 mM phosphate buffer containing 1% Brij 35, pH 6.8) via an ultrasonicator in an ice bath. After 1 h, the cells were centrifuged and the supernatants were used for the enzyme assay. The cell-originated tyrosinase activity assay required 150 μg of protein (Fuller et al., 2000). The tyrosinase activity was determined by measuring the rate of oxidation of L-DOPA (Takahashi and Parsons, 1990). For the DOPA oxidation assay, 40 μl of each sample and 120 μl L-DOPA (8.3 mM in 80 mM phosphate buffer, pH 6.8) were mixed with proteins and incubated in a dark place for 20 min at 37°C. The tyrosinase activity was calculated at 490 nm with a microplate reader. The tyrosinase specific inhibitor, kojic acid, was used as a positive control (Cabanes et al., 1994).

Cell-originated DCT activity assay
Melan-a cells were seeded with approximately 5×10⁵ cells in 100-mm culture dishes. When the cells were confluent, they were detached from the dishes and gathered. The cells were then disrupted with a hypotonic medium (80 mM phosphate buffer containing 1% Brij 35, pH 6.8) via an ultrasonicator in an ice bath. After 1 h, the cells were centrifuged and the supernatants were used for the enzyme assay. The cell-originated DCT activity assay used 7 mg/ml of protein (Fuller et al., 2000). DOPAchrome was obtained by mixing cold L-DOPA and silver oxide. L-DOPA (0.5 mg) was dissolved in 1 ml 80 mM phosphate buffer (pH 6.8), and 3 mg silver oxide was dissolved in 1 ml L-DOPA solution. Then 80 μl of the mixture was added to each well. The formazan formation was measured by absorbance at 570 nm in a microplate reader.

Western blot analysis
To detect the protein levels of tyrosinase, TRP-1, DCT, and MITF, melan-a cells were treated with SAR and DCT. The co-immunoprecipitation assay (Moon et al., 2007) was used to detect the protein expression levels. The expression levels of tyrosinase, TRP-1, and DCT were measured by Western blot analysis. The membranes were blocked with 5% non-fat skim milk in Tris-buffered saline-T and incubated overnight with the primary antibodies at 4°C. The membranes