Microphthalmia Transcription Factor and Melanocyte Stimulation Hormone Mediate Cytotoxicity to Reactive Oxygen Species in Human Melanocytes

Douglas Nguyen, Prashiela Manga, Raymond Boissy

University of Cincinnati College of Medicine, Department of Dermatology

**Background and Purpose:** Two melanocyte specific genes, Tyrp1 (tyrosinase-related protein 1) and P protein (Pink-eyed dilution) may mediate the cytotoxic action of phenol derivatives on melanocytes; Tyrp1 by facilitating the generation of reactive oxygen species (ROS) and P protein by disrupting cellular antioxidant response. The expression of Tyrp1 and P are regulated by Microthalmia Transcription Factor (MITF), the “master regulator” of melanocytes. We propose that MITF expression is up-regulated in the presence of α-Melanocyte Stimulating Hormone (MSH), and the increase in MITF expression should up-regulate the expression of Tyrp1 and P protein, leading to an increase in oxidative stress. The aim of our current study was to confirm that exposure to 4-terbutylphenol (4-TBP), a phenolic compound, resulted in the generation of ROS and to characterize the cellular response to oxidative stress. This initial study is necessary to identify candidate pathways that are dysfunctional in contact vitiligo, a depigmentary disorder, which may result from compromised antioxidant responses that lead to premature death of melanocytes in the skin.

**Methods:**

- **Detection of ROS.** The Image-iT Live Green Reactive Oxygen Species Detection Kit from Invitrogen was used to detect ROS in human melanocytes.
- **Western Blotting.** A 10% SDS-page was ran for 4 hours and the protein were transferred onto a PVDF membrane. A 1:1000 dilution of catalase or MITF antibody was added to the membrane and allowed to hybridize overnight. The membrane was washed and the antibodies detected by chemiluminescence.

**Results and Conclusions:** Phenolic compounds such as 4-TBP induce oxidative stress via the production of ROS in cultured human melanocytes in a time-dependent manner. However, the amount of ROS begins to diminish after 6 hours of exposure to 4-TBP. The reduction in ROS may be attributed to a 2-fold increase in catalase expression. The addition of 300mU of exogenous catalase was efficacious at reducing the level of ROS produced. In addition, MSH enhances the production of ROS in melanocytes after 4-TBP exposure. We propose that MSH enhances ROS production via up-regulation of Microthalmia Transcription Factor (MITF) expression and therefore up-regulates Tyrp1 and P protein expression.