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Melanogenesis inhibits respiration in B16-F10 melanoma cells whereas enhances mitochondrial cell content

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ABSTRACT

Melanoma is a rare and aggressive skin tumor; the survival of patients diagnosed late is fairly low. This high mortality rate is due to the characteristics of the cells that allow them to be resistant to radiotherapy and conventional chemotherapy, besides of being able to evade the immune system. Melanin, the pigment responsible for skin, hair and eye color, seems to be involved in this resistance. The main function of melanin is to protect the cells against ultraviolet (UV) light by absorbing this radiation and reactive oxygen species (ROS) scavenging. But this pigment may have also a role as photosensitizer, because when it is irradiated with UVA light (320-400 nm), the generation of ROS was detected. Besides, the melanogenesis stimulation on B16-F10 cells resulted in cell cycle arrest, induction of a quiescent state, change in the expression of several proteins and alterations on ADP/ATP ratio. The present study aimed to investigate the influence of melanogenesis stimulation in mitochondrial function of B16-F10 melanoma cells. Therefore, we analyzed cells respiration, mitochondrial membrane potential ($\Delta\psi_m$) and mitochondria mass in B16-F10 melanoma cells stimulated with 0.4 mM L-tyrosine and 10 mM NH_4Cl. Our results showed that the induction of melanin synthesis was able to reduce significantly the oxygen consumption after 48 h of stimulation, without changes of mitochondrial membrane potential when compared to non-stimulated cells. Despite of respiration inhibition, the mitochondria mass was higher in cells with melanogenesis stimulation. We suggest that the stimulation in the melanin synthesis might be promoting the inhibition of electrons transport chain by some intermediate compound from the synthesis of the pigment and this effect could contribute to explain the entry in the quiescent state.

1. Introduction

Melanoma is a rare and aggressive skin tumor, representing 2% of cases of skin cancers, but due to its aggressiveness, survival of patients diagnosed late is fairly low [1]. The melanoma originates from the melanocytes, dendritic cells located near to the epidermal basal membrane and responsible to produce the melanin, a pigment which confers color to skin, hair and eyes [8].

Melanin is a very heterogeneous polymer, synthesized by melanosomes through hydroxylation of tyrosine or oxidation of 3,4-dihidroxiphenilalanine (DOPA) by tyrosinase, consisting of different oxidative states of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) units and pyrrole units derived from their peroxidative cleavage [35]. The main function of melanin is to protect the cell from damage caused by ultraviolet (UV) radiation, both by absorbing part of this radiation as through reactive oxygen species (ROS) scavenging [39].

However, melanin can play several roles in the cells. Besides of its photoprotective activity, melanin can act as a photosensitizer producing ROS after UV irradiation [26] or exposed to visible light [7]. This pigment is also involved in calcium metabolism, interferes in the ADP/ATP ratio, promotes cell cycle arrest and the intermediates of their synthesis, DHICA, promotes DNA breakage and its interaction with the DNA prevents the action of repair enzymes [20,31,38,9].

Melanogenesis stimulation with L-tyrosine and ammonium chloride (NH_4Cl) was also able to change the protein profile of B16-F10 melanoma cells, leading to increased levels of some proteins related to metabolism, cytoskeleton and cell cycle [9] and promoted an increase in activity of complex II of hyperpigmented FM55 cells [6].

Mitochondria, through oxidative phosphorylation, are the main source of energy in form of ATP for cells. The importance of this organelle in studies with tumor cells, especially aiming antitumor therapies, is based on the fact that this organelle participates in cell death events such as apoptosis and autophagy. Furthermore, it is known that mitochondrial dysfunction results in increased ROS levels,

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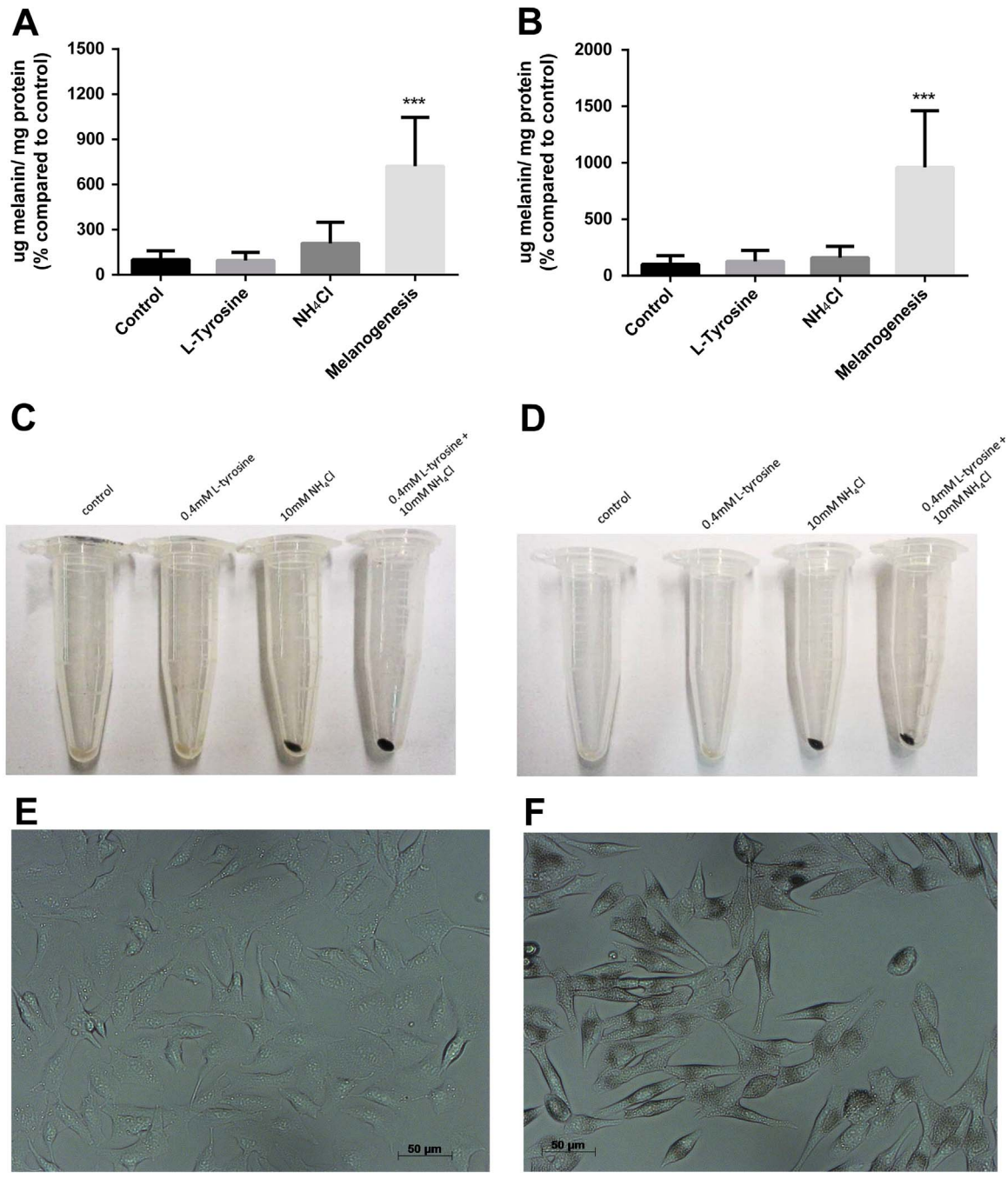

Fig. 1. Melanin synthesis induced by L-tyrosine and ammonium chloride: (A-B) Spectrophotometric analysis of intracellular melanin concentrations in cell lysates after 24 h (A) and 48 h (B) of stimulation. Data are shown as the means ± SD of three independent experiments and the results are expressed as µg of melanin/ mg of protein (***p≤0.001). (C-D) Representative images of cell pellet after 24 h (C) and 48 h (D) of stimulus, shown the browning of B16-F10 melanoma cells after treatment with L-tyrosine and/or ammonium chloride. Representative images of B16-F10 cells obtained using optical microscopy without induction (E) and after 48 h of melanogenesis stimulus with 0.4 mM L-tyrosine and 10 mM ammonium chloride (F).

leading to a condition of oxidative stress and cell death [27,36,4].

Regarding the effect of melanin on mitochondria, some studies have pointed this interaction, as demonstrated by Daniele et al. [10] that observed the physical relationship between mitochondria and melanosome mediated by mitofusin-2 (Mfn2) in melanocytes. Kim et al. [22] studied the effect of mitochondrial dynamics on melanogenesis and found that mitochondrial fusion enhances melanin synthesis whereas mitochondrial fission suppresses hyperpigmentation induced by α-melanocyte-stimulating hormone (α-MSH), via the ROS-ERK signaling pathway, which leads to proteasomal degradation of microphthalmia-associated transcription factor (MITF).

Based on the early studies that have pointed a possible interaction between the induction of melanin synthesis and cell metabolism alterations, the aim of this study was to investigate the effect of melanogenesis stimulation on mitochondrial parameters, as oxygen consumption and mitochondrial membrane potential (ψ_m).

2. Materials and methods

2.1. Chemical and reagents

B16-F10 cell line was obtained from Dr. Roger Chammas (Medicine School of São Paulo University, Brazil). RPMI 1640 medium was purchased from Cultilab (Campinas, SP, Brazil). Fetal Bovine Serum