Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Activation of apoptosis and G0/G1 cell cycle arrest along with inhibition of melanogenesis by humic acid and fulvic acid: *BAX/BCL-2* and *Tyr* genes expression and evaluation of nanomechanical properties in A375 human melanoma cell line

Mitra Salehi [1], Hossein Piri [2, 3*], Alireza Farasat [3], Babak Pakbin [4], Nematollah Gheibi [3*]

[1] Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran
[2] Department of Biochemistry and Genetics, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
[3] Cellular and Molecular Research Center, Research Institute for Prevention of Non-Communicable Diseases, Qazvin University of Medical Sciences, Qazvin, Iran
[4] Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

ARTICLE INFO

Article type:
Original

Article history:
Received: Sep 28, 2021
Accepted: Apr 18, 2022

Keywords:
Apoptosis
BAX
BCL-2
Fulvic acid
Humic acid
Melanoma
Tyrosinase

ABSTRACT

Objective(s): Humic acid (HA) and Fulvic acid (FA) are major members of humic substances, which are extracted from organic sources including soil and peat. The pro-apoptotic and anti-melanogenic effects of HA and FA at the cellular and molecular levels in the A375 human melanoma cell line were examined in this study.

Materials and Methods: The cytotoxicity effect of HA and FA were evaluated by cell viability assay. Apoptosis and cell cycle were investigated by flow cytometry. Real-time PCR was carried out to measure the expression of *BAX*, *BCL-2*, and *Tyr* genes. Moreover, the changes in nanomechanical properties were determined through atomic force microscopy (AFM).

Results: It was found that HA and FA decrease cell viability with an IC_{50} value of 50 µg/ml (dose-dependent) for 14 hr, arrested cells in the G0/G1 phase, and increased the sub-G1 phase (induce apoptosis). Based on the AFM analysis, Young's modulus and adhesion force values were increased, also ultrastructural characteristics of cells were changed. Results of Real-time PCR revealed that HA and FA lead to a decrease in the expressions of *BCL-2* and *Tyr* genes, and increase the *BAX* gene expression.

Conclusion: These results exhibited that HA and FA possess pro-apoptotic effects through increasing the *BAX/ BCL-2* expression in A375 cells. These molecular reports were confirmed by cellular nanomechanical assessments using AFM and flow cytometry. In addition, HA and FA inhibited melanogenesis by decreasing the expression of the *Tyr* gene. It is worthwhile to note that, HA and FA can be regarded to design new anti-cancer and anti-melanogenesis products.

▶ Please cite this article as:
Salehi M, Piri H, Farasat AR, Pakbin B, Gheibi N. Activation of apoptosis and G0/G1 cell cycle arrest along with inhibition of melanogenesis by humic acid and fulvic acid: *BAX/BCL-2* and *Tyr* genes expression and evaluation of nanomechanical properties in A375 human melanoma cell line. Iran J Basic Med Sci 2022; 25:489-496. doi: https://dx.doi.org/10.22038/IJBMS.2022.60651.13444

Introduction

Malignant melanoma is the most aggressive type of skin cancer (1). In 2021, the American Cancer Society appraised 106,110 new cases of this cancer in the USA. (2). The mortality rate of malignant melanoma accounted for more than 80% of all types of skin cancer; it has been revealed that current treatments of melanoma (localized radiotherapy, surgical resection, immunotherapy, and chemotherapy) are far from adequacy; this problem is related to metastasis to vital organs through blood vessels, resistance to current treatments, and poor early diagnosis (3). Therefore, it is necessary to prevent metastasis through early diagnosis and targeted therapies. In recent years, drug-resistance to targeted therapies for malignant melanoma has hindered researchers' efforts (4).

One of the most important molecular markers in malignant melanoma is the tyrosinase enzyme; its structure contains carbohydrates (glycoprotein enzyme) (5). This enzyme restricted the oxidation of tyrosine in the melanogenesis pathway. The function of tyrosinase is regulated by a Microphthalmia-associated transcription factor or MITF that is an oncogene in the malignancy process of melanoma. Tyrosinase is downstream of MITF (6). To date, numerous studies have reported the anti-melanogenic effects of the natural compounds for inhibition of melanogenesis through decreasing the expression of *Tyr* and MITF genes. The previous study has revealed that targeting of the *Tyr* gene could be a strategy for the regulation of hyperpigmentation in melanoma cells (7, 8).

Scientific evidence has confirmed that targeting intracellular signaling pathways such as RAS/BRAF/MAPK and PI3K/AKT in the treatment of malignant melanoma has not been adequate (3). Studies have suggested that targeting the *BCL-2* family is a proper strategy to control

*Corresponding authors: Hossein Piri. Department of Biochemistry and Genetics, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran; Cellular and Molecular Research Center, Research Institute for Prevention of Non-Communicable Diseases, Qazvin University of Medical Sciences, Qazvin, Iran. Email: hpiri@qums.ac.ir; Nematollah Gheibi. Cellular and Molecular Research Center, Research Institute for Prevention of Non-Communicable Diseases, Qazvin University of Medical Sciences, Qazvin, Iran. Tel: +98-2813330534; Fax: +98-2813324970; Email: gheibi_n@yahoo.com; ngheibi@qums.ac.ir