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Research Article

Resistance to ursolic acid-induced apoptosis through involvement of melanogenesis and COX-2/PGE_2 pathways in human M4Beu melanoma cancer cells

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ABSTRACT

Melanoma is one of the most aggressive forms of cancer with a continuously growing incidence worldwide and is usually resistant to chemotherapy agents, which is due in part to a strong resistance to apoptosis. Previously, we had showed that B16-F0 murine melanoma cells undergoing apoptosis are able to delay their own death induced by ursolic acid (UA), a natural pentacyclic triterpenoid compound. We had demonstrated that tyrosinase and TRP-1 up-regulation in apoptotic cells and the subsequent production of melanin were implicated in an apoptosis resistance mechanism. Several resistance mechanisms to apoptosis have been characterized in melanoma such as hyperactivation of DNA repair mechanisms, drug efflux systems, and reinforcement of survival signals (PI3K/Akt, NF-κB and Raf/MAPK pathways). Otherwise, other mechanisms of apoptosis resistance involving different proteins, such as cyclooxygenase-2 (COX-2), have been described in many cancer types. By using a strategy of specific inhibition of each ways, we suggested that there was an interaction between melanogenesis and COX-2/PGE_2 pathway. This was characterized by analyzing the COX-2 expression and activity, the expression of tyrosinase and melanin production. Furthermore, we showed that anti-proliferative and proapoptotic effects of UA were mediated through modulation of multiple signaling pathways including Akt and ERK-1/2 proteins. Our study not only uncovers underlying molecular mechanisms of UA action in human melanoma cancer cells but also suggest its great potential as an adjuvant in treatment and cancer prevention.

1. Introduction

Despite the deployment of targeted therapies, the incidence and mortality rates of cutaneous melanoma is increasing very fast making it a pre-eminent public health threat [1]. Melanoma derived from malignant transformation of melanocytes, cells responsible for the production of the skin pigment melanin. The melanin synthesis, commonly named melanogenesis, occurs within vesicles called melanosomes and is carried out by the tyrosinase family of proteins, including tyrosinase and the tyrosinase-related proteins TRP-1 and TRP-2, also known as dopachrome tautomerase (DCT). Briefly, tyrosinase is the first key enzyme, which is responsible for the limiting step in melanogenesis: the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) followed by oxidation of L-DOPA to DOPAquinone. Then, the synthesis is divided into either the eumelanogenesis, which involves both TRP-1 and TRP-2, or the phaeomelanogenesis by spontaneously reactions [2–4].

For melanoma development, the predominant environmental risk factor appears to be chronic exposure to sunlight [5]. UV radiation generates harmful free radicals, which cause DNA damage [6]. In this context, cutaneous pigmentation is described as a major physiologic defense against UV radiation. However, melanin overproduction and accumulation in melanoma cells display strong photo- and radioprotective properties [7]. Indeed, it is well established that melanogenesis represents a factor that diminishes response to conventional radiotherapy and chemotherapy since pigmented melanoma cells over their unpigmented counterparts were less susceptible to apoptosis [8]. Several resistance mechanisms to apoptosis have been characterized in melanoma such as hyperactivation of DNA repair mechanisms, drug efflux systems, reinforcement of survival signals (PI3K/Akt, NF-κB and Raf/MAPK

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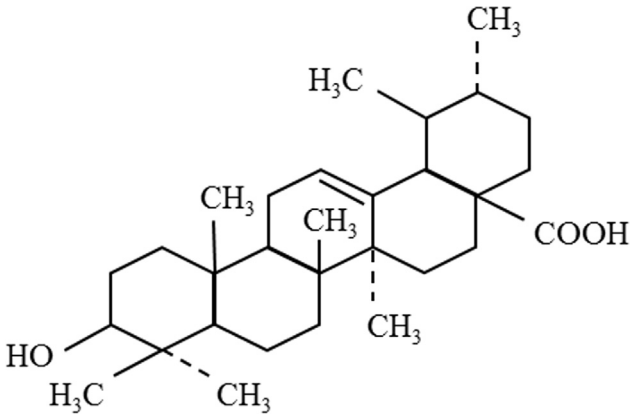

Fig. 1. Chemical structure of ursolic acid.

pathways), altered expression of oncogenes (tumor suppressor p53, RAS, BRAF) and up-regulation of some antiapoptotic factors (Bcl-2, survivin, Mel-IAP, FLIPs) [9–11]. In order to reduce melanoma frequency, the identification of targets, involved in apoptosis resistance mechanisms, is essential for the discovery and the development of new potent anticancer drugs. Previously, we showed that B16-F0 murine melanoma cells undergoing apoptosis are able to delay their own death induced by ursolic acid (UA), a natural pentacyclic triterpenoid compound (Fig. 1). We demonstrated that tyrosinase and TRP-1 up-regulation in apoptotic cells and the subsequent production of melanin were implicated in an apoptosis resistance mechanism [12].

Otherwise, other mechanisms of apoptosis resistance involving different proteins, such as cyclooxygenase-2 (COX-2), have been described in many cancer types [13,14]. COX-2 is the inducible form of cyclooxygenases, enzymes responsible for the formation of prostaglandins (PGs) from arachidonic acid [15]. COX-2 is induced in response to multiple mitogenic and inflammatory stimuli, including cytokines, growth factors, hypoxia and UV light. Several studies reported that COX-2 upregulation and abundant production of its main metabolite prostaglandin E_2 (PGE_2), were involved in tumor promotion, progression, invasion and metastasis [16]. Concerning the role played by COX-2, and particularly in apoptosis resistance, we showed in a previous study that human colorectal cancer cells, even when engaged in apoptosis, are able to trigger a resistance mechanism by upregulating COX-2 via $P2Y_2$/Src/p38 pathway [17,18]. In literature, the role of COX-2 has been extensively studied in colorectal cancer, but COX-2 has been also

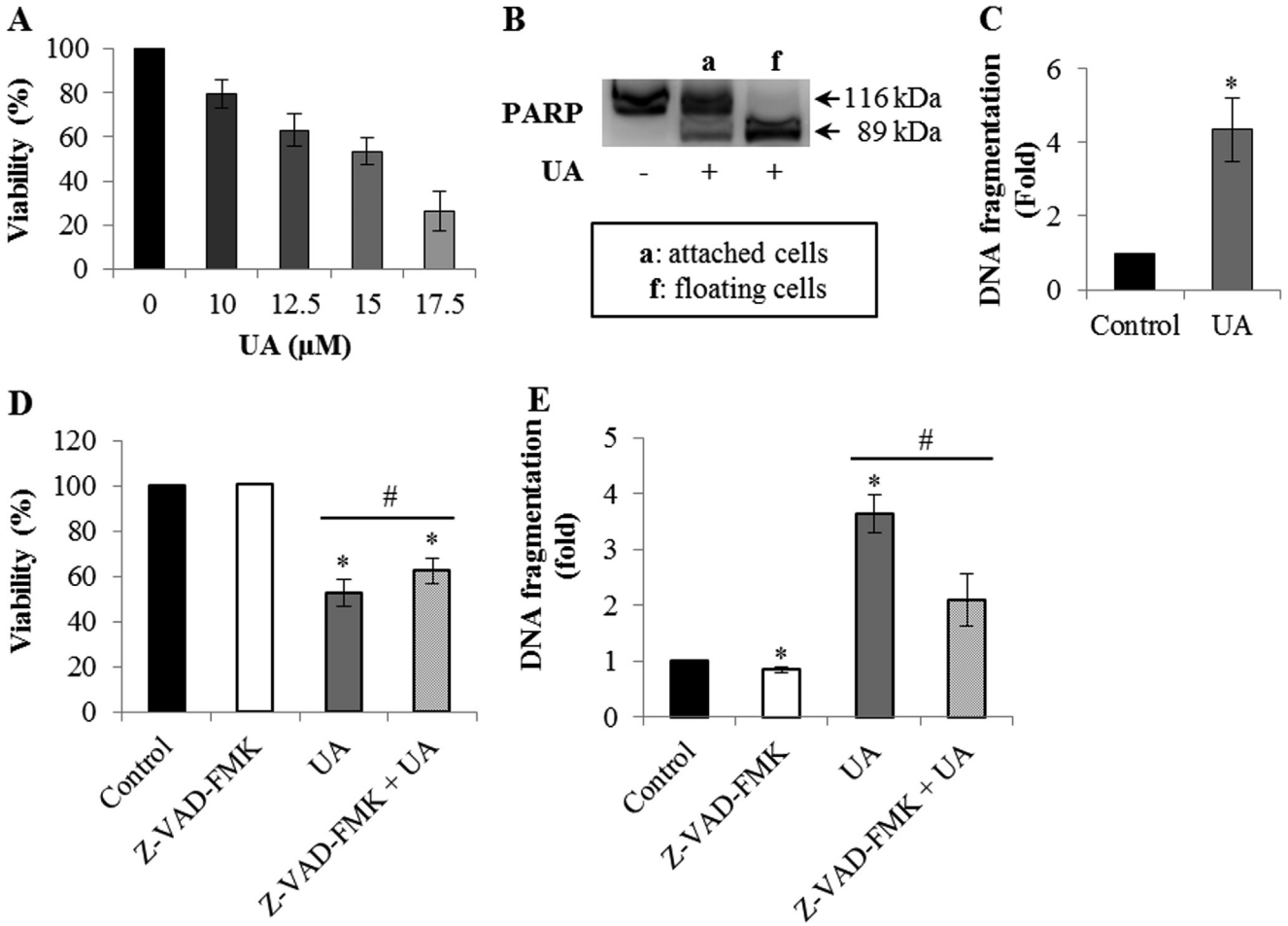

Fig. 2. UA effects on cell proliferation and apoptosis in M4Beu melanoma cells. Cells were treated for 48 h with UA at indicated concentrations concerning viability assay and with or without 15 μM (IC_{50}) for all following experiments. **(A)** Relative cell viability was assessed by Trypan blue dye exclusion method. Results were expressed as percentage of proliferation compared to control. Each value represents the mean ± SD of three separate experiments. **(B)** PARP protein expression in attached (a) and floating (f) cells was determined using western blot analysis. The blot shown is representative of three separate experiments. **(C)** DNA fragmentation (ELISA) was quantified in the total cell pool lysates. Results were reported as n-fold compared to control. Each value represents the mean ± SD of three separate experiments. * $p < 0.05$ *vs. control.* **(D)** M4Beu cells were pretreated for 2 h with 50 μM Z-VAD-FMK, a general caspase inhibitor, prior to exposure or not to 15 μM UA for 48 h. Relative cell viability was assessed by Trypan blue dye exclusion method. Results were expressed as percentage of proliferation compared to control. Each value represents the mean ± SD of three separate experiments. * $p < 0.05$ *vs. control,* # $p < 0.05$ *vs. UA.* **(E)** DNA fragmentation (ELISA) was quantified in the total cell pool lysates. Results were reported as n-fold compared to control. Each value represents the mean ± SD of three separate experiments.* $p < 0.05$ *vs. control,* # $p < 0.05$ *vs. UA.*