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Maslinic acid, a triterpenic anti-tumoural agent, interferes with cytoskeleton protein expression in HT29 human colon-cancer cells

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ABSTRACT

Maslinic acid (MA) is an anti-tumoural agent which shows potent anti-proliferative properties against the HT29 colon-cancer cells. To shed light upon the active mechanism of MA we have investigated its effects upon the cytoskeleton. We used a proteomics procedure based on two-dimensional gel electrophoresis, mass analysis and peptide mass fingerprinting. The incubation of HT29 cells with MA led to G1 cell-cycle arrest. After 24 hours' exposure to 3.7 μM ($IC_{50/8}$) and 30 μM (IC_{50}) MA fourteen differentially expressed cytoskeletal proteins could be discerned. One group of these proteins, made up of keratin 2, keratin 8, keratin type II cytoskeletal 8, keratin type I cytoskeletal 9, keratin type I cytoskeletal 18, cytokeratins 18 and 19, and β-actin, exert a structural function, whilst another group, made up of lamin B1, gelsolin 1, septin 2, villin 1, actin-related protein 2 and moesin, is related to the nucleation of actin and cytoskeleton formation. Changes in the expression of moesin, villin 1 and β-actin identified by the proteomics techniques were corroborated by Western blotting. This is the first evidence obtained of the regulatory effects of MA on the cytoskeleton, which may prove to be one of the bases of its anti-proliferative effect against colon-cancer cells.

Biological significance
In this paper we describe the changes in the expression of different cytoskeleton proteins identified by the proteomics techniques and corroborated by Western blotting. This is the first evidence obtained of the regulatory effects of MA on the cytoskeleton, which may prove to be one of the bases of its anti-proliferative effect against colon-cancer cells.

1. Introduction

In recent decades colon cancer has become one of the leading causes of cancer-related deaths [1]. Conventional anticancer therapies have not yet achieved significant improvement in preventing the progression of colon-cancer. Maslinic acid (2-α, 3-β-dihydroxyolean-12-en-28-oic acid) (MA) is a pentacyclic triterpenic compound abundantly present in the olive (*Olea europaea* L.) [2] (Fig. 1A). In olive tree of the Picual variety, the concentration reported of MA is 1.5 g/kg of dry weight in the fruit [3], 4.8 g/kg of dry weight in the leaf [3], and 17.03 mg/kg of virgin olive oil [4]. MA has important properties with regard

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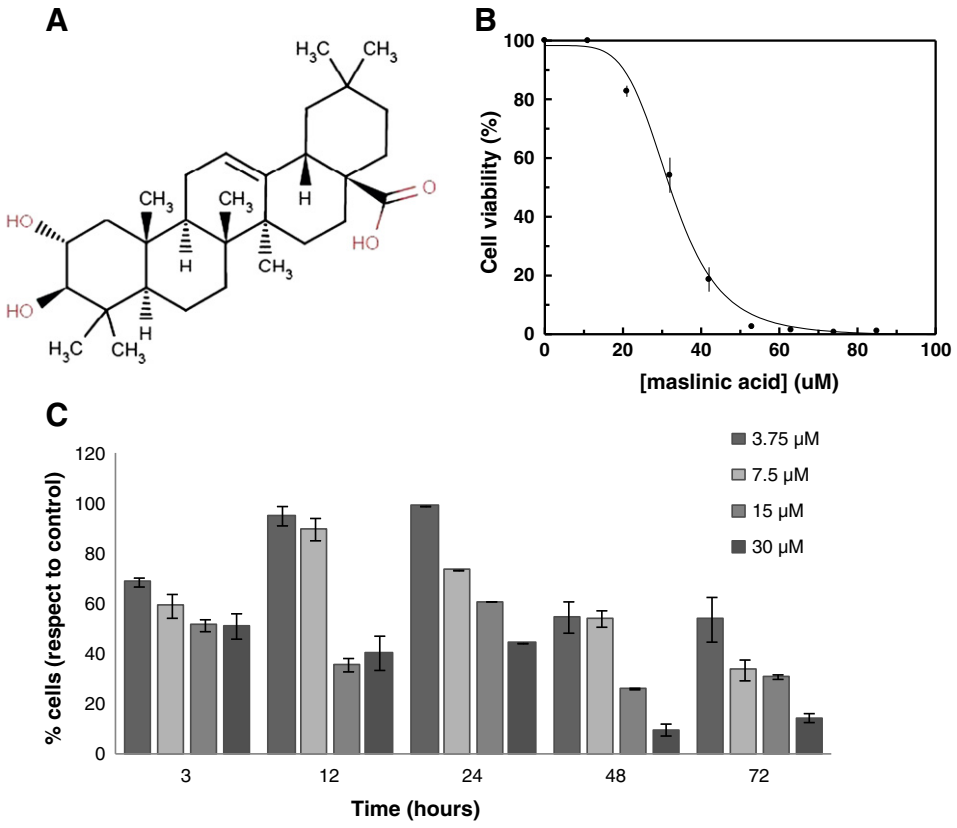

Fig. 1 – A. Structure of maslinic acid. B. Effect of maslinic acid on HT29 human colon-cancer cell proliferation. HT29 cells (6×10^3 cells/well) were seeded in 96-well tissue-culture plates followed by treatment with the indicated concentrations of MA for 24 h. Cell proliferation was determined by MTT assay. C. HT29 cells were treated with 3.75, 7.5, 15 and 30 µM of maslinic acid for 3, 12, 24, 48 and 72 h. Cell viability was determined in a Neubauer counting chamber and cell viability was expressed as a percentage compared to the untreated control. Results are mean value ± SEM (n = 6) $p < 0.05$.

to maintaining cellular growth and health [5–8] and also preventing certain diseases [9,10]. Within this context it has recently been demonstrated that MA may act as an anti-tumoural agent. It has potent differentiating and anti-proliferation properties against HT29 and Caco2 colon-cancer cell lines, inducing cell-cycle arrest in the G0 phase and apoptosis through caspase activation in these cancer cells but not in normal intestinal cell lines. It induces an apoptotic process characterized by caspase-3 activation by a mechanism that appears to be independent of p53, occurring as it does via mitochondrial disturbances and cytochrome c release [11,12].

The cytoskeleton is considered to be the backbone of a cell as it provides the cell with its shape and structure [13]. In most eukaryotic cells, the cytoskeleton is made up of three major components: actin microfilaments, with a diameter of around 6 nm, which are essential for maintaining the shape of the cell and inter- and intracellular transport; microtubules, with a diameter of around 23–25 nm, which contain tubulin protein and are important for intracellular transport and cell division, during which they form spindle fibres; and intermediate filaments, with a diameter of around 10 nm, which constitute a family of proteins including keratin, desmin and peripherin, among others, all of which play a role in maintaining cell shape and its internal organisation [14,15]. One critical step in the neoplastic process is the acquisition by tumour cells of a motile, invasive phenotype. Invasive cancer cells harbour protrusive actin structures known as lamellipodia and filopodia. These extensions depend largely upon a local dynamic reorganisation of the actin cytoskeleton, which in turn is finely tuned by multiple actin-binding proteins [16–18]. Some reports indicate that there are alterations in expression and interaction among several cytoskeletal proteins during early carcinogenesis [19]. It has recently been reported that the expression of some actin-binding proteins, such as gelsolin, villin, moesin and septin, increases concomitantly with the growth of colon-cancer cells [20–22].

Our aim therefore has been to investigate the effects of MA on cytoskeleton protein levels in HT29 cells according to the hypothesis that the anti-carcinogenic properties of MA may reside in its interference with the structure and function of the cytoskeleton. To this end we used the HT29 cell line as an experimental model to study colon adenocarcinoma. The tumourogenic capacity of these cells has been shown in nude mice, in which they form moderately well differentiated adenocarcinomas consistent with primary colon (grade II). On reaching confluence the cells express enterocyte differentiation characteristics. Doubling time is around 62 h (ATCC: