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Chemical proteomics reveals HSP70 1A as a target for the anticancer diterpene oridonin in Jurkat cells

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ABSTRACT

Oridonin, an ent-kaurane diterpene isolated from well known Chinese medicinal plant *Isodon rubescens*, has been shown to have multiple biological activities. Among them, the anticancer activity has been repeatedly reported by many research groups. The chemopreventive and antitumor effects of oridonin have been related to its ability to interfere with several pathways which are involved in cell proliferation, cell cycle arrest, apoptosis and/or autophagy. Despite the number of studies performed on this diterpene, the molecular mechanism underlying its cellular activity remains to be elucidated. Hence, we tried to mine target protein(s) of oridonin by employing a mass spectrometry-based chemical proteomics approach, providing evidences that oridonin is able to directly bind the multifunctional, stress-inducible heat shock protein 70 1A (HSP70 1A). Oridonin/HSP70 complex formation was confirmed in leukemia-derived Jurkat cells. The characterization of HSP70 inhibition by oridonin was performed using chemical and biological approaches. Moreover, the binding site of oridonin on the chaperone was identified by a mass-based approach combined with Molecular Dynamics simulations.

Biological significance

Although natural products showed high efficiency and several of these agents have now entered in clinical trials, information concerning the mechanisms of action at a molecular level of many of them is very poor or completely missed. Nevertheless, the identification of the molecular target of a drug candidate has several advantages. The most significant is the ability to set up target-based assays and to allow structure–activity relationship studies to guide medicinal chemistry efforts towards lead optimization. The knowledge of drug targets can also facilitate the identification of potential toxicities or side effects, if there is any precedent of toxicities for the identified target. Achieving this in an effective, unbiased and efficient manner subsists as a significant challenge for the new era in drug discovery and optimization. In the present study, we used a chemical proteomic approach aimed to define the possible protein target of the ent-kaurane diterpene oridonin. This natural compound has drawn a rising attention for cancer biologists due to its remarkable anti-tumor activities: accumulating evidence has suggested that oridonin is able to hamper the progression of tumor, mitigate tumor burden and alleviate cancer syndrome, which may improve greatly the survival rates of cancer patients; however molecular mechanisms by which this compound exerts its anti-tumor activities still remained to be discovered. We

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identified the molecular chaperone HSP70 1A as an oridonin target in Jurkat cells, thus suggesting a mechanism of action for the diterpene consistent with the multiple biological activities described for it. HSP70 inhibition by oridonin might indeed simultaneously result in the impairment of some of client proteins, thus in turn affecting several molecular pathways. Shedding light on the molecular basis of the biological activity of oridonin, our findings may be relevant for possible therapeutic applications of oridonin, such as its use in combination and the design of new therapeutic approaches. In addition, this research demonstrates the effectiveness of chemical proteomic approaches in drug discovery studies and in orphan drug molecular target identification.

1. Introduction

Chemical proteomics is a compound-centric approach aimed to describe the mechanism of action of bioactive small molecule at a molecular level; it is a powerful mass spectrometry (MS)-based affinity chromatography approach for identifying proteome-wide small molecule–protein interactions; mapping these interactions on signaling and metabolic pathways could comprehensively characterize drug targets, profile toxicity of known drugs, and lead to the identification of possible off-target activities [1]. Recently, there is an increasing interest in application of chemical proteomics experiments to bioactive natural products. Indeed, even if natural products have been the single most productive source of leads for the development of drugs [2], information concerning mechanism of action at a molecular level of many of them is very poor or completely missed.

Kaurane diterpenes, a very important class of natural compounds identified from numerous medicinal plants, have been shown to possess several biological activities, including plant growth regulators, antimicrobial, antiviral, inflammation, and antitumoral activity [3]. Kaurane diterpene oridonin (Fig. 1), the principal active metabolite of *Rabdosia rubescens* (Hemsl.) Hara (Donglingcao), Hook. f. (Leigongteng), has long been used in Chinese medicine to treat several diseases [4,5]. It has aroused high interest especially in cancer researchers due to its potential to be developed into tumor chemotherapeutic drug [6,7 and the references therein]. The ability to inhibit tumor cell growth either in in vitro or in in vivo experimental models has indeed been repeatedly confirmed by many research groups. Inhibition of tumor cell growth by oridonin was ascribed to the ability of the drug to affect cell cycle progression and/or to promote cell death by apoptosis and autophagy [8 and the references therein]. Depending on cell type, oridonin has been shown to induce cell cycle arrest in G1/S, S/G2 or G2/M and to modulate the expression/activity of different cell cycle regulatory proteins [9–11]. Again, depending on the cell type and the experimental conditions used, oridonin has been shown to modulate the expression of proteins implicated in either death receptor-mediated (i.e. FAS, FAS ligand) [12] or mitochondria-dependent apoptotic pathways (i.e. increased BAX/Bcl2 ratio [13]). Moreover oridonin was reported to negatively modulate several pro-survival signaling proteins, such as NF-kB, MAPKs and PI3K/Akt [9,14,15]. Despite the large number of proteins whose activity and/or expression have been shown to be modulated by oridonin, the primary target of this diterpene has not been yet identified. In the present study, we have tried to mine target proteins of oridonin in leukemia-derived Jurkat cells by employing a chemical proteomics approach. Among the identified four potential partners of oridonin we focused on the multifunctional, stress-inducible molecular chaperone HSP70 1A given its importance as an anticancer drug target. The binding of oridonin to HSP70 1A and the resulting inhibition of this chaperone were confirmed by SPR and biochemical assays. Remarkably, the ability of oridonin to target HSP70 also inside Jurkat cells was demonstrated.

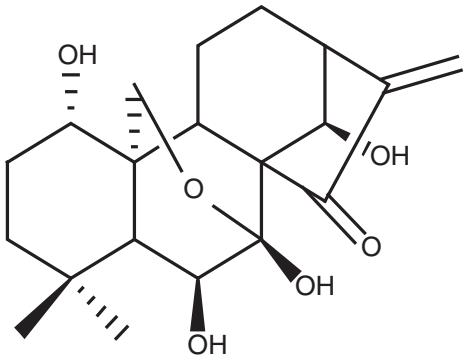

Fig. 1 – Structure of oridonin.

2. Materials and methods

2.1. Cells and protein extracts

Jurkat (human leukemia, T cell lines) obtained from American Type Cell Culture (ATCC, Sesto San Giovanni, Italy), were maintained in RPMI 1640 medium supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 2 mM L-glutamine and antibiotics at 37 °C in humidified 5% CO_2 atmosphere. To ensure logarithmic growth, cells were sub-cultured every 2 days. All experiments were performed using cells at 2×10^5 cells/mL density. Under given experimental conditions, untreated cells were able to double in number in less than 24 h. For protein extracts, control or treated cells were harvested by using a solution of trypsin–EDTA and washed three times with phosphate buffer saline