

## **A multiple approach allowed unveiling the mechanism of action of the anti-cancer natural compound oridonin**

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Plant metabolites are a very broad and heterogeneous set of intrinsically (bio)active compounds. Unfortunately, the actual targets of several among these compounds are partially unknown; thus, a profound understanding of their mechanism of action is a very ambitious aim for researchers working in different scientific fields. Oridonin is a promising anti-cancer ent-kaurane diterpene; its ability to induce apoptosis and/or autophagy in tumor cells, either in vitro or in vivo, has been extensively demonstrated (Li et al., 2016). This compound has been reported to modulate activity and/or expression level of many oncoproteins, but its specific intracellular targets have not been identified yet. This lack of information has limited oridonin use as a drug, and hampered the design of optimized derivatives of this molecule.

To ride out this problem, we have used two orthogonal compound-centric proteomics approaches (Rix et al., 2009) to define oridonin targets. The data we obtained have indicated that the molecular chaperone Hsp70 and the multifunctional protein Nucleolin (Ncl) are the main cell targets of oridonin.

Afterwards, we set up several tests in vitro and in cells, which confirmed the effective interaction of oridonin with these proteins. In particular, confocal fluorescence measurements and CETSA (Cellular Thermal Shift Assay, (Jafari et al., 2014)) experiments were performed using both Jurkat (immortalized T lymphocyte cells) and HeLa (Human cervical cancer cells) cell lines. Kinetic and thermodynamic parameters of oridonin interaction with HSP70 and Ncl were obtained which showed that the diterpene strongly affected in vitro the ATPase and chaperone activities of Hsp70, and inhibited both the mRNA chaperone and the ribosome formation activities of Ncl in the cell.

Our results shed light on the mechanism of action of oridonin, showing that this molecule may inhibit at the same time two proteins that are crucial for cancer development and progression. This finding can finally explain the efficiency of oridonin as antitumor agent and its ability in interfering with many different cellular pathways.

Li et al. (2016) *Int J Mol Sci.* 17, pii: E1395

Rix et al. (2009) *Nat Chem Biol.* 9, 616–24.

Jafari et al. (2014) *Nat Protoc.* 9, 2100-22.