DOCTORAL THESIS

Molecular mechanisms of oridonin-induced cytotoxicity and apoptosis in HepG2 cells

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ABSTRACT

Oridonin, one of the main active constituents of *Rabdosia rubescens*, has anti-hepatocarcinoma activity in experimental and clinical settings. Although laboratory and clinical data showed beneficial effect for oridonin in treating hepatocellular carcinoma (HCC), detailed pharmacological activities and molecular mechanisms of oridonin have not been fully elucidated. In this study, I evaluated anticancer effects of oridonin in HepG2 cells and identified relevant molecular mechanisms.

Results showed that oridonin treatment for 24 or 48 h resulted in a time- and dose-dependent decrease in cell viability. IC_{50} values were determined to be 38.86 μM and 24.90 μM for 24-h and 48-h treatments, respectively. Flow cytometric analysis showed that a 24-h treatment of 40 μM oridonin induced G2/M cell cycle arrest and apoptosis. Typical apoptotic nucleus alterations were observed with fluorescence microscope after DAPI staining. Further investigations showed that MAPK and p53 pathways were involved in oridonin-induced G2/M cell cycle arrest and apoptosis of HepG2 cells. Our data showed that in HepG2 cells oridonin activated JNK and p38 pathways which in turn activated p53, and subsequently the expressions of p21 and cyclin-B1/p-Cdc2, which caused G2/M cell cycle arrest. Alterations in mitochondrial functions and p53-dependent activation of caspases as well as the down-regulation of p-ERK were involved in oridonin-induced apoptosis.

Using 2-DE based proteomic approach, 11 up-regulated proteins, namely Grp78, Hsp70.1, Hsc70, Sti1, Prdx2, trifunctional purine biosynthetic protein adenosine-3, Strap, PPase, TCTP, HP1 beta, and GlyRS, as well as 4 down-regulated proteins, namely hnRNP-C1/C2, hnRNP-E1, p27K, and CK18 were identified in HepG2 cells treated with 40 μM oridonin for 24 h. Alteration patterns of Grp78, Hsp70.1 and hnRNP-E1 were verified by Western blotting. Expression patterns of mRNA as determined by qPCR for Hsp70.1, Grp78, Sti1, hnRNP-E1 and hnRNP-C1/C2 were comparable to their protein expression patterns. Functional analyses by exogenous expression or RNA interference demonstrated that hnRNP-C1/C2 was not involved in the anticancer activity of oridonin. Knockdown of hnRNP-E1 expression with specific siRNA significantly decreased cell viability and increased apoptosis. Knockdown of

Hsp70.1 expression with specific shRNA significantly increased cell death. These data provide evidence for the involvement of hnRNP-E1 in apoptosis and the protective effect of Hsp70.1 in oridonin-treated HepG2 cells.

Immunoblot analysis for the endoplasmic reticulum (ER) stress mediators revealed increased expressions of p-PERK and CHOP, and decreased expressions of PERK, ATF-6 and IRE-1. These findings suggest that oridonin activated ER stress pathway. Knockdown of PERK or IRE-1 with specific siRNA increased oridonin-induced cell death, while suppression of CHOP expression showed no effect on cell viability. These results demonstrated that oridonin-induced ER stress played a protective role in HepG2 cells. Moreover, oridonin treatment for 24 h resulted in reactive oxygen species (ROS) generation, and ROS scavenger N-acetylcysteine (NAC) completely inhibited ROS production and restored cell viability, suggesting that ROS generation contributed to oridonin-induced HepG2 cell death.

In conclusion, this study provides further insight into the anticancer activities and molecular targets of oridonin in cultured HepG2 cells. Findings of this work advance our understanding about the molecular mechanisms of oridonin in liver cancer intervention.

TABLE OF CONTENTS