

DOCTORAL THESIS

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

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**Molecular Mechanisms of Oridonin-induced Cytotoxicity and
Apoptosis in HepG2 Cells**

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for the degree of

Doctor of Philosophy

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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 μ M and 24.90 μ M for 24-h and 48-h treatments, respectively. Flow cytometric analysis showed that a 24-h treatment of 40 μ M 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 μ M 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

DECLARATION	i
ABSTRACT	ii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xii
Chapter 1. Introduction.....	1
1.1 Hepatocellular carcinoma	2
1.1.1. Epidemiology of hepatocellular carcinoma	2
1.1.2. Prevention and surveillance of hepatocellular carcinoma.....	3
1.1.3. Diagnosis of hepatocellular carcinoma	4
1.1.4. Molecular pathogenesis of hepatocellular carcinoma.....	6
1.1.4.1. p53 signaling pathway	9
1.1.4.2. Wnt/ β -catenin signaling pathway	10
1.1.4.3. TGF- β signaling pathway	11
1.1.4.4. PI3K/Akt/mTOR signaling pathway.....	12
1.1.5. Current management of hepatocellular carcinoma	13
1.1.5.1. Surgical treatments	13
1.1.5.2. Locoregional treatments	15
1.1.5.3. Molecular targeted treatments	16
1.2. MAPK pathways and HCC.....	19
1.2.1. ERK pathway.....	20
1.2.2. JNK pathway	21
1.2.3. p38 pathway.....	22
1.3. Traditional Chinese medicine (TCM).....	24
1.3.1. TCM in cancer therapy	24
1.3.2. TCM in HCC management	25
1.4. Oridonin.....	27
1.4.1. <i>Rabdosia rubescens</i> (Hemsl.) Hara	27
1.4.2. Anticancer active constituents of <i>R. rubescens</i>	28
1.4.2.1. Polysaccharides.....	28
1.4.2.2. Ponicidin.....	29
1.4.2.3. Oridonin.....	29
1.5. Objectives of this study	31
Chapter 2. Anticancer effects of oridonin in HepG2 cells	33
2.1. Introduction	34
2.2. Materials and methods.....	36
2.2.1. Oridonin and reagents.....	36
2.2.2. Cell culture and drug treatment	36
2.2.3. Determination of cell viability.....	36
2.2.4. Flow cytometric analysis of cell cycle distribution	37

2.2.5. Flow cytometric analysis of apoptosis.....	38
2.2.6. Fluorescent microscopic analysis of apoptosis.....	39
2.2.7. Data analysis.....	39
2.3. Results and discussion.....	40
2.3.1. Oridonin inhibited HepG2 cell proliferation.....	40
2.3.2. Oridonin induced G2/M cell cycle arrest in HepG2 cells.....	40
2.3.3. Oridonin induced apoptosis in HepG2 cells.....	41
2.4. Conclusion.....	44
Chapter 3. Involvement of p53 and MAPK pathways in the apoptotic and G2/M arresting activities of oridonin.....	45
3.1. Introduction.....	46
3.1.1. Interactions between p53 and MAPK pathways.....	46
3.1.2. p53 pathway and apoptosis.....	46
3.1.3. p53 pathway and G2/M phase cell cycle arrest.....	48
3.1.4. Effects of oridonin on MAPK pathways.....	48
3.2. Material and methods.....	50
3.2.1. Reagents.....	50
3.2.2. Observation of mitochondrial membrane potential (MMP).....	50
3.2.3. Western blot analysis.....	50
3.3. Results and discussion.....	52
3.3.1. Oridonin increased p-JNK, p-p38 and p-p53, and decreased p-ERK protein expression levels.....	52
3.3.2. Oridonin increased the expression of p21 and the inactive form of cyclin B1/Cdc2 complex.....	53
3.3.3. Oridonin inhibited Bcl-2 expression, promoted cytochrome c release, decreased MMP, and activated caspases.....	54
3.4. Conclusion.....	58
Chapter 4. Proteomic identification of differentially expressed proteins in oridonin-treated HepG2 cells.....	59
4.1. Introduction.....	60
4.1.1. Proteomics.....	60
4.1.2. Oncoproteomics of HCC.....	63
4.1.2.1. Cell lines.....	63
4.1.2.2. Tumor tissues.....	63
4.1.2.3. Sera.....	64
4.1.2.4. Animal models.....	65
4.1.3. Quantitative real-time PCR (qPCR).....	65
4.2. Materials and methods.....	69
4.2.1. Reagents.....	69
4.2.2. Proteomic sample preparation and 2-DE.....	69
4.2.3. Silver staining and image analysis.....	70
4.2.4. In-gel digestion and MALDI-TOF-MS/MS analysis.....	71
4.2.5. Western blot analysis.....	72
4.2.6. qPCR.....	72

4.3. Results and discussion	74
4.3.1. Differentially expressed proteins in oridonin-treated HepG2 cells.....	74
4.3.2. Validation of the expression patterns of the identified proteins.....	81
4.3.3. Stress response proteins.....	82
4.3.4. Nucleic acid biosynthesis/binding proteins	85
4.3.5. PPase.....	88
4.3.6. Strap.....	88
4.3.7. Cytoskeleton and cytoskeleton associated proteins	89
4.3.8. Proteins related to protein biosynthesis and degradation.....	90
4.3.9. HP1 β	90
4.4. Conclusion.....	92
Chapter 5. Roles of proteins hnRNP-C1/C2, hnRNP-E1 and Hsp70.1 identified by proteomics in oridonin activities	94
5.1. Introduction	95
5.2. Materials and methods.....	96
5.2.1. Reagents	96
5.2.2. HnRNP-C1/C2 and hnRNP-E1 PCR amplification, digestion and DNA fragments ligation.....	96
5.2.3. Bacterial competent cell preparation and DNA transformation.....	98
5.2.4. Verification of hnRNP-E1 and hnRNP-C1/C2 clones	99
5.2.5. HnRNP-C1/C2 or hnRNP-E1 plasmid transient transfection.....	100
5.2.6. HnRNP-E1 siRNA transient transfection	100
5.2.7. Hsp70 shRNA transient transfection.....	101
5.3. Results and discussion	102
5.3.1. Plasmid construction.....	102
5.3.2. Role of hnRNP-E1 in the anti-proliferative effect of oridonin	105
5.3.3. Over-expression of hnRNP-C1/C2 did not affect the anti-proliferative activity of oridonin.....	108
5.3.4. Hsp70.1 knockdown increased the anti-proliferative effect of oridonin.....	110
5.4. Conclusion.....	112
Chapter 6. Involvement of ER stress and oxidative stress in the anticancer effects of oridonin in HepG2 cells	113
6.1. Introduction	114
6.1.1. ER stress: life or death.....	114
6.1.2. Oxidative stress: cell survival or apoptosis.....	116
6.2. Materials and methods.....	118
6.2.1. Reagents	118
6.2.2. Western blot analysis of ER stress related proteins	118
6.2.3. PERK, IRE-1, or CHOP siRNA transient transfection	119
6.2.4. Measurement of intracellular ROS production.....	119
6.3. Results and discussion	120
6.3.1. Oridonin activated ER stress in HepG2 cells	120
6.3.2. Effects of knockdown of IRE-1, PERK or CHOP on the anti-proliferative effect of oridonin in HepG2 cells.....	122

6.3.3. Oridonin-induced ROS production was responsible for cell death in HepG2 cells	127
6.4. Conclusion	131
Chapter 7. Summary and future plan	132
7.1. Summary	133
7.1.1. MAPK and p53 pathways were involved in oridonin-induced apoptosis and G2/M cell cycle arrest in HepG2 cells	133
7.1.2. Differentially expressed proteins identified by proteomics in oridonin- treated HepG2 cells	133
7.1.3. Roles of hnRNP-E1, hnRNP-C1/C2 and Hsp70.1 in the activities of oridonin in HepG2 cells	134
7.1.4. Oridonin induced ER stress and oxidative stress in HepG2 cells	135
7.2. Future plan	137
7.2.1. Over-expression of hnRNP-E1 and further characterization for its roles in the activities of oridonin	137
7.2.2. Investigation on the interaction between ROS and MAPK pathway in medicating the anticancer effect of oridonin	137
7.2.3. Confirmation and functional analysis of other identified proteins in my proteomic analysis	137
REFERENCES	139
PUBLICATIONS	167
CURRICULUM VITAE	172