

**A Dissertation for the Degree of Doctor of Philosophy**

**Effects of metformin on Sirt1, Nrf2 and AhR  
expression in cancer cells**

**Department of Pharmacy  
Graduate School  
Chungnam National University**

**By**

**Minh Truong Do**

**Advisor Hye Gwang Jeong**

**August 2014**

**Effects of metformin on Sirt1, Nrf2 and AhR expression  
in cancer cells**

**Advisor Hye Gwang Jeong**

**Submitted to the Graduate School  
in Partial Fulfillment of the Requirements  
for the Degree of  
Doctor of Philosophy**

**April, 2014**

**Department of Pharmacy  
Graduate School  
Chungnam National University**

**By**

**Minh Truong Do**

To Approve the Submitted Dissertation  
for the Degree of Doctor of Philosophy

**By Minh Truong Do**

**Effects of metformin on Sirt1, Nrf2 and AhR  
expression in cancer cells**

**June, 2014**

**Committee Chair**

**Sang Kyum Kim**



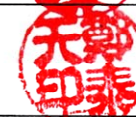
**Committee**

**Bong Hee Kim**



**Committee**

**Tae Cheon Jeong**



**Committee**

**Kwang Youl Lee**



**Committee**

**Hye Gwang Jeong**



**Graduate School**

**Chungnam National University**

# Contents

<b>Contents</b> .....	i
<b>List of Figures</b> .....	vi
<b>List of Abbreviations</b> .....	x
<b>Abstract</b> .....	1
<b>I. Introduction</b> .....	6
1. Metformin and reduced risk of cancer .....	6
2. Role of the AhR, CYP1A1 and CYP1B1 in carcinogenesis and mechanisms of regulation of gene expression .....	6
3. Regulation of gene expression and role of Nrf2 and HO-1 in tumorigenesis and chemoresistance .....	9
4. Role of Sirt1 in tumorigenesis and chemoresistance .....	11
<b>II. Materials &amp; Methods</b> .....	15
1. Materials .....	15
2. Cell culture and treatment .....	16
3. Measurement of cell viability and cytotoxicity .....	17
4. BrdU incorporation assay .....	18

5. RNA preparation and reverse transcription-polymerase chain reaction (RT-PCR) -----	19
6. Quantitative real-time RT-PCR (qRT-PCR) -----	20
7. Luciferase and $\beta$ -galactosidase assays -----	22
8. Western blotting -----	23
9. Preparation of nuclear and cytosolic extracts -----	24
10. Immunoprecipitation (IP) -----	24
11. Chromatin immunoprecipitation (ChIP) -----	25
12. Small interfering RNA transfection -----	26
13. Sp1, HO-1, Sirt1, Pgc-1 $\alpha$ and PPAR $\gamma$ overexpression -----	26
14. miR-34a inhibitor and mimic transfection -----	27
15. Measurement of intracellular reactive oxygen species (ROS) -----	27
16. Statistical analysis -----	28
<b>III. Results</b> -----	<b>29</b>
1. Metformin suppresses CYP1A1 and CYP1B1 expression in breast cancer cells by down-regulating aryl hydrocarbon receptor expression -----	29
1.1. Metformin inhibits CYP1A1 and CYP1B1 expression in breast cancer cells -----	29
1.2. Down-regulation of AhR expression is required for the	

suppression of CYP1A1 and CYP1B1 by metformin -----	33
1.3. Down-regulation of Sp1 by metformin inhibits AhR transcriptional activity in breast cancer cells -----	38
1.4. Inhibition of CYP1A1 and CYP1B1 expression by metformin is independent of ER $\alpha$ -----	41
1.5. Metformin suppresses endogenous AhR-ligand-induced CYP1A1 and CYP1B1 expression by reducing TDO expression in breast cancer cells -----	43
1.6. Metformin suppresses TDO expression by down-regulating the Sp1/glucocorticoid receptor (GR) signaling pathway -----	47
2. Metformin inhibits heme oxygenase-1 expression in cancer cells through inactivation of Raf/ERK/Nrf2 signaling and AMPK-independent pathways -----	52
2.1. Metformin suppresses HO-1 expression in cancer cells -----	52
2.2. Metformin suppresses Nrf2 expression through a Keap1- independent mechanism in cancer cells -----	54
2.3. Metformin suppresses Nrf2 expression in cancer cells <i>via</i> Raf-ERK inactivation -----	58
2.4. Down-regulation of HO-1 expression by metformin is independent of AMPK -----	61

2.5. Reduction of HO-1 contributes to anti-proliferative effects of metformin in cancer cells -----	65
3. Metformin induces microRNA-34a to down-regulate Sirt1/Pgc-1 $\alpha$ /Nrf2 pathway leading to increased susceptibility of cancer cells to oxidative stress and therapeutic agents -----	71
3.1. Metformin suppresses Sirt1 expression in p53 wild-type cancer cells -----	71
3.2. p53-dependent induction of miR-34a is required for the reduction of Sirt1 by metformin -----	73
3.3. Down-regulation of Sirt1 by metformin inhibits Nrf2 expression and increases susceptibility of wild-type p53 cancer cells to oxidative stress -----	77
3.4. Metformin inhibits Nrf2 expression mediated by suppression of Pgc-1 $\alpha$ -----	83
3.5. Metformin suppresses Nrf2 expression by inhibiting PPAR $\gamma$ transcriptional activity and attenuating PPAR $\gamma$ binding to the Nrf2 promoter -----	86
3.6. Up-regulation of DR5 expression by metformin sensitizes wild-type p53 cancer cells to TRAIL-induced apoptosis -----	90

<b>IV. Discussion</b>	97
<b>V. Conclusion</b>	118
<b>VI. References</b>	120
<b>Abstract in Korean</b>	147
<b>Appendix</b>	150



## List of Figures

1. Metformin suppresses CYP1A1 and CYP1B1 expression in breast cancer cells by down-regulating aryl hydrocarbon receptor expression
- Fig. 1. Metformin down-regulates CYP1A1 and CYP1B1 transcription in MCF-7 breast cancer cells ----- 31
- Fig. 2. Metformin down-regulates AhR expression in MCF-7 breast cancer cells ----- 35
- Fig. 3. Down-regulation of AhR expression is required for the reduction of CYP1A1 and CYP1B1 by metformin in MCF-7 cells ----- 37
- Fig. 4. The reduction in Sp1 protein levels mediated by metformin suppresses AhR transcriptional activity in MCF-7 breast cancer cells ----- 39
- Fig. 5. Metformin down-regulates CYP1A1 and CYP1B1 expression in ER $\alpha$ -negative MDA-MB-231 breast cancer cells ----- 42
- Fig. 6. Metformin attenuates endogenous AhR ligand-induced CYP1A1 and CYP1B1 expression by reducing tryptophan-2,3-dioxygenase expression in MCF-7 breast cancer cells ----- 45
- Fig. 7. The down-regulation of TDO expression by metformin is mediated *via* down-regulation of Sp1 and GR proteins ----- 49

Fig. 8. Proposed signaling pathways underlying the effects of metformin on down-regulation of CYP1A1 and CYP1B1 expression in breast cancer cells -----	51
2. Metformin inhibits heme oxygenase-1 expression in cancer cells through inactivation of Raf/ERK/Nrf2 signaling and AMPK-independent pathways	
Fig. 9. Metformin down-regulates HO-1 expression in various cancer cells -----	53
Fig. 10. Effects of metformin on Nrf2 and Keap1 expression in cancer cells -----	56
Fig. 11. Inactivation of Raf-ERK signaling by metformin is required for down-regulation of Nrf2 expression in cancer cells -----	59
Fig. 12. Metformin suppresses HO-1 expression in cancer cells in an AMPK-independent manner -----	63
Fig. 13. Effects of metformin on proliferation of cancer cells -----	67
Fig. 14. Role of HO-1 suppression in anti-proliferative effect of metformin in cancer cells -----	69
Fig. 15. Proposed signaling pathways underlying the effects of metformin on down-regulation of HO-1 expression in cancer cells -----	70