

DOCTORAL THESIS

Role of epidermal growth factor receptor (EGFR) and mitogen-activated protein kinases (MAPKs) signaling pathways in Zn-BC-AM photodynamic therapy-induced apoptosis of the well-differentiated nasopharyngeal carcinoma cell

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Date of Award:
2009

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**Role of Epidermal Growth Factor Receptor (EGFR) and
Mitogen-activated Protein Kinases (MAPKs) Signaling Pathways in
Zn-BC-AM Photodynamic Therapy-induced Apoptosis of the
Well-differentiated Nasopharyngeal Carcinoma Cells**

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A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Prof. MAK Nai Ki

Hong Kong Baptist University

February 2009

ABSTRACT

Nasopharyngeal carcinoma (NPC) has a high prevalence in Hong Kong, southern China and Southeast Asia. Due to the complexity of the disease, new treatment methods for locoregionally advanced or metastatic NPC are currently under development. Photodynamic therapy (PDT), a recently developed method for effective treatment for cancer, may be used as an alternative to treat the recurrent and advanced NPC. In the present study, the biological actions of Zinc (II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin amidinium salt (Zn-BC-AM) PDT on the well differentiated NPC cells were investigated.

Zn-BC-AM PDT was found to induce irreversible cell death of the well differentiated HK-1 NPC cells. Zn-BC-AM PDT was found to activate the intrinsic apoptotic cell death pathway and the sequence of activation included the induction of mitochondrial membrane potential depolarization, externalization of phosphatidylserine, proteolytic cleavage of caspase-9 and -3, chromatin condensation and formation of apoptotic cells. The expression of anti-apoptotic proteins Bcl-2/ Bcl-xL was decreased immediately after Zn-BC-AM PDT treatment while the expression of pro-apoptotic proteins Bax/ Bid was not affected. Addition of singlet oxygen ($^1\text{O}_2$) scavenger L-histidine or overexpression of Bcl-2 was found to reduce Zn-BC-AM PDT-induced apoptosis, indicating that the apoptotic process was initiated through the generation of $^1\text{O}_2$ and Bcl-2 appeared to play a cyto-protective role in retarding the cell death process.

Combination therapy with anti-epidermal growth factor receptor (EGFR) drug (or anti-EGFR antibody) and chemoradiotherapy is emerging as a novel approach for treatment of advanced NPC patients. We hypothesized that inhibition of EGFR signaling pathway might also increase the efficacy of Zn-BC-AM PDT in HK-1 cells. It is found that EGFR, Akt and ERK were constitutively activated in HK-1 cells and the activities could be inhibited by EGFR inhibitor AG1478. Inhibition of EGFR, Akt or ERK by their specific inhibitors was found to enhance Zn-BC-AM PDT-induced formation of apoptotic cells and reduced the expression of Bcl-2. The results indicate that the efficacy of Zn-BC-AM PDT may further be increased through the inhibition of EGFR signaling pathways in NPC cells.

Mitogen-activated protein kinases (MAPKs) are a group of protein kinase highly sensitive to oxidative stress. The role of MAPKs in Zn-BC-AM PDT-induced HK-1 cell death was also investigated. Zn-BC-AM PDT was found to induce persistent activation of p38 MAPK and JNK, while ERK was transiently activated after PDT treatment. Inhibition of JNK with SP600125 had no effect on Zn-BC-AM PDT-induced cell death. Transient genetic knock down of specific p38 isoforms with siRNA revealed that inhibition of p38 β but not p38 α nor p38 δ would increase Zn-BC-AM PDT-induced cell death and apoptosis. Zn-BC-AM PDT-activated p38 β appears to play a role to counteract the PDT-induced cell death.

Pro-inflammatory cytokines and chemokines produced by PDT-treated tumour have been implicated to play a role in the indirect tumour destruction. Using ELISA method, we found that PDT induced the production of IL-1 α and IL-1 β . In contrast, IL-8 (a cytokine with both neutrophil chemotact and angiogenic activities) was

downregulated in HK-1 cells at 24 hours after Zn-BC-AM PDT, suggesting that Zn-BC-AM PDT might indirectly reduce tumour growth through the reduction of tumour angiogenesis.

In conclusion, multiple signaling pathways are involved in Zn-BC-AM PDT-induced apoptosis of HK-1 cells. Combination therapy with Zn-BC-AM PDT and EGFR/MAPKs inhibitors may further be developed for the treatment of advanced NPC.

TABLE OF CONTENTS

DECLARATION	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	xix
Chapter 1	1
Literature Review	1
1.1 Photodynamic Therapy	1
1.1.1 Introduction	1
1.1.2 Photosensitizers	3
1.1.2.1 <i>An Ideal Photosensitizer</i>	3
1.1.2.2 <i>Development of Photosensitizers</i>	4
1.1.3 Photochemistry and Photophysics	5
1.1.4 Light Source and Delivery for PDT	7
1.1.5 Effects of PDT on Tumour	8
1.1.5.1 <i>Direct Tumour Cell Damage</i>	8
1.1.5.2 <i>Vasculature Damage</i>	9
1.1.5.3 <i>Activation of Immune Response</i>	9
1.2 Overview of Cell Death	10
1.2.1 Apoptosis	10
1.2.1.1 <i>Caspases Activation</i>	11
1.2.1.2 <i>Extrinsic Apoptotic Pathway</i>	12
1.2.1.3 <i>Intrinsic Apoptotic Pathway</i>	12

1.2.1.4	<i>Cross-talk between Extrinsic and Intrinsic Apoptotic Pathways</i>	13
1.2.1.5	<i>Bcl-2 Family Proteins</i>	13
1.5.1.6	<i>Caspase-independent Apoptosis</i>	14
1.5.1.7	<i>Induction of Apoptosis as a Strategy for Cancer Treatment</i> ..	15
1.2.2	Necrosis	15
1.2.3	Autophagy	16
1.3	EGFR and MAPKs Signaling Pathways in Cancers	17
1.3.1	Epidermal Growth Factor Receptor (EGFR) Signaling Pathways	17
1.3.1.1	<i>Ras/ERK</i>	18
1.3.1.2	<i>PI3K/Akt</i>	18
1.3.1.3	<i>JAK/STAT</i>	19
1.3.1.4	<i>Association of EGFR Signaling to Cancers</i>	19
1.3.2	Mitogen-activated Protein Kinase (MAPKs) Signaling Pathways	20
1.3.2.1	<i>p38 MAPK</i>	21
1.3.2.2	<i>c-Jun N-terminal Kinases (JNK)</i>	22
1.3.2.3	<i>Extracellular Signal Regulated Protein Kinases (ERK)</i>	22
1.3.2.4	<i>MAPKs Signaling Pathways as Chemotherapeutic Targets</i> ..	23
1.4	Nasopharyngeal Carcinoma (NPC)	23
1.4.1	Epidemiology of NPC	23
1.4.2	Classification	24
1.4.3	Aetiology	24
1.4.3.1	<i>Environmental Factors</i>	24
1.4.3.2	<i>Genetic Factors</i>	25
1.4.3.3	<i>Latent Infection with Epstein Barr Virus (EBV)</i>	25
1.4.4	Molecular Alternations of Apoptosis and Growth Signalings in NPC ...	26
1.4.4.1	<i>Bcl-2</i>	26
1.4.4.2	<i>EGFR</i>	27
1.4.5	PDT on NPC	28
1.5	Aims and Scopes of the Studies	28

Chapter 2	37
Materials and Methods	37
2.1 Materials.....	37
2.1.1 Photosensitizer.....	37
2.1.2 Cell Lines.....	37
2.1.3 Reagents for Cell Culture.....	37
2.1.3.1 <i>Fetal Bovine Serum (FBS)</i>	38
2.1.3.2 <i>Antibiotics</i>	38
2.1.3.3 <i>Roswell Park Memorial Institute (RPMI) Medium 1640</i>	38
2.1.3.4 <i>Trypsin-EDTA (1X)</i>	38
2.1.3.5 <i>Dulbecco's Phosphate Buffered Saline (PBS)</i>	39
2.1.4 Fluorescent Probes for Organelle Localization.....	39
2.1.4.1 <i>Mitochondrial Probe</i>	39
2.1.4.2 <i>Endoplasmic Reticulum Probe</i>	39
2.1.4.3 <i>Lysosome Probe</i>	39
2.1.5 Reagents for the Determination of Cell Viability.....	39
2.1.5.1 <i>Trypan Blue Staining Dye</i>	39
2.1.5.2 <i>Propidium Iodide (PI) Staining Solution</i>	40
2.1.5.3 <i>Crystal Violet Staining Dye (0.5%)</i>	40
2.1.6 Fluorescent Probe for Reactive Oxygen Species (ROS) Detection.....	40
2.1.7 Antioxidants.....	40
2.1.7.1 <i>L-histidine</i>	40
2.1.7.2 <i>D-mannitol</i>	40
2.1.7.3 <i>N-acetyl-L-cysteine (NAC)</i>	41
2.1.8 Reagents for Determination of Mitochondrial Functions.....	41
2.1.8.1 <i>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium</i> <i>Bromide (MTT) Solution</i>	41
2.1.8.2 <i>Ethyl Ester Tetramethylrhodamine (TMRE) Solution</i>	41
2.1.9 Reagents for Annexin V Binding Assay.....	42
2.1.9.1 <i>AnnexinV-FITC</i>	42
2.1.9.2 <i>Binding Buffer</i>	42
2.1.10 Reagents for Nuclear Staining.....	42

2.1.11	Pharmacological Inhibitors	42
2.1.12	Reagents for Transient Transfections	43
	2.1.12.1 <i>Transfecting Agent</i>	43
	2.1.12.2 <i>Bcl-2 pcDNA3 Plasmid</i>	43
	2.1.12.3 <i>Small-interfering RNA (siRNA)</i>	43
2.1.13	Reagents for Total Cell Lysates Extraction	43
	2.1.13.1 <i>Tris-HCl (pH 8.0)</i>	43
	2.1.13.2 <i>Sodium Chloride (NaCl) Solution</i>	43
	2.1.13.3 <i>Cell Lysis Buffer</i>	44
	2.1.13.4 <i>Phosphatase Inhibitor</i>	44
	2.1.13.5 <i>Protease Inhibitor</i>	44
	2.1.13.6 <i>Cell Lysis Buffer Working Solution</i>	44
2.1.14	Reagents for Protein Determination	44
2.1.15	Reagents and Materials for Western Blotting	44
	2.1.15.1 <i>30% Acrylamide/bis Solutions</i>	45
	2.1.15.2 <i>1.5 M Tris-HCl (pH 8.8)</i>	45
	2.1.15.3 <i>0.5 M Tris-HCl (pH 6.8)</i>	45
	2.1.15.4 <i>10% Sodium Dodecyl Sulfate (SDS) Solution</i>	45
	2.1.15.5 <i>10% Ammonium Persulphate (APS) Solution</i>	45
	2.1.15.6 <i>N, N, N', N'- tetramethylethylenediamine (TEMED)</i>	45
	2.1.15.7 <i>SDS Sample Buffer (5X)</i>	46
	2.1.15.8 <i>Protein Sample Markers</i>	46
	2.1.15.9 <i>Electrode Buffer</i>	46
	2.1.15.10 <i>Transblotting Buffer</i>	46
	2.1.15.11 <i>PolyScreen[®] Polyvinylidene Fluoride (PVDF) Hybridization</i> <i>Transfer Membrane</i>	47
	2.1.15.12 <i>Filter Paper and Transblotting Fiber Pads</i>	47
	2.1.15.13 <i>Tris Buffered Saline (TBS) (10X)</i>	47
	2.1.15.14 <i>Tris Buffered Saline-Tween 20 (TBST) (1X)</i>	48
	2.1.15.15 <i>5% Non-fat Milk Blotto</i>	48
	2.1.15.16 <i>Stripping Buffer</i>	48
	2.1.15.17 <i>Chemiluminescent Detection Reagent</i>	48
	2.1.15.18 <i>X-ray Film</i>	48
	2.1.15.19 <i>Developer and Fixer</i>	48

2.1.16	Antibodies for Western Blotting.....	49
	2.1.16.1 Primary Antibodies	49
	2.1.16.2 Secondary Antibodies.....	49
2.1.17	Reagents for Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR).....	49
	2.1.17.1 Reagents for RNA Extraction.....	49
	2.1.17.2 Reagents for First-strand DNA (cDNA) Preparation	50
	2.1.17.3 Reagents for PCR.....	50
	2.1.17.4 Reagents for DNA Gel Electrophoresis.....	51
	2.1.17.5 Ethidium Bromide Solution.....	51
2.1.18	Human Cytokines ELISA Kits	51
2.2	Methods	52
2.2.1	Cell Cultures	52
2.2.2	Trypan Blue Exclusion Method.....	52
2.2.3	Cellular Uptake of Zn-BC-AM.....	53
2.2.4	Determination of Subcellular Localization of Zn-BC-AM.....	53
2.2.5	PDT Treatment of NPC Cells	54
2.2.6	Determination of Cell Viability	54
	2.2.6.1 PI Exclusion Assay.....	54
	2.2.6.2 Clonogenicity Assay.....	55
2.2.7	Detection of ROS Production	55
2.2.8	Determination of Mitochondrial Function.....	56
	2.2.8.1 MTT Assay	56
	2.2.8.2 Detection of Mitochondrial Membrane Potential Depolarization	57
2.2.9	Annexin V Binding Assay	57
2.2.10	Transient Transfection	58
	2.2.10.1 Plasmid DNA Transfection	58
	2.2.10.2 siRNA Transfection	58
2.2.11	Western Blotting.....	59
	2.2.11.1 Total Cell Lysates Preparation and Protein Extraction.....	59
	2.2.11.2 Protein Determination	59
	2.2.11.3 Preparation of Loading Samples	60

2.2.11.4	<i>Sodium Dodecyl Sulfate- Polyacrylamide Gels Electrophoresis (SDS-PAGE)</i>	60
2.2.11.5	<i>Transblotting of Protein to Membrane</i>	61
2.2.11.6	<i>Antibody Probing</i>	61
2.2.11.7	<i>Stripping and Re-probing</i>	62
2.2.11.8	<i>Analysis of Protein Band Intensity</i>	62
2.2.12	Nuclear Staining	62
2.2.13	Reverse Transcription-Polymerase Chain Reaction (RT-PCR).....	62
2.2.14	Cytokine ELISA Analysis	63
2.2.15	Statistical Analysis.....	63
 Chapter 3		72
 Characterization of Cell Death Induced by Zn-BC-AM PDT in HK-1 Cells		72
3.1	Introduction	72
3.2	Results	73
3.2.1	Cellular Uptake of Zn-BC-AM by HK-1 Cells	73
3.2.2	Subcellular Localization of Zn-BC-AM in HK-1 Cells.....	73
3.2.3	Induction of Cell Death by Zn-BC-AM PDT	74
3.2.4	Changes in Nuclear Morphology after Zn-BC-AM PDT	75
3.2.5	Disruption of Mitochondrial Membrane Potential and Inhibition of Mitochondrial Enzyme Activities.....	76
3.2.6	Externalization of Phosphatidylserine	77
3.2.7	Western Blotting Analysis of Caspase Activation.....	77
3.2.8	Expression of Bcl-2 Family Proteins in Zn-BC-AM PDT-treated HK-1 Cells	77
3.2.9	Involvement of ROS in Zn-BC-AM PDT-induced Cell Death	78
3.2.10	Effects of L-histidine on Zn-BC-AM PDT-induced Cell Death.....	78
3.2.11	Effects of L-histidine on Zn-BC-AM PDT-induced Apoptosis.....	79

3.2.12	L-histidine Attenuated the Loss of Anti-apoptotic Bcl-2 Family Proteins	79
3.2.13	Effects of Overexpression of Bcl-2 on Zn-BC-AM PDT-induced Cell Death	80
3.3	Discussion.....	81
3.3.1	Mode of Zn-BC-AM PDT-induced Cell Death	81
3.3.2	Role of Bcl-2 and Other Bcl-2 Family Proteins in Zn-BC-AM PDT-induced Cell Death.....	84
Chapter 4	114
EGFR Inhibitor Enhances Zn-BC-AM PDT-induced Apoptosis in HK-1 Cells	114
4.1	Introduction	114
4.2	Results	115
4.2.1	Effects of EGFR Inhibitor AG1478 on HK-1 Cells	115
4.2.2	Effects of EGFR Inhibition on Akt, ERK Phosphorylation in HK-1 Cells	115
4.2.3	Effects of AG1478 on Zn-BC-AM PDT-treated HK-1 Cells	116
4.2.4	Enhancement of Zn-BC-AM PDT-induced Apoptosis by AG1478.....	117
4.2.5	Effects of AG1478 and Zn-BC-AM PDT on Akt and ERK Protein Expressions	117
4.2.6	Effects of Pharmacological Inhibition of EGFR Downstream Pathways on Zn-BC-AM PDT-induced Cell Death in HK-1 Cells.....	118
4.2.7	Effects of Inhibition of EGFR, PI3K/Akt and MEK/ERK Pathways on the Zn-BC-AM PDT-induced Apoptosis.....	118
4.3	Discussion.....	119
4.3.1	EGFR Inhibitor Enhances Zn-BC-AM PDT-induced Apoptosis in HK-1 Cells	119

4.3.2	Involvement of PI3K/Akt and MEK/ERK Pathways in Zn-BC-AM PDT- induced Apoptosis.....	120
4.3.3	Bcl-2 is the Target of EGFR and Zn-BC-AM PDT	122
Chapter 5.....		140
Role of Mitogen-activated Protein Kinase (MAPKs) Signaling Pathways in Zn-BC-AM PDT-induced Apoptosis on HK-1 Cells.....		140
5.1	Introduction	140
5.2	Results	141
5.2.1	Zn-BC-AM PDT Induced Activation of MAPKs.....	141
5.2.2	Effects of Pharmacological Inhibitors on Zn-BC-AM PDT-induced Cell Death	141
5.2.3	Effects of Pharmacological Inhibitors on the Formation of Apoptotic Cells	142
5.2.4	Effects of p38 MAPK Inhibitors on Colony Formation	142
5.2.5	Effects of Genetic Knockdown of p38 MAPK Isoforms on Zn-BC-AM PDT-induced Cell Death.....	143
5.2.6	Effects of Genetic Knockdown of p38 MAPK Isoforms on Zn-BC-AM PDT-induced Apoptosis.....	143
5.3	Discussion.....	145
5.3.1	Activation of MAPKs by Zn-BC-AM PDT	145
5.3.2	Role of p38 MAPK Isoforms in Zn-BC-AM PDT-treated HK-1 Cells	147
5.3.3	Protective Effects of PD169316	148
Chapter 6.....		164
Zn-BC-AM PDT-induced Production of Cytokines by Epstein-Barr Virus-infected HK-1 Cells		164

6.1	Introduction	164
6.2	Results	165
6.2.1	Effects of Zn-BC-AM PDT on the Growth of HK-1-EBV Cells	165
6.2.2	Expression of Cytokines mRNA in Zn-BC-AM PDT-treated NPC Cells	165
6.2.3	Zn-BC-AM PDT Up-regulated Production of IL-1	166
6.2.4	Production of IL-1 β is Mediated via Interleukin-1 β Converting Enzyme (ICE)/ Caspase-1 Independent Mechanism	166
6.2.5	Detection of IL-6 Production in HK-1 and HK-1-EBV Cells	167
6.2.6	Inhibition of IL-8 Production by Zn-BC-AM PDT	167
6.3	Discussions	167
6.3.1	Effect of Zn-BC-AM PDT on the Expression and Production of IL-1 in HK-1 and HK-1-EBV Cells.....	167
6.3.2	Downregulation of IL-8 in Zn-BC-MA PDT-treated NPC Cells	169
Chapter 7.....		178
General Discussions and Conclusions		178
7.1	General Discussions	178
7.2	Summary.....	179
7.2.1	Zn-BC-AM PDT Induced Apoptosis in HK-1 Cells	180
7.2.2	The Efficacy of Zn-BC-AM PDT in HK-1 Cells Could be Enhanced through the Inhibition of the EGFR Signaling Pathways	181
7.2.3	Contributions of MAPKs Signaling in Zn-BC-AM PDT	181
7.2.4	Modulation of Pro-inflammatory Cytokines and Chemokines by Zn-BC-AM PDT	182
7.3	Perspectives	182
7.3.1	Other Potential Mechanisms Involved in Zn-BC-AM PDT-induced Apoptosis	182

7.3.2	Studies on the Effects of EGFR Inhibition <i>in vivo</i>	183
7.3.3	Modulation of Pro-inflammatory Cytokines and Chemokines by MAPKs Signaling	183
7.3.4	Identification of PD169316-sensitive Pathway(s) in Zn-BC-AM PDT-induced Cell Death.....	184
REFERENCES.....		186
LIST OF PUBLICATIONS		213
CURRICULUM VITAE		214