ANTI-INVASIVE EFFECTS OF 2,6-BIS-(4-HYDROXYL-3METHOXYBENZYLIDINE) CYCLOHEXANONE AND ITS MOLECULAR TARGETS ASSOCIATED WITH INVADOPODIA FORMATION IN MDA-MB-231 HUMAN BREAST CANCER CELLS

SITI NOR AINI BINTI HARUN

FPSK(M) 2018 21
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By

SITI NOR AINI BINTI HARUN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2018
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

ANTI-INVASIVE EFFECTS OF 2,6-BIS-(4-HYDROXYL-3METHOXYBENZYLIDINE) CYCLOHEXANONE AND ITS MOLECULAR TARGETS ASSOCIATED WITH INVADOPODIA FORMATION IN MDA-MB-231 HUMAN BREAST CANCER CELLS

By

SITI NOR AINI BINTI HARUN

May 2018

Chair : Nur Fariesha binti Md Hashim, PhD
Faculty : Medicine and Health Sciences

Cancer metastasis is a pathological event occurred in cancer patients where it can colonize the distant organs. In order to metastasize, the tumor cells need to migrate and invade the surrounding tissues. Invadopodia are thought to be specialized actin-rich protrusions formed by the highly invasive cells to degrade the dense meshwork of the extracellular matrix (ECM). This is the initial step employed to drive cancer invasion. Cancer metastasis is deadliest and has affected the survival rate of cancer patients. Current cancer treatment has also produced side effects. Chemotherapy has given rise to the invasion and metastasis while radiotherapy has caused the recurrence of cancer. Identification of compound(s) capable to disrupt the metastasis of cancer especially for hindering the invadopodia formation is important so as to provide anti-metastasis targeted therapy. Curcumin has been demonstrated to produce significant effect as an anti-cancer compound. However, due to its poor bioavailability, some analogues have been formulated. A curcuminoid analogue known as 2,6-bis-(4-hydroxyl-3-methoxybenzylidine) cyclohexanone or BHMC has shown good potential in inhibiting inflammation and hyperalgesia. It also possesses anti-tumor effects on 4T1 murine breast cancer cells in vivo. However, there is still lack of empirical evidence on how BHMC works in preventing human breast cancer invasion. In this study, we pursued to investigate the role of BHMC on MDA-MB-231 breast cancer cells its underlying mechanism of action to prevent breast cancer invasion especially on the formation of invadopodia.
Analysis revealed that treatment of BHMC at 12.5 μM and below did not interfere with the proliferation of MDA-MB-231 cells. By using scratch migration assay, transwell migration and invasion assays, we found that BHMC at 12.5 μM reduces the percentage of the migration and invasion of MDA-MB-231 cells. The gelatin degradation assay showed that BHMC reduces the number of cells forming invadopodia. Analysis of the proteins involved in invasion showed that there is significant reduction in the expression of Rho guanine nucleotide exchange factor 7 (β-PIX), matrix metalloproteinase-9 (MMP-9) and membrane type 1-MMP (MT1-MMP) in the present of BHMC treatment at 12.5 μM. It can be postulated that BHMC at 12.5 μM is the optimal concentration to prevent the invasiveness of breast cancer cells.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ANTI PENYERANGAN 2,6-BIS-(4-HYDROXYL-3METHOXYBENZYLIDINE) CYCLOHEXANONE DAN SASARAN MOLEKUL BERKAITAN PEMBENTUKAN INVADOPODIA TERHADAP SEL KANSER PAYUDARA MDA-MB-231

Oleh

SITI NOR AINI BINTI HARUN

Mei 2018

Chair : Nur Fariesha binti Md Hashim, PhD
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perawatan BHMC pada kepekatan 12.5 μM dan ke bawah tidak memberikan
esan terhadap tahap pertambahan sel MDA-MB-231. Walaupun begitu,
eksperimen pergerakan migrasi dan esei migrasi dan invasi transwell, BHMC
pada kepekatan 12.5 μM mengurangkan migrasi dan invasi sel MDA-MB-231.
Esei degradasi gelatin pula menunjukkan BHMC mengurangkan bilangan sel
yang membentuk invadopodia. Analisis kesan BHMC terhadap protein yang
berkaitan penyerangan kanser pula menunjukkan BHMC mengurangkan
ekspresi Rho guanine nucleotide exchange factor 7 (β-PIX), matrix
metalloproteinase-9 (MMP-9) dan membrane type 1-MMP (MT1-MMP) pada
kadar kepekatan 12.5 μM dan menyimpulkan bahawa BHMC pada kepekatan
12.5 μM merupakan kepekatan optimum untuk menghalang penyerangan sel
kanser payudara.
ACKNOWLEDGEMENTS

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I certify that a Thesis Examination Committee has met on 14 May 2018 to conduct the final examination of Siti Nor Aini binti Harun on her thesis entitled "Anti-Invasive Effects of 2,6-Bis-(4-Hydroxyl-3methoxybenzylicine) Cyclohexanone and Its Molecular Targets Associated with Invadopodia Formation in MDA-MB-231 Human Breast Cancer Cells" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

2D  Two-dimensional  
3D  Three-dimensional  
β-arr  β-arrestin  
ADAM  A disintegrin and metalloproteinase  
ANGPTL  Angiopoietin-like  
ANOVA  Analysis of variance  
AP 1  Activator protein 1  
Arf 6  ADP-ribosylation factor 6  
Arp  Actin-related protein  
ATP  Adenosine triphosphate  
b-FGF  Basic fibroblast growth factor  
BCA  Bicinchoninic acid  
BHMC  2,6-bis-(4-hydroxyl-3methoxybenzylidine)cyclohexanone  
BRCA1  Breast cancer gene 1  
BRCA2  Breast cancer gene 2  
BSA  Bovine serum albumin  
BSC  Bio safety cabinet  
CD63  CD63 antigen  
CD9  CD9 antigen  
Cdc42  Cell division cycle 42  
CIP  Cdc42-interacting protein  
CLP  Caecal-ligation puncture  
COPD  Chronic Obstructive Pulmonary Disease  
CRM  Chromosome region maintenance  
CSF  Colony stimulating factor  
DMEM  Dulbecco’s modified eagle’s medium  
DMOG  Dimethyloxaloylglycine  
DMSO  Dimethylsulfoxide  
DNA  Deoxyribonucleic acid  
DNA  Deoxyribonucleic acid  
ECL  Enhanced chemiluminescent  
ECM  Extracellular matrix  
EDTA  Ethylenediaminetetraacetic acid  
EGF  Epidermal growth factor  
EGFR  Epidermal growth factor receptor  
EMT  Epithelial mesenchymal transition  
EOC  Epithelial ovarian cancer  
ER/PR  Estrogen/progesterone receptor  
ERRB2  Epidermal growth factor receptor 2  
ET  Endothelin  
EV  Extracellular vesicles  
FA  Focal adhesion  
FAK  Focal adhesion kinase  
F-BAR  Fer/CIP4 homology-Bin/Amphiphysin/Rvs  
FBS  Fetal bovine serum  
GAPDH  Glyceraldehyde 3-phosphate dehydrogenase  
GDP  Guanosine diphosphate
<table>
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<td>GLUT</td>
<td>Glucose transporter</td>
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<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
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<td>HB</td>
<td>Heparin binding</td>
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<td>HER2</td>
<td>Human epidermal growth factor receptor 2</td>
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<td>HGF</td>
<td>Hepatocyte growth factor</td>
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<td>HIF</td>
<td>Hypoxia inducible factor</td>
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<td>HNSCC</td>
<td>Head and neck squamous cell carcinoma</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>HR</td>
<td>Homology region</td>
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<td>HRP</td>
<td>Horseradish peroxidase</td>
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<td>IGF</td>
<td>Insulin-like growth factor</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MAPK</td>
<td>p-38 and mitogen-activated protein kinase</td>
</tr>
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<td>MCP</td>
<td>Monocyte chemotactic protein</td>
</tr>
<tr>
<td>MLCK</td>
<td>Myosin like-chain kinase</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>Membrane type-1 matrix metalloproteinase</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian target of rapamycin</td>
</tr>
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<td>MTT</td>
<td>3-(4,5-Dimethylthiazol-2-yl)-2,5 diaphenyltetrazolium bromide</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor κB</td>
</tr>
<tr>
<td>NHE</td>
<td>Sodium/hydrogen exchanger</td>
</tr>
<tr>
<td>NIC</td>
<td>Notch 1 fragment</td>
</tr>
<tr>
<td>N-WASP</td>
<td>Neural-Wiskott Aldrich Syndrome</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
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<td>PDZ-RhoGEF</td>
<td>Postsynaptic density protein 95/disc-large/zonula</td>
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<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PIX</td>
<td>PAK-interacting exchange factor</td>
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<td>PKC</td>
<td>Protein kinase C</td>
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<td>PTEN</td>
<td>Phosphatase and tensin</td>
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<td>PVDF</td>
<td>Polyvinylidene difluoride</td>
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<tr>
<td>RIPA</td>
<td>Radioimmunoprecipitation assay</td>
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<td>ROCK</td>
<td>Rho-associated coiled-forming kinase</td>
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<td>RSV</td>
<td>Rous sarcoma virus</td>
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<tr>
<td>SDF</td>
<td>Stromal cell derived factor</td>
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<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
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<td>SDS-PAGE</td>
<td>SDS-polyacrylamide gel electrophoresis</td>
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<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>SH</td>
<td>Sulphydryl</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Transforming growth factor α</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor β</td>
</tr>
<tr>
<td>TIMP</td>
<td>Tissue inhibitor of metalloproteinase</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNBC</td>
<td>Triple negative breast cancer</td>
</tr>
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<td>TNF</td>
<td>Tumor necrosis factor</td>
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<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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</tr>
<tr>
<td>TOM1L</td>
<td>Target of MYB1-like protein</td>
</tr>
<tr>
<td>uPAR</td>
<td>Urokinase plasminogen activator receptor</td>
</tr>
<tr>
<td>US FDA</td>
<td>United State Food and Drug Administration</td>
</tr>
<tr>
<td>VCA</td>
<td>Verprolin-cofilin-acidic</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VHL</td>
<td>von Hippel-Lindau</td>
</tr>
<tr>
<td>WAVE</td>
<td>WASP family verprolin-homologous protein</td>
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<tr>
<td>WIP</td>
<td>WASP-interacting protein</td>
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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Breast cancer is a form of disorder in which the cancer cells can grow either at the lobular, medullary, ductal or sebaceous of the breast tissue. The causes of breast cancer include the mutations of the genes such as epidermal growth factor receptor 2 (ERBB2) (also known as human epidermal growth factor receptor 2 (HER2)), breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) (Lukong, 2017). There are six hallmarks of cancer that have been proposed which encompass of the ability of the cancer cells to sustain the proliferative signaling, resist cell death, induce angiogenesis, evade the growth suppressors, enable the replicative immortality and activate invasive metastasis (Hanahan & Weinberg, 2011). Most of the patients whom endure breast cancer are likely to face mortality once the cancer cells have metastasized to other parts of body. Triple negative breast cancer patients, whom are clinically having less or absent of HER2 receptor (-HER2) and estrogen/progesterone receptor (-ER/PR) are the highly invasive cancer incidence among the patients (Bianchini et al., 2016). These types of patients are having increased likelihood of distant recurrence and believed to be more aggressive compared to other types of breast cancer (Bianchini et al., 2016).

1.2 Problem Statement

Metastatic breast cancer is the major problem in the population as it can affect the survival of breast cancer patients (Lukong, 2017). The ability of the tumor cells to disseminate to other parts of the body such as lungs, brain and bones and only can be diagnosed at an advanced stage has been troublesome as the tumor cells cannot be eradicated totally from the body thus causing the recurrence of cancer (Scully et al., 2012). Once the invasive cancer cells have metastasized, the treatment to reduce the growth of cancer would be challenging as many cancer genes have been up-regulated and also reduce the sensitivity of cancer cells to the treatment (Massagué et al., 2017). There are several treatments used to treat breast cancer including the application of surgery, radio- and chemotherapy. radio- and chemotherapy (Lukong, 2017). However, besides killing the tumor cells, especially radio- and chemotherapy, both radio- and chemotherapy have caused side effects to the patients such as thrombocytopenia, diarrhea, infertility and others (Lukong, 2017).

Curcumin, a diferuloylmethane is a potential compound to target and modulate many proteins linked to cancer (Hasima & Aggarwal, 2012). Curcumin has been reported to have entered the clinical trials but the poor bioavailability
problem that curcumin had has been questioned (Anand et al., 2007). Having promising abilities to target multiple proteins related to cancer has urged the researchers to modify the structure of curcumin but still preserving the methyl and hydroxyl group which are responsible for the anti-proliferation and anti-oxidant properties of curcumin (Nagahama et al., 2016). One of the analogues of curcumin which is 2,6-bis-(4-hydroxyl-3methoxybenzylidine) cyclohexanone or BHMC has been studied in inflammation and hyperalgesic pain (Ming-Tatt et al., 2013; Tham et al., 2010). Recently, BHMC has shown to possess anti-tumor activity in 4T1 murine breast cancer (Razak et al., 2017). However, BHMC is yet to be studied in human breast cancer. It will be interesting to investigate the effects of BHMC on the migration and invasion of MDA-MB-231 human breast cancer cells and specifically in the formation of invadopodia and their targeted proteins.

1.3 Objectives

This research was conducted to investigate the anti-invasive effects of BHMC and its molecular target(s) on MDA-MB-231 human breast cancer cells.

The specific objectives of the study include the following:
1. To determine the effect of BHMC on the proliferation of MDA-MB-231 human breast cancer cells
2. To determine the effect of BHMC on the migration and invasion of MDA-MB-231 human breast cancer cells
3. To determine the effect of BHMC on the formation of invadopodia in MDA-MB-231 human breast cancer cells
4. To identify the effect of BHMC on expressions of the proteins involved in invasion and invadopodia such as MMP-9, MT1-MMP and β-PIX

1.4 Hypothesis

It is hypothesized that BHMC at non-cytotoxic concentrations will reduce the migration and invasion of MDA-MB-231 human breast cancer cells. In addition, it is expected that BHMC would reduce the number of cells forming invadopodia via down-regulation of MMP-9, MT1-MMP and β-PIX expressions.
REFERENCES


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