



UNIVERSITI PUTRA MALAYSIA

**OVER-EXPRESSION OF BIOMOLECULES
IN PHOSPHATIDYLINOSITOL-3-KINASE/IAKT SIGNALING PATHWAY
IN BREAST CANCER**

LOH HUI WOON

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BREAST CANCER**

By

LOH HUI WOON

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

November 2005



Specially dedicated to,

The one who had given me the strength to complete this course.....

For their invaluable love, understanding, tolerance, sacrifice and moral support.

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

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Chairman : Professor Seow Heng Fong, PhD

Faculty: Medicine and Health Science

Breast cancer is the leading cancer among women in Malaysia. Genetics, experimental and epidemiological data suggest that breast cancer develops from complex interaction between inherited susceptibility and environmental factors. Accumulating evidence suggests that the PI3K/Akt signaling pathways play a causative role in tumorigenesis of breast cancer.

By employing the immunohistochemical method, the expression of several key regulators or related biomolecules of the PI3K/Akt signaling pathways in 43 archived formalin fixed, paraffin embedded tissues of surgically resected breast carcinoma specimens from 1999 to 2002, were studied. A functional assay was performed to determine the expression of Akt related molecules when treated with SDF-1 α recombinant protein.

The results showed that: 1) The expression rates in tumour tissue of ER α , ER β , c-erbB2, p-Akt^{T308}, p-Akt^{S473}, p-BAD^{S136}, SDF-1 and Ki67 were 25.6%, 4.7%, 51.2%, 81.4%, 48.8%, 67.4%, 93.0% and 26.8%, respectively. In contrast, in the apparently normal adjacent tissue, the expression rates of these molecules were 23.1%, 53.8%, 0%, 7.7%, 7.7%, 53.8%, 92.3% and 15.4%, respectively. 2) Correlation of biomolecules with tumour tissues and apparently normal adjacent tissues was seen in the following biomolecules: ER β (p=0.001), c-erbB2 (p<0.001), p-Akt^{T308} (p<0.001) and Ki67 (p<0.001). 3) In tumour tissue, significant correlation was found between ER β with p-BAD^{S136} (p=0.004), p-Akt^{S473} with p-BAD^{S136} (p=0.006), c-erbB2 with p-Akt^{T308} and Ki67 (p=0.014 and p=0.000 respectively) and c-erbB2 with SDF-1 (p=0.047). In the apparently normal adjacent tissue, a significant correlation was found between ER α with p-BAD^{S136} (p=0.042), ER β with p-Akt^{S473} and p-BAD^{S136} (p=0.009 and p=0.001 respectively), and c-erbB2 with p-Akt^{T308} (p=0.042).

Our study also showed that SDF-1 α protein had a different effect on the expression of biomolecules, namely p-Akt^{T308}, p-Akt^{S473} and p-BAD^{S136}. In this functional assay, we found that SDF-1 α could possibly induce cell survival by inducing phosphorylation of Akt at Thr308 and Ser473 as well as phosphorylation of BAD at Ser136 which are anti-apoptotic signals. Similar patterns were observed with all three cell lines, namely MCF-7, MDA-MB-231 and MCF10A but the level of expression differed from each other.

This study had provided three important information for researchers and clinicians in terms of: (1) evidence of the involvement of the SDF-1 α in the PI3K/Akt signaling pathway in breast carcinoma tumourigenesis with detection of p-Akt. 2) For the first

time, we found c-erbB2 was inversely correlated with SDF-1 α expression. 3)

Identification of potential targets for therapeutic intervention of breast carcinoma.

On the basis of our data, we conclude that PI3K/Akt signalling pathway is involved in tumorigenesis of breast cancer. To the best of our knowledge, this is the first report from Malaysia. PI3K/Akt pathway might act with upstream molecules such as estradiol, SDF-1 α , c-erbB2 independently in promoting tumour growth and inhibition of apoptosis. This study has also provided useful information for the search or design of antitumour interventions.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**EKSPRESI BERLEBIHAN BIOMOLEKUL-BIOMOLEKUL YANG
TERDAPAT DALAM LALUAN ISYARAT PHOSPHATIDYLINOSITOL-3-
KINASE/AKT PADA BARAH PAYU DARA**

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November 2005

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Barah payudara adalah barah yang paling biasa di Malaysia. Data dari segi genetik, eksperimen dan epidemiologi menunjukkan bahawa barah payudara berlaku apabila terdapat komunikasi yang kompleks antara faktor kejadian dan alam sekitar. Terdapat bukti yang mengatakan bahawa laluan isyarat PI3K/Akt memainkan peranan dalam kejadian barah payudara.

Hubungan antara beberapa pengawal-aturl atau biomolekul yang berkaitan dengan lintasan isyarat PI3K/Akt telah dikaji dalam 43 tisu yang diperolehi daripada pesakit barah payudara dari tahun 1999 hingga 2002 telah diblokkan di dalam paraffin. Eksperimen fungsian telah dijalankan untuk menentukan ekspresi biomolekul Akt yang berhubungan dengan protein SDF-1 α .

Keputusan kajian menunjukkan bahawa: 1) ekspresi biomolekul ER α , ER β , c-erbB2, p-Akt^{T308}, p-Akt^{S473}, p-BAD^{S136}, SDF-1 and Ki67 terdapat di tisu barah dalam peratus 25.6%, 4.7%, 51.2%, 81.4%, 48.8%, 67.4%, 93.0% and 26.8% masing-masing. Manakala ekspresi biomolekul tersebut pada tisu selkeliling yang kelihatan biasa adalah 23.1%, 53.8%, 0%, 7.7%, 7.7%, 53.8%, 92.3% and 15.4% masing-masing. 2) Kolorasi biomolekul dengan tisu selkeliling yang kelihatan biasa adalah seperti berikut: ER β (p=0.001), c-erbB2 (p<0.001), p-Akt^{T308} (p<0.001) dan Ki67 (p<0.001). 3) Di tisu barah, kolerasi yang mencukupi terdapat di antara ER β dengan p-BAD^{S136} (p=0.004), di antara p-Akt^{S473} dengan p-BAD^{S136} (p=0.006), di antara c-erbB2 dengan p-Akt^{T308} dan Ki67 (p=0.014 dan p=0.000 masing-masing), di antara c-erbB2 dengan SDF-1 (p=0.047). Pada tisu selkeliling yang kelihatan biasa, kolerasi yang mencukupi terdapat di antara ER α dengan p-BAD^{S136} (p=0.042), di antara ER β dengan p-Akt^{S473} dan p-BAD^{S136} (p=0.009 dan p=0.001 masing-masing), di antara c-erbB2 dengan p-Akt^{T308} (p=0.042).

Kajian ini juga menunjukkan bahawa protein SDF-1 α mempunyai rangsangan yang berbeza terhadap ekspresi pelbagai biomolekul seperti p-Akt^{T308}, p-Akt^{S473} and p-BAD^{S136}. Dalam kajian fungsi ini, kami mendapati SDF-1 α boleh merangsang kehidupan sel melalui penfosforilasi Akt pada Thr 308 dan Ser473 yang akan menfosforilasi BAD pada Ser136 dan lalu menutup laluan apoptotic. Terdapat kesamaan antara ekspresi biomolekul pada tiga jenis sel yang lain, MCF-7, MDA-MB-231 dan MCF10A dengan perbezaan tahap ekspresi yang berlainan.

Kajian kami menghasilkan sekurang-kurangnya tiga maklumat yang penting kepada para penyelidik dan perubatan dari segi: 1) bukti pembabitan SDF-1 α dalam laluan

isyarat PI3K/Akt dalam barah payudara. 2) Buat kali pertama, kami menunjukkan bahawa c-erbB2 berkolerasi terbalik dengan ekspresi SDF-1. 3) Pengenalan sasaran-potensi bagi intervensi barah payudara terapi maju. Berdasarkan keputusan ini, kami mencadangkan bahawa SDF-1 α boleh dianggap sebagai sasaran terapeutik yang berpotensi untuk rawatan immuno barah payudara.

Berdasarkan keputusan yang diperolehi, kami membuat kesimpulan bahawa lintasan isyarat PI3K/Akt adalah berkait dengan tumorigenesis barah payudara di Malaysia. Lintasan isyarat ini adalah saling berhubungan dengan molekul awalan seperti estradiol, SDF-1 α dan c-erbB2 walaupun mereka juga boleh bertindak secara berasingan untuk menggalakkan pertumbuhan barah dan perencatan apoptosis. Kajian ini telah memberi maklumat yang berguna kepada para penyelidik dan perubatan dalam penemuan dan perekaan cara anti-barah yang lebih baik.

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LIST OF ABBREVIATIONS

APES	3-Aminopropyltrimethoxysilane
BSA	Bovine serum albumin
°C	Celsius degree
CaCl ₂	Calcium chloride
DAB	3,3'-Diminobenzidine
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
DTT	1,4-Dithiothreitol
EDTA	Ethylenediaminetetracetic acid
g	Gram
h	Hour(s)
HCl	Hydrochloric acid
mg	Milligram
MgCl ₂	Magnesium chloride
min(s)	Minute(s)
ml	Milliliter
mM	Millimolar
n	Nano
NaCl	Sodium chloride
NaOH	Sodium hydroxide
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI3K	Phosphatidylinositol-3 Kinase
PMSF	Phenylmethylsulfonyl fluoride

RNA	Ribonucleic acid
rpm	Revolutions per minute
s	Second(s)
TAE	Tris acetate EDTA buffer
Taq	Thermus aquaticus
μl	Microlitre
μg	Microgram
v/v	Volume per unit volume

CHAPTER 1

INTRODUCTION

Cancer is a disease that involves the dysfunction of the immune system. Transformation of normal cells to abnormal cells usually leads to apoptosis of that transformed cells. Cancer occurs when the immune system lost its ability to do surveillance to destroy those abnormal cells. The cancer cells could then proliferate uncontrollably into a mass. Loss of their normal function may interfere with the other body systems.

Breast cancer is the commonest cancer among women all around the world and it is a significant global disease burden. In Malaysia, there were 4337 cases reported by the National Cancer Registry Malaysia 2002. Worldwide, the ratio of mortality to incidence is about 36% which, compared to other cancer types, represents a relatively good prognosis. However, it remains the leading cause of cancer mortality in women and its treatment is often associated with toxicity and unfavourable cosmetic outcome that impacts greatly on quality of life.

After several decades of cancer research focusing only on the tumour cell itself, we are just realizing that cancer is not only a group of abnormally growing cells, but it is an abnormal mass with multiple cell types communicating with each other (Polyak, 2001).