

**OVER-EXPRESSION OF BIOMOLECULES IN
PHOSPHATIDYLINOSITOL-3-KINASE/AKT SIGNALING PATHWAY IN
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

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

By

LOH HUI WOON

November 2005

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

Oleh

LOH HUI WOON

November 2005

Pengerusi: Profesor Seow Heng Fong, PhD

Fakulti: Perubatan dan Sains Kesihatan

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 protin 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 protin 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.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my main supervisor, Prof. Dr. Seow Heng Fong for giving me the chance to work on this most interesting project. Her generous guidance, endless help and encouragement throughout the period of this project have been the base and driving force for this thesis. Her careful review and constructive criticism have been crucially important for this thesis.

I would also like to acknowledge and thank my co-supervisors, Assoc. Prof. Dr. Hairuszah Ithnin and Dr. Thuaibah Hashim, for their endless help, advice and comments throughout the entire progress of this project.

I am grateful to the staff of Pathology and Surgery, Hospital Universiti Kebangsaan Malaysia, especially Mr. Rohaizak and Ms. Nakayah, for generously providing tissue specimens and collection of the clinical information. Not forgetting all the staff of Histopathology Unit, Dept of Clinical Laboratory Science, UPM, especially Mrs. Juita and Pn. Normah for kindly allowing me access to their laboratory facilities.

I am indebted to my labmates, especially Dr. Maha Abdullah, Mr. Khor Tin Oo, Mr. Yip Wai Kien, Ms. Lim Pei Ching, Ms. Leong Pooi Pooi, Ms. Ong Hooi Tin, Ms. Cheah Hwen-Yee, Ms. Masriana Hassan and Mr. Anthonysamy for their collaboration and sharing everyday joys and miseries in the making of science.

My family, Mom, Dad, Sisters (Hui Ling and Hui Yee) who had given me endless support in completion of this thesis.

I certify that an Examination Committee met on 29th November 2005 to conduct the final examination of Loh Hui Woon on her Master of Science thesis entitled "Over-Expression of Biomolecules in Phosphatidylinositol-3-Kinase/Akt Signaling Pathway in Breast Cancer" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Elizabeth George, MBBS(Mal), FAMM, DCP(Lond), FRCPA(Aust), MD(S'pore), FRCPE(E'din)

Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Foo Yoke Ching, PhD

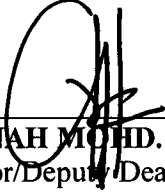
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Zaridah Hambali, PhD

Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Cheong Soon Keng, PhD

Professor
Clinical School
International Medical University
(External Examiner)



HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: **19 JAN 2006**

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

SEOW HENG FONG, PhD

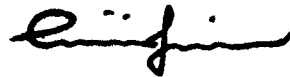
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

HAIRUSZAH ITHNIN, MD (UKM), M. Path (UKM), AM (Malaysia)

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

THUAIBAH HASHIM, LRCP & SI, MB BCh BAO (Ireland), M. Path (Malaysia)

Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: **07 FEB 2006**

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Loh Hui Woon

LOH HUI WOON

Date: *10/01/2008*



TABLE OF CONTENTS

DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxiii
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	4
2.1 Epidemiology	
2.2 Risk Factors of Breast Cancer	6
2.2.1 Host Factors	6
2.2.2 Environmental Factors	7
2.3 Breast Cancer Pathogenesis	10
2.3.1 Oestrogen and Receptors Signaling Pathway	11
2.3.2 ErbB-2 Signaling Pathway	12
2.3.3 Phosphatidylinositol-3 Kinase (PI3-Kinase)/Akt-signaling Pathway	14
2.3.4 Involvement of Chemokines in the Immune Regulation in Breast Cancer	16
2.4 Pathologic Types and Tumour Staging	18
2.4.1 Pathologic Types	18
2.4.2 Histological Grade	19
2.4.3 Tumour Staging	20
2.5 Methods of Diagnosis	21
2.5.1 Needle Biopsy	22
2.5.2 Radiological Diagnosis	26
2.6 Treatment of Breast Cancer	28
2.6.1 Surgical Management	28
2.6.2 Adjuvant Endocrine and Chemotherapy	30
2.6.3 Radiotherapy	32
2.6.4 New Strategies for Breast Cancer Treatment	32
2.7 Prevention	33
3. MATERIAL AND METHODS	34
3.1 Tissue Specimens	34
3.1.1 Tissue Specimens for Immunohistochemistry Staining	34
3.2 Immunohistochemistry	34
3.2.1 Preparation of Sections	34
3.2.2 Standard Procedure for Immunohistochemical Staining	35
3.2.2.1 Immunohistochemical Staining of ER α Protein	36



3.2.2.2	Immunohistochemical Staining of ER β Protein	37
3.2.2.3	Immunohistochemical Staining of C-erbB2 Protein	37
3.2.2.4	Immunohistochemical Staining of Phospho-Akt 1/2/3 (Thr308) Protein	37
3.2.2.5	Immunohistochemical Staining of Phospho-Akt 1 (Ser473) Protein	38
3.2.2.6	Immunohistochemical Staining of Phospho-BAD (Ser136) Protein	38
3.2.2.7	Immunohistochemical Staining of SDF-1 Protein	38
3.2.2.8	Immunohistochemical Staining of Ki67	39
3.2.3	Evaluation of Immunohistochemical Staining	39
3.2.4	Statistical Analysis	40
3.3	SDF-1 Recombinant Protein Functional Assay	41
3.3.1	Cell Culture	41
3.3.2	Treatment with SDF-1 α Recombinant Protein	41
3.3.3	Harvesting and Processing of Treated Cell Lines	41
3.3.4	Immunohistochemical Staining on Cell Lines	42
3.3.5	Evaluation of Immunohistochemical Staining	42
3.3.6	Analysis of Expression of Biomolecules in Response to SDF-1 Treatment by Western Blotting	43
3.3.6.1	Protein Extraction	43
3.3.6.2	Protein Concentration Determination	43
3.3.6.3	Western Blot	43
4.	RESULTS	45
4.1	Clinicopathological Data of Patients	45
4.2	Biomarkers of Breast Carcinoma	48
4.2.1	Detection of ER α Expression in Breast Tissues	48
4.2.2	Detection of ER β Expression in Breast Tissues	51
4.2.3	Detection of C-erbB2 Expression in Breast Tissues	53
4.2.4	Association of Biomarkers in Breast Tumour Tissues	55
4.3	PI3-Kinase/Akt Pathways in Relation to Breast Carcinoma	58
4.3.1	Detection of Phospho-Akt 1/2/3 (Thr308) Expression in Breast Tissues	58
4.3.2	Detection of Phospho-Akt 1 (Ser473) Expression in Breast Tissues	60
4.3.3	Detection of Phospho-BAD (Ser136) Expression in Breast Tissues	62
4.3.4	Detection of Ki67 Expression in Breast Tissues	64
4.3.5	Association of p-Akt ^{T308} , p-Akt ^{S473} and p-BAD ^{S136} Biomolecules in Relation to Breast Carcinoma	66
4.4	Expression of Chemokines in Breast Carcinoma	68
4.4.1	Detection of SDF-1 Expression in Breast Tissues	68
4.4.2	Association of SDF-1 with Biomolecules in Breast Tumour	70
4.5	Correlation among the Expression of PI3-K/Akt Signaling Pathway-Related Biomolecules	72
4.6	Correlation of Molecules Studied with Clinicopathological Data	75
4.6.1	Association Between Total Biomolecule Score with Age and Race	75
4.6.2	Association Between Total Biomolecules Score with Histological Grade and Pathological Stage	78
4.7	Alternation of Expression of Biomolecules in SDF-1 α Treated Cell Lines	81
4.7.1	Expression of Biomolecules in SDF-1 α Treated MCF-7 Cell Line	81

4.7.1.1	Detection of Expression of Biomolecules in SDF-1 α Treated MCF-7 Cell Line Using Immunohistochemistry Staining	81
4.7.1.2	Detection of Expression of PI3K/Akt Signaling Pathway Related Biomolecules in SDF-1 α Treated MCF-7 Cell Lines Using Western Blot	85
4.7.2	Expression of Biomolecules in SDF-1 α Treated MDA-MB-231 Cell Line	88
4.7.2.1	Detection of Expression of Biomolecules in SDF-1 α Treated MDA-MB-231 Cell Line Using Immunohistochemistry Staining	88
4.7.2.2	Detection of Expression of PI3K/Akt Signaling Pathway Related Biomolecules in SDF-1 α Treated MDA-MB-231 Cell Lines Using Western Blot	91
4.7.3	Expression of Biomolecules in SDF-1 α Treated MCF10A Cell Line	95
4.7.3.1	Detection of Expression of Biomolecules in SDF-1 α Treated MCF10A Cell Line Using Immunohistochemistry Staining	95
4.7.3.2	Detection of Expression of PI3K/Akt Signaling Pathway Related Biomolecules in SDF-1 α Treated MCF10A Cell Lines Using Western Blot	98
5.	DISCUSSION	102
5.1	Clinical Data	102
5.2	Immunohistochemistry Studies	103
5.2.1	Biomarkers of Breast Carcinoma	103
5.2.2	PI3-Kinase/Akt Pathways in Relation to Breast Carcinoma	105
5.2.3	Expression of Chemokines in Breast Carcinoma	108
5.3	Functional Assay Studies	110
6.	CONCLUSION AND RECOMMENDATION	113
6.1	Conclusion	113
6.2	Recommendation of Future Work	114
	REFERENCES	115
	APPENDICES	122
	BIODATA OF THE AUTHOR	137



LIST OF TABLES

Table	Page
2.1 Factors associated with increased risk of breast cancer	7
2.2 Histologic types of breast cancer	19
2.3 Modified Bloom and Richardson System for histological grading of carcinoma of the breast	20
2.4 Stage grouping for breast cancer	21
2.5 Agents commonly used for hormonal management of metastatic breast cancer.	30
2.6 Prognostic factors in node-negative breast cancer	31
3.1 Primary antibodies used in this study	36
3.2 Scoring system	40
4.1 Clinicopathological data of patients	46
4.2 Number of cases and correlation matrices among the clinicopathological factors (n=43)	47
4.3 Immunohistochemical staining for the expression of various biomolecules	50
4.4 Mean and standard deviation for the expression of various biomolecules	49
4.5 Percentage of cases with coexpression of biomolecules namely, ER α , ER β and c-erbB2 in tumour tissues	56
4.6 Correlation between ER β with ER α , c-erbB2 and Ki67 in tumour tissues	56
4.7 Correlation between ER α , ER β , c-erbB2 with histopathological parameters	57
4.8 Coexpression of ER α , ER β , c-erbB2, p-Akt ^{T308} and p-Akt ^{S437} in tumour samples	67
4.9 Comparison of p-Akt ^{T308} , p-Akt ^{S437} and p-BAD ^{S136} expression in tumour samples	68
4.10 Coexpression of SDF-1 with ER α , ER β , c-erbB2, p-Akt ^{T308} , p-Akt ^{S437} and p-BAD ^{S136} in tumour samples	71



4.11	Correlation among the total scores of biomolecules in breast tumour tissues	73
4.12	Correlation among the total scores of biomolecules in apparently normal adjacent tissues	74
4.13	Correlation between age and total scores of biomolecules in apparently normal adjacent tissues.	75
4.14	Correlation between age and total scores of biomolecules in breast tumour tissues.	76
4.15	Correlation between race and total scores of biomolecules in apparently normal adjacent tissues.	76
4.16	Correlation between race and total scores of biomolecules in tumour cancer tissues.	77
4.17	Correlation between histological grade and total scores of biomolecules in breast tumour tissues.	79
4.18	Correlation between pathological stage and total scores of biomolecules in breast tumour tissues.	79
4.19	Normalised integrated density values of various biomolecules using β -actin as baseline value for SDF-1 α treated MCF-7 cell line	86
4.20	Normalised intergrated density values of various biomolecules using β -actin as baseline value for MCF-7 cell lines without treatment	87
4.21	Normalised intergrated density values of various biomolecules using β -actin as baseline value for SDF-1 α treated MDA-MB-231 cell lines	93
4.22	Normalised intergrated density values of various biomolecules using β -actin as baseline value for MDA-MB-231 cell lines without treatment	94
4.23	Normalised intergrated density values of various biomolecules using β -actin as baseline value for SDF-1 α treated MCF10A cell lines	100
4.24	Normalised intergrated density values of various biomolecules using β -actin as baseline value for MCF10A cell lines without treatment	101

LIST OF FIGURES

Figure		Page
2.1	Classes of molecules regulating growth and differentiation of the normal mammary gland.	5
2.2	Dimerization and downstream signaling of the HER (EGFR) family.	12
2.3	Activation of growth factor receptor protein tyrosine kinases results in autophosphorylation on tyrosine residues.	15
2.4	Evaluation of breast masses in postmenopausal women.	24
2.5	Evaluation of breast masses in premenopausal women.	25
4.1	Representative areas showing the immunohistochemical staining of ER α .	49
4.2	Immunoreactivity of ER α in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	51
4.3	Representative areas showing the immunohistochemical staining of ER β .	52
4.4	Immunoreactivity of ER β in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	53
4.5	Representative areas showing the immunohistochemical staining of c-erbB2.	54
4.6	Immunoreactivity of c-erbB2 in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	55
4.7	Representative areas showing the immunohistochemical staining of phospho-Akt 1/2/3 (Thr308).	59
4.8	Immunoreactivity of p-Akt ^{T308} in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	60
4.9	Representative areas showing the immunohistochemical staining of phospho-Akt 1 (Ser473).	61

4.10	Immunoreactivity of p-Akt ^{S473} in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	62
4.11	Representative areas showing the immunohistochemical staining of phospho-BAD (Ser136).	63
4.12	Immunoreactivity of p-BAD ^{S136} in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	64
4.13	Representative areas showing the immunohistochemical staining of Ki67.	65
4.14	Immunoreactivity of Ki67 in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	66
4.15	Representative areas showing the immunohistochemical staining of SDF-1.	69
4.16	Immunoreactivity of SDF-1 in 43 breast carcinoma tissues and 13 apparently normal adjacent tissues (Score >3 = positive staining).	70
4.17	Percentage of immunopositive samples according to age group and race in tumour and apparently normal adjacent tissues.	77
4.18	Percentage of immunopositive samples according to histological grade and pathological stage in tumour tissues.	80
4.19	Immunohistochemical staining on various biomolecules on MCF-7 cell lines treated with 100 ng/ml of human SDF-1 α and harvested at 0, 2, 6 and 24 hours.	82
4.20	Immunohistochemical staining on various biomolecules on MCF-7 cell lines without treatment of human SDF-1 α and harvested at 0, 2, 6 and 24 hours.	83
4.21	Scores for immunoreactivity of various biomolecules namely ER α , c-erbB2, p-Akt ^{T308} , p-Akt ^{S473} , p-BAD ^{S136} , SDF-1 α and Ki67 in MCF-7 cell lines.	84
4.22	Western blot of various biomolecules on MCF-7 cell lines treated with 100 ng/ml of human SDF-1 α and harvested at 0, 2, 6 and 24 hours.	86
4.23	Western blot of various biomolecules on MCF-7 cell lines without any treatment and harvested at 0, 2, 6 and 24 hours.	86

4.24	Comparison line chart on the normalized level of expression of various biomolecules as compared to base line β -actin with treated and untreated MCF-7 cell lines with SDF-1 α .	87
4.25	Immunohistochemical staining on various biomolecules on MDA-MB-231 cell lines treated with 100 ng/ml of human SDF-1 α and harvested at different time.	89
4.26	Immunohistochemical staining on various biomolecules on MDA-MB-231 cell lines without treatment of human SDF-1 α and harvested at different time.	90
4.27	Scores for immunoreactivity of various biomolecules namely ER α , c-erbB2, p-Akt ^{T308} , p-Akt ^{S473} , p-BAD ^{S136} , SDF-1 α and Ki67 in MDA-MB-231 cell lines.	91
4.28	Comparison of western blot on various biomolecules on MDA-MB-231 cell lines treated with 100 ng/ml of human SDF-1 α and harvested at 0, 2, 6 and 24 hours.	93
4.29	Comparison of western blot on various biomolecules on MDA-MB-231 cell lines without any treatment and harvested at 0, 2, 6 and 24 hours.	93
4.30	Comparison line chart on the normalized level of expression of various biomolecules as compared to base line β -actin with treated and untreated MDA-MB-231 cell lines with SDF-1 α .	94
4.31	Immunohistochemical staining on various biomolecules on MCF10A cell lines treated with 100 ng/ml of human SDF-1 α and harvested at different time.	96
4.32	Immunohistochemical staining on various biomolecules on MCF10A cell lines without treatment of human SDF-1 α and harvested at different time.	97
4.33	Scores for immunoreactivity of various biomolecules namely ER α , c-erbB2, p-Akt ^{T308} , p-Akt ^{S473} , p-BAD ^{S136} , SDF-1 α and Ki67 in MCF10A cell lines.	98
4.34	Western blot on various biomolecules on MCF10A cell lines treated with 100 ng/ml of human SDF-1 α and harvested at 0, 2, 6 and 24 hours.	100
4.35	Western blot on various biomolecules on MCF10A cell lines without any treatment and harvested at 0, 2, 6 and 24 hours.	100

4.36	Comparison line chart on the normalized level of expression of various biomolecules as compared to base line β -actin with treated and untreated MCF10A cell lines with SDF-1 α .	101
5.1	A new mechanism by which oestrogen promotes proliferation of ER-positive ovarian epithelial cancer cells. (Adapted from Hall and Korach, 2003)	110
5.2	Phenotypic signaling in breast cancer cells. (Adapted from Kumar and Hung, 2005)	112



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
μ l	Microlitre
μ g	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).



Many methods of early detection and treatment of breast cancer had been developed, but they are still not enough to fully and successfully treat all breast cancer patients. Intensive research efforts have been conducted to find the cause of this disease, but unfortunately, the causative factor of the disease has still not been found.

Oestrogen receptor α (ER α) belongs to the superfamily of steroid nuclear receptor transcriptional factors. It regulates the proliferation and differentiation of many tissues, especially reproductive tissues. On binding to specific DNA sequences such as estrogen responsive elements (EREs), oestrogen-ER α complexes activate or repress target gene transcription. The biological activity of oestrogen is now realized to be more complex than initially thought, with the discovery of a second oestrogen receptor (ER) named ER β (Girault *et al.*, 2003). ERs utilize the membrane epidermal growth factor receptor (EGFR) to rapidly signal through various kinase cascades that influence both transcriptional and non-transcriptional actions of estrogen in breast cancer cells (Levin *et al.*, 2003). Recent evidence suggests that common adaptations which occur during resistance to both tamoxifen and oestrogen deprivation use various signal transduction pathways, often involving cross-talk with a retained and functional ER protein (Johnston *et al.*, 2003). Oh and colleagues (2001) found that hyperactivation of mitogen-activated protein kinase (MAPK) could induce loss of ER α expression in breast cancer cells. This might be one of the causes of resistance to antioestrogen drugs in ER α positive cells. Studies of forced c-erbB2 overexpression in animals and cell lines have demonstrated the oncogenic potential of c-erbB2, and spontaneous homodimerization leading to tyrosine kinase activation is most likely an important mechanism for the oncogenicity of c-erbB2 overexpression (Siegel and Muller, 1996). Lindberg and colleagues identified the