UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF CANDIDATE PROTEINS INVOLVED IN CALRETICULIN-DEPENDENT INVASION USING BREAST CANCER TISSUE BIOPSIES

ZAHRA YEKTA MOGHADDAM NOUDEHI

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By

ZAHRA YEKTA MOGHADDAM NOUDEHI

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

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Chairman: **Professor Rozita Rosli, PhD**

Faculty: **Medicine and Health Sciences**

Breast cancer is one of the most common malignancies among women and accounts for more than one-fifth of all female cancer deaths. Invasive ductal carcinoma is the largest sub-type of invasive breast cancers. Tumor cells can alter their ability to adhere to both surrounding cells and the extracellular matrix during invasion. The delineation of the molecular networks and pathways that affect invasion has been a central target in biomedical research. A large number of proteins have been implicated in breast cancer invasion and one important protein is calreticulin (CRT). CRT is a high-capacity calcium-binding protein found in the endoplasmic reticulum (ER) lumen and has been implicated in a variety of functions outside of the ER, including cell adhesion and integrin-dependent calcium signaling. CRT has been found to be over-expressed in cancers including breast cancer. It is postulated to have an important role in the development and progression of cancers through the enhancement of cell motility and anoikis resistance.

It is hypothesised that the effect of CRT over-expression on cell adhesion is due to the effect of CRT-dependent cellular signaling pathways. There are many proteins that CRT is linked to and hypothesised to contribute to the conferment, maintenance and increment of the invasive potential of breast cancer cells. Previous studies have shown that there correlations between CRT over-expression and some of the proteins involved in cancer cell invasion exist. The protein candidates investigated in this study are found to be implicated with a cancer invasive phenotype and potentially CRT-dependent. Hence the main objective of this study was to determine the expression and localization patterns of MUC1, TSP-1, CTTN, E-cadherin, and HER-2/neu in breast cancer tissues and corresponding non-malignant tissues.

In this study, immunohistochemistry (IHC) analysis was employed to evaluate the expression patterns of the selected proteins in 46 formalin-fixed paraffin-embedded breast cancer and non-malignant tissues. Semi-quantitative immunohistochemical analyses demonstrated differing degrees of differential expression patterns of MUC1, TSP-1, CTTN, E-cadherin and HER-2/neu proteins in breast cancer tissues. MUC1 protein was over-expressed in the malignant tissues as compared to non-malignant tissues ($p<0.05$), and also showed positive correlation with CRT expression. TSP-1 over-expression was not significant between non-malignant and malignant tissues. Nevertheless TSP-1 was over-expressed in a subset of stage 4 and grade 3 tumors. There was a correlation of expression between TSP-1 and CRT. CTTN protein showed a significant decrease in expression in malignant tissues as compared to non-malignant tissues ($p<0.05$). Comparison of CTTN and CRT expression also yielded no correlation in expression patterns. E-cadherin expression was decreased in stage 2 tumors but when compared between malignant and non-malignant tissues, there

was no significant loss of expression. However, there was an inverse correlation between E-cadherin and CRT expression patterns. HER-2/neu analyses showed significant over-expression in malignant lesions as compared to non-malignant ($p<0.05$), but there was direct correlation between HER-2/neu and CRT expression patterns. In conclusion, this study identified a positive association between CRT and HER-2/neu, MUC1, and TSP-1 and a negative association between CRT and E-cadherin in breast cancer. This body of work will form the basis for future validation studies of expression patterns as well as the molecular dissection of CRT-dependent pathways that are linked to invasive breast cancers. HER-2/neu and MUC1 proteins over-expression can potentially be used as molecular markers for early detection of breast cancer.

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

PENCIRIAN PROTEIN YANG BERPOTENSI TERLIBAT DALAM SERANGAN BARAH PAYUDARA YANG BERGANTUNG PADA CALRETICULIN DENGAN MENGGUNAKAN BIOPSI TISU

Oleh

ZAHRA YEKTA MOGHADDAM NOUDEHI

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Barah payudara ialah salah satu kejadian malignan yang biasa di kalangan wanita dan menjadi punca satu perlima daripada seluruh kematian wanita akibat barah. Karsinoma duktus invasif ialah jenis barah payudara invasif yang paling banyak dihidapi. Sel tumor boleh berubah bagi membolehkan pelekatan dengan sel di persekitaran dan matriks di luar sel semasa serangan. Penggambaran rangkaian dan laluan molekular yang boleh memberi kesan terhadap serangan menjadi sasaran utama dalam penyelidikan biomedik. Sebilangan besar protein telah dikenal pasti terlibat dalam serangan barah payudara dan salah satu protein yang utama ialah kalretikulin (CRT). CRT ialah protein penambat kalsium berkapasiti tinggi yang didapati di dalam lumen retikulum endoplasma (ER) dan dijangka terlibat dalam pelbagai fungsi di luar ER, yang merangkumi pelekatan sel serta pengisyaratan kalsium yang bergantung pada integrin. CRT didapati terlebih ekspresi dalam barah termasuk barah payudara. CRT dipostulat mempunyai peranan penting dalam

perkembangan barah melalui peningkatan gerakan sel dan ketahanan terhadap anoikis.

Kesan CRT dengan ekspresi berlebihan terhadap pelekatan sel dihipotesis disebabkan oleh laluan pengisyaratan sel yang bergantung pada CRT. Terdapat banyak protein yang berhubung kait dengan CRT dan dihipotesis membantu penyumbangan, pengekalan dan peningkatan potensi sel barah payudara. Kajian terdahulu telah menunjukkan bahawa terdapat hubung kait antara ekspresi berlebihan CRT dengan sebilangan protein yang terlibat dalam serangan sel barah. Calon protein yang dikaji dalam penyelidikan ini didapati terbabit sebagai ciri fenotip barah invasif dan berkemungkinan bergantung pada CRT. Oleh itu, objektif utama kajian ini ialah bagi menentukan corak ekspresi dan penyetempatan MUC1, TSP-1, CTTN, E-cadherin dan HER-2/neu dalam tisu barah payudara dan tisu bukan malignan yang berpadanan.

Dalam kajian ini, analisis imunohistokimia digunakan bagi menilai corak ekspresi protein yang terpilih dalam 46 tisu barah payudara dan tisu bukan malignan yang diawet dengan formalin dan dibenamkan di dalam parafin. Analisis imunohistokimia separa kuantitatif menunjukkan tahap berbeza-beza bagi corak ekspresi MUC1, TSP-1, CTTN, E-cadherin dan HER-2/neu dalam tisu barah payudara. Protein MUC1 terlebih ekspresi di dalam tisu malignan berbanding dengan tisu bukan malignan ($p<0.05$), dan turut menunjukkan korelasi positif dengan ekspresi CRT. Ekspresi berlebihan TSP-1 antara tisu malignan dengan yang bukan malignan adalah tidak signifikan. Walau bagaimanapun, TSP-1 terlebih ekspresi di dalam tumor peringkat 4 dan gred 3. Terdapat korelasi ekspresi antara TSP-1 dengan CRT. Protein CTTN

menunjukkan pengurangan signifikan ekspresi di dalam tisu malignan berbanding di dalam tisu bukan malignan ($p<0.05$). Perbandingan antara CTTN dan CRT juga menunjukkan tiada korelasi dari segi corak ekspresi. Ekspresi E-cadherin berkurang dalam tumor peringkat 2 tetapi tiada pengurangan yang signifikan apabila corak ekspresi dibandingkan antara E-cadherin dan CRT. Analisis HER-2/neu menunjukkan ekspresi berlebihan signifikan di dalam tisu malignan berbanding di dalam tisu bukan malignan ($p<0.05$), tetapi terdapat korelasi langsung antara corak ekspresi HER-2/neu dan CRT. Kesimpulannya, kajian ini mengenal pasti hubungan positif antara CRT dengan HER-2/neu, MUC1 dan TSP-1 serta hubungan negatif antara CRT dengan E-cadherin dalam barah payudara. Hasil kajian ini boleh dijadikan asas kajian pengesahan pada masa akan datang dari segi corak ekspresi dan juga kajian molekular laluan yang bergantung pada CRT yang berkaitan dengan barah payudara invasif. Ekspresi berlebihan protein HER-2/neu and MUC1 ini berpotensi untuk digunakan sebagai penanda molekul untuk barah payudara pada peringkat awal.

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I certify that a Thesis Examination Committee has met on 30 August 2012 to conduct the final examination of Zahra Yekta Moghaddam Noudehi on thesis entitled "Characterization of Candidate Proteins Involved in Calreticulin-dependent Invasion Using Breast Cancer Tissue Biopsies'' in accordance with the Universities and University College 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.

Member of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements of the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

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

ZAHRA YEKTA MOGHADDAM NOUDEHI

Date: 30 August 2012

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