UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERISATION OF FLOTILLIN-2 AS A MOLECULAR TARGET IN BREAST AND BLADDER CANCERS

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By

NARGES JAFARZADEH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

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DEDICATION

This thesis is dedicated to my parents.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

IDENTIFICATION AND CHARACTERISATION OF FLOTILLIN-2 AS A MOLECULAR TARGET IN BREAST AND BLADDER CANCERS

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Chairman : Abhimanyu Veerakumarasivam, PhD

Faculty : Bioscience

Invasive breast and bladder cancers are associated with poor clinical outcome and are characterised by a genotype that is distinct from superficial disease. Predicting the invasive and metastatic potential of tumours at the time of diagnosis remains a major challenge in cancer management. Exploiting a multi-component data-mining *in silico*strategy, genes associated with an invasive phenotype were targeted. These bioinformatics screens were conducted on previously published and publicly available datasets derived from various high-throughput genome-wide experimental data based on gene expression arrays and array-based comparative genomic hybridisation. Differentially expressed genes between high and low-risk cancers were functionally annotated by gene ontology and compared to available expression datasets. Overexpression of a lipid raft associated protein, Flotillin 2 (*FLOT2*) in invasive cancers was identified. The *FLOT2* locus (17q11-q12) was associated with copy number gains in 15% of tumours.Flotillin-2 is an important lipid raft marker and is predicted to be involved in cell-matrix adhesion, cell migration and signal

transduction. Immunohistochemistry was used to evaluate flot2 expression and localisation in formalin fixed paraffin-embedded malignant and non-malignant breast cancers. Flot2 localisation varied from a cytoplasmic distribution in normal cells to a more cell-cell contact distribution in malignant cells. A correlation was found between flot2 overexpression in the invasive compartments of tumour tissues and clinical stage. The staining intensity in the invasive compartment increased with cancer progression. Flot2 protein expression was tested in an independent bladder cancer tissue microarray series by immunohistochemistry. Flot2 protein expression increased with bladder cancer progression as well. Subsequently,*FLOT2*was knockdown in bladder and breast cancer cells *in vitro* by siRNA. Migration and invasion assays were employed to determine the phenotypic effects of *FLOT2* inhibition. The inhibition of*FLOT2* expression in knockdown cells was confirmed by RT-qPCR and Western blotting. Knockdown of *FLOT2* led to a significant reduction in the invasive and migratory cellular phenotypes. The precise mode of action of flot2 remains to be elucidated but it is predicted to play an important role in transmembrane signal transduction, cell adhesion and endocytosis. Incidentally, *FLOT2* overexpression has also been shown to enhance the spreading of cells, formation of filopodia as well as melanoma progression and metastasis. This study identifies and confirms flot2 overexpression as a common feature of invasive breast and bladder cancers.In addition, the functional targeting experiments and gene-dosage dependent *FLOT2* overexpression in invasive breast and bladder cancers confirm a link between *FLOT2* and a pro-invasive cancer phenotype.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGENALPASTIAN DAN PENCIRIAN FLOTILLIN-2 SEBAGAI SASARAN MOLEKUL DALAM ARAH PAYUDARA DAN PUNDI KENCING

Oleh

NARGES JAFARZADEH

January 2013

Pengerusi : Abhimanyu Veerakumarasivam, PhD

Fakulti : Biosains

Barah payudara dan pundi kencing yang invasif dikaitkan dengan diagnosis yang kurang memuaskan dan dicirikan dengan genotip yang berbeza daripada barah yang tidak invasif.Kebolehan untuk meramal potensi invasif dan metastatik tumor semasa diagnosis masih menjadi satu cabaran utama dalam pengurusan pesakit barah.Melalui penggunaan strategi perlombongan data pelbagai komponen secara '*in silico*', gen-gen yang dikaitkan dengan fenotip invasif telah disiasat.Penyaringan bioinformatik telah dijalankan terhadap dataset-dataset umum dan yang diterbitkan sebelum ini, hasil daripada pelbagai data eksperimen seluruh genom pemprosesan tinggi berdasarkan tatasusunan ekspresi gen dan perbandingan hibridisasi genomik berasaskan tatasusunan.Pembezaan ekspresi gen-gen diantara barah berisiko tinggi dan rendah dipadankan kepada fungsi molekular secara ontologi dan dibandingkan kepada dataset ekspresi yang sedia ada.Kami mengenal pasti ekspresi berlebihan protein yang berkaitan dengan rakit lipid, Flotillin 2 (*FLOT2*) dalam barah invasif.Lokus *FLOT2* (17q11-q12) dihubungkaitkan dengan pernambahan bilangan salinan dalam 15% tumor.*FLOT2* merupakan penanda rakit lipid yang penting dan

dijangka terlibat dalam pelekatan sel-matriks, perpindahan sel dan transduksi isyarat.Imunohistokimia digunakan bagi menilai ekspresi flot2 di dalam tisu barah payudara malignan dan bukan malignan yang diawet dengan formalin dan dibenamkan di dalam parafin.Penyentempatan flot2 berbeza-beza daripada taburan sitoplasma di dalam tisu normal kepada taburan yang mempunyai lebih banyak pertemuan antara sel di dalam tisu malignan.Suatu perkaitan didapati antara ekspresi berlebihan flot2 di dalam bahagian invasif tisu tumor dengan peringkat klinikal.Tahap pewarnaan di dalam bahagian invasif semakin meningkat apabila peringkat barah semakin meningkat.Ekspresi protein flot2 diuji di dalam satu siri mikroatur tisu pundi kencing.Ekspresi protein flot2turut meningkat dengan perkembangan barah pundi kencing juga.Berikutan itu, ekspresi *FLOT2* dilenyapkan di dalam sel barah pundi kencing dan payudara secara *'in vitro'*denganmenggunakan siRNA.Ujian perpindahan dan pencerobohan digunakan bagi mengenal pasti kesan perencatan *FLOT2* dari segi fenotip.Perencatan ekspresi *FLOT2* di dalam sel disahkan melalui RT-qPCR dan blot Western.Pelenyapan ekspresi *FLOT2*menyebabkan pengurangan signifikan fenotip sel yang berciri invasif.Cara tindakan khusus flot2 masih belum dicirikan tetapi diramalkanberperanan penting dalam transduksi isyarat merentasi membran, pelekatan sel dan endositosis.Ekspresi berlebihan *FLOT2* juga didapati meningkatkan penyebaran sel, pembentukan filopodia, dan juga perkembangan melanoma dan metastasis.Kajian ini mengenalpasti dan mengesahkan ekspresi berlebihan flot2 sebagai ciri subset barah payudara dan barah pundi kencing yang invasif.Di samping itu, eksperimen-eksperimen yang mensasarkan fungsi dan ekspresi berlebihan *FLOT2* yang bergantung pada gen-dos mengesahkan hubungan antara *FLOT2* dengan fenotip barah proinvasif.

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I certify that a Thesis Examination Committee has met on September 2012 to conduct the final examination of Narges Jafarzadeh on her thesis entitled "**IDENTIFICATION AND CHARACTERISATION OF FLOTILLIN-2 AS A MOLECULAR TARGET IN BREAST AND BLADDER CANCER**" 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 relevant degree of Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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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, and is not concurrently, submitted for other degree at Universiti Putra Malaysia or at any other institution.

NARGES JAFARZADEH
Date: 4 January 2013

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