UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN HUMAN BLADDER CANCER USING OLIGONUCLEOTIDE MICROARRAY

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By

NIK NORLIZA BT NIK HASSAN

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

June 2006

Al-Fatihah

This thesis is specially dedicated to beloved mother, Allahyarhamah Hajjah Nik Noh Bt Nik Man, who succumbed for Ischaemic Heart Disease. She passed away peacefully on Wednesday 9 June, 2004 at 12.20 am at Intensive Care Unit, Hospital Universiti Sains Malaysia. Our thoughts are always with you. In life, we love you dearly; in death we love you still. They say time heals everything, but we know this isn't so. Because it hurts as much today as it did a year ago. Loving you, forever and always.

Amin

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

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NIK NORLIZA BT NIK HASSAN

June 2006

Chairman: **Associate Professor Rozita Rosli, PhD**

Faculty: **Faculty of Medicine and Health Sciences**

Bladder cancer is one of the most common malignancies in developed countries while transitional cell carcinomas (TCC) are the origin of more than 90% of diagnosed bladder cancers. Patients diagnosed with bladder cancer basically belong to two clinically distinct groups, namely non-muscle invasive (which are papillary pattern) and muscle invasive (which are solid). These carcinomas pose the greatest clinical problems due to the high recurrence of non-muscle invasive tumors even after transurethral resection of the tumors. At present, there are no clinically useful markers available for identifying bladder cancer patients with a high risk of disease recurrence or progression.

Multiple molecular events take place when normal epithelial cells are transformed into tumor tissues. These can now be monitored simultaneously, by using oligonucleotide microarrays and the expression patterns of three different grades of TCC namely TCC WHO Grade I, TCC WHO Grade II and TCC WHO Grade III (according to WHO classification) can be established. In this study, individual cell suspensions were

prepared from bladder tumor biopsies. Pools of cells were also prepared from non-cancerous tissues. Total RNA was isolated and reverse transcribed into cDNA. *In vitro* transcription into cRNA was carried out with the incorporation of Cy3 and Cy5 dyes. Labeled cRNA probes were co-hybridized to the microarray slide containing 1,853 cancer related genes. Following hybridization procedure, scanning of the array slides was carried out to identify the gene expression levels in each of the sample investigated.

To determine the co-expression patterns displayed between different stages of the bladder cancer, hierarchical clustering analysis that group tumors according to similarity in their expression profile was used. Hierarchical clustering demonstrated an unambiguous separation of TCC WHO Grade I from Grades II and III of these urothelial tumors. In addition, based on the gene function, nine clusters of genes were identified. These genes are associated with cell adhesion molecules, protein synthesis, oncogenes, apoptosis markers, growth factors, immunology, cell cycle regulators, transcription factors and angiogenesis. Fold-change analysis of gene expression revealed 106, 49 and 51 genes that are over-expressed and 13, 186 and 132 that are suppressed in TCC Grades I, II and III, respectively. A gene is considered differentially expressed if its relative expression is two-fold or greater.

Because of the inherent limitations in the reliability of microarray, genes identified as differentially expressed were validated with a PCR-based method. Real-Time PCR was able to confirmed 75% of the microarray data. These results were in concordance to that previously reported. This study indicates that gene expression patterns can be identified in bladder cancer by combining oligonucleotide arrays and Real-Time PCR

analysis. These data combined with other data from similar research, could provide insights into the extent of expression differences underlying malignancy of the bladder cancer and reveal genes that may prove useful as diagnostic or prognostic markers.

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

IDENTIFIKASI DAN ANALISIS EKSPRESI GEN BERBEZA DALAM KANSER PUNDI KENCING MENGGUNAKAN MIKROATUR OLIGONUKLEOTIDA

Oleh

NIK NORLIZA BT NIK HASSAN

Jun 2006

Pengerusi: **Professor Madya Rozita Rosli, PhD**

Fakulti: **Fakulti Perubatan Dan Sains Kesihatan**

Kanser pundi kencing merupakan kanser yang paling kerap berlaku di negara membangun. Kebanyakan neoplasma adalah dari jenis 'transitional cell carcinoma' (TCC). Secara klinikal, TCC terbahagi kepada dua kumpulan iaitu yang dipanggil sebagai 'non-muscle invasive' dan 'muscle invasive'. Karsinoma tersebut memberikan masalah klinikal yang tinggi kerana biasanya pesakit dengan kanser 'non-muscle invasive' mengalami masalah serangan ulangan yang kerap walaupun setelah dilakukan prosedur 'transurethral resection'. Buat masa ini, masih tiada penanda klinikal yang berpotensi untuk mengenalpasti pesakit kanser pundi kencing terutamanya yang mempunyai risiko serangan ulangan atau berpotensi untuk berkembang keperingkat yang lebih teruk.

Pelbagai perubahan molekul berlaku apabila sel epithelial normal berubah kepada sel kanser yang kemudiannya berportensi menjadi invasif. Keadaan ini boleh diperhatikan

secara serentak dengan adanya kaedah terbaru yang dikenali sebagai teknologi DNA mikroatur di mana corak pengekspresan gen-gen bagi tiga gred TCC yang dinamakan sebagai TCC WHO Gred I, TCC WHO Gred II dan TCC WHO Gred III (berdasarkan kepada klasifikasi WHO) dapat dikenalpasti. Dalam kajian in, suspensi sel daripada biopsi kanser pundi kencing disediakan. Suspensi dari sel bukan kanser turut disediakan. RNA diekstrakan dan dilakukan transkripsi berbalik untuk menghasilkan cDNA. Transkripsi *in vitro* kemudiannya dilakukan bagi mendapatkan cRNA yang secara serentak di labelkan dengan pewarna Cy3 dan Cy5. cRNA berlabel di hibridisasikan bersama ke atas slid mikroatur yang mengandungi 1,853 gen berkaitan dengan kanser. Pengimbasan slaid dilakukan bagi mengenalpasti aras hibridisasi yang mencerminkan aras ekspresi gen dalam setiap sampel kajian.

Untuk mengenalpasti corak ko-ekspresi yang dipamirkan antara gred kanser pundi kencing yang berlainan, analisis kelompok hiraki yang menggumpulkan kanser berdasarkan persamaan profil ekspresi telah digunakan. Kelompok hiraki telah menunjukkan pengasingan yang jelas antara TCC WHO Gred I dengan TCC WHO Gred II dan TCC WHO Gred III. Berdasarkan kepada fungsi gen, sebanyak sembilan kelompok gen telah dikenalpasti. Mereka adalah gen yang berkaitan dengan sel molekul pelekap, sintesis protin, onkogen, penanda apoptosis, faktor transkripsi, pengawalatur kitaran sel, faktor pertumbuhan, imunologi dan angiogenesis. Analisis perubahan pengekspresan gen telah mendapati 106, 49 dan 51 gen menunjukan corak pengekspresan melebihi dua kali ganda manakala 13, 186 and 132 gen yang menunjukan corak perencatan dalam TCC Gred I, II and III, masing-masing. Gen dianggap menunjukkan pengekspresan berbeza apabila ekspresi relatifnya adalah dua kali lebih dari sampel kawalan.

Oleh kerana terdapat faktor penghad dalam reliabiliti teknik mikroatur, gen yang dikenalpasti menunjukkan pengekspresan yang berbeza telah disahkan semula menggunakan kaedah Real-Time PCR. Kaedah ini telah mengesahkan 75% daripada data mikroatur. Keputusan ini adalah sama dengan apa yang telah dilaporkan sebelum ini. Kajian ini telah mencadangkan corak pengekspresan gen boleh dikenalpasti dengan mengabungkan kaedah mikroatur dan kaedah Real-Time PCR. Pergabungan data yang dihasilkan dalam kajian ini serta hasil penemuan penyelidik lain dalam kajian yang berkaitan dapat memberikan informasi mengenai perbezaan ekspresi gen yang berlaku dalam setiap proses perkembangan kanser pundi kencing dan seterusnya mendedahkan gen yang berpotensi sebagai penanda diagnostik dan prognostik.

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In the Name of Allah, Most Gracious, Most Merciful

"Over the knowledgeable, is Allah the most knowledgeable"

All praises and gratitude is due to Allah, to whom every single creature in the heaven and the earth belongs to. Thank you Allah for giving me the strength and patient during this trying times. May peace and blessings be on the leader of all creations, the prophet Muhammad S.A.W, his family and companions.

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I certify that an Examination Committee has met on 29 Jun 2006 to conduct the final examination of Nik Noriza Nik Hassan on her Doctor of Philosophy thesis entitled "Identification of Differentially Expressed Genes in Human Bladder Cancer Using Oligonucleotide Microarray" 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:

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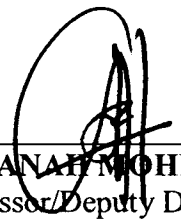

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DECLARATION

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 or concurrently submitted for any other degree at UPM or other institutions.

NIK NORLIZA BT NIK HASSAN

Date: 6/10/08

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LIST OF ABBREVIATIONS

ADRB2	Adrenergenic beta-2 receptor
ALL	Acute lymphoblastic leukemia
AML	Acute myloid leukemia
Bax	Bcl2-associated x protein
bp	Base pair
BCG	Bacille Calmette-Guerin
bFGF	Basic fibroblast growth factor
BPH	Benign prostate hyperplasia
Casp10	Caspase 10
cDNA	Complementary deoxyribonucleic acid
Cdh15	Cadherin 15
Cdh3	Cadherin 3
Cdk4	Cyclin-dependent kinase 4
Cdk6	Cyclin-dependent kinase 6
Cdk7	Cyclin-dependent kinase 7
Cdk8	Cyclin-dependent kinase 8
C/EBP	Ccaater enhancer binding protein cebp delta
CGH	Comparative genomic hybridization
Cis	Carcinoma *in situ*
CNS	Central nervous system
Cp	Ceruloplasmin
cRNA	Complementary ribonucleic acid
CSF1R	Colony stimulating factor 1

CSV	Comma separated value
Ct	Crossing threshold
CYP1A2	Cytochrome p450 1A2
DAG1	Dystrogylcan 1
DD-PCR	Differential display polymerase chain reaction
DEPC	Diethypyrocarbonate
DLBCL	Diffuse large B-cell lymphoma
dNTPs	Dioxynucleotide triphosphate
DNA	Deoxyribonucleic acid
dsDNA	Double stranded deoxyribonucleic acid
Dvl	Disheveled
EDTA	Ethylenediamine tetra acetate
EF1G4	Eukaryotic translation initiation factor gamma 1
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGF16	Epidermal growth factor –like protein 6
ERK	Extracellular signal-regulated kinase
ERα	Estrogen receptor α
EST	Express sequence taq
EtBr	Ethidium bromide
Fez 1	Zygin isoform 2
FGFR	Fibroblast growth factor receptor
FGF	Fibroblast growth factor
FOI	Frequency of incorporation
FRET	Fluorescent resonance energy transfer

Fz	frizelled
g	gram
G-CSF	Granulocyte colony stimulating factor
GSK-3β	Glycogen synthesis kinase-3β
GSTM1	Glutathione S transferase M1
h	hour
H & E	Hematoxylin and eosin
HKB	Hospital Kota Bharu
hPim-2	Human Pim-2
HPSF	High purity salt free
Hspa 1a	Heat shock 70kd protein
HUSM	Hospital Universiti Sains Malaysia
IL-6	Interleukin 6
IL8ra	Interleukin 8 receptor alpha
IL9r	Interleukin 9 receptor
Itgb4	Integrin beta 4
IVT	*In vitro* transcription
Jun b	Jun b proto-oncogene
KRT 19	Keratin 19
L	Liter
LCM	Laser microdissection
LEF-1	Lymphoid enhancer binding factor
LOH	Loss of heterozygosity
M	molar
MAPK	Mitogen activated protein kinase

Mcl-1	Myeloid leukemia Bcl-2 related
M-CSF	Macrophage colony stimulating factor
MDM2	Minute double murine 2
min	minutes
mg	milligram
mL	milliliter
MGB	Minor groover binder
mM	Millimolar
MMP	Matrix metalloproteinase
Mmp14	Matrix metalloproteinase 14
Mmp3	Matrix metalloproteinase 3
mRNA	Messenger ribonucleic acid
Myo5a	Myosin
NaOH	Sodium hydrogen
NAT2	N-acetyltransferase
NDN	Necdin
ng	nanogram
NMP22	Nucler matrix protein 22
NM23b	Non-metastatic cell protein
nM	Nanometer
NQO1	NADPH menandione oxidoreductase dioxin-inducible
NTC	Non-template control
PAI-1	Plasminogen activator inhibitor 1
PCR	Polymerase chain reaction
Pim-2	Pim-2 oncogene

pmol	picomol
PPARBP	Thyroid hormone receptor interactor
Rab 21	Rab 2 member ras oncogenes family-like
Rb	Retinoblastoma
RNA	Ribonucleic acid
RPL5	Ribosomal protein L5
RPL15	Ribosomal protein L15
RPS3	Ribosomal protein S3
RPS5	Ribosomal protein S5
RPS8	Ribosomal protein S8
RPS9	Ribosomal protein S9
RPS19	Ribosomal protein S19
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcriptase polymerase chain reaction
RT-	No-RT control
s	Second
SAGE	Serial analysis of gene expression
Scyb14	Small inducible cytokine 14
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SNCG	Synuclein gamma breast cancer
SNP	Single nucleotide polymorphism
ssDNA	Single stranded deoxyribonucleic acid
SOMs	Self organizing maps
TCC	Transitional cell carcinoma