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"IDENTIFICATION OF *RB1* GERM-LINE MUTATIONS IN PATIENTS WITH RETINOBLASTOMA"

Field of Study: **MOLECULAR GENETICS**

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ABSTRACT

Background: Retinoblastoma (*RB1*; OMIM#180200) is the most common intraocular tumor in early childhood. The malignancy mainly affects children under five years of age, with a prevalence of 1:15,000 to 1:20,000. *RB1* or retinoblastoma susceptibility gene is the gene associated with retinoblastoma and is located on chromosome 13q14.2. The whole coding region of *RB1* has 27 exons. The gene encodes a tumor suppressor, the retinoblastoma protein (pRB). Retinoblastoma develops when both alleles of the tumor suppressor gene are inactivated by loss-of-function mutations. The hereditary predisposition to the disease is caused by germ-line mutations in *RB1*. Since *RB1* is a tumor suppressor gene, the survivors of hereditary retinoblastoma also possess high risk of developing non-ocular cancers in their subsequent life. The identification of oncogenic germ-line mutations in the *RB1* gene of patients with retinoblastoma is significant for genetic counselling. There are very few reports on *RB1* mutations identified in Malaysian patients with retinoblastoma although several studies have been published on the prevalence and clinical features of the disease.

Aims: This was a hospital-based retrospective study to screen the entire coding region of *RB1* for *de novo* germ-line mutations in patients with no reported family history of retinoblastoma.

Methods: This study involved a cohort of six unrelated cases of sporadic retinoblastoma with different laterality of disease: three unilateral and three bilateral cases. Genomic DNA was extracted from peripheral blood of healthy control and patients with retinoblastoma. Polymerase chain reaction (PCR) was carried out to amplify the 27 exons of *RB1*. The purified PCR amplicons were then subjected to bidirectional DNA sequencing. Sequence data was compared with *RB1* DNA sequence

of two healthy controls of Chinese and Malay ethnicity and *RB1* reference sequence (GenBank accession no. L11910.1) to identify mutations. This exon-by-exon mutation analysis was conducted *in silico*.

Results: Analysis of DNA from blood leukocytes revealed heterozygous and heritable mutations in the *RB1* gene of two patients with bilateral retinoblastoma. The mutations were identical single base substitutions which caused a C to T transition: g.162237C>T. The nonsense mutation which was found in exon 23 changed codon 787 from wild-type CGA to mutant TGA (p.Arg787X), resulting in a premature stop codon. All three patients with unilateral retinoblastoma did not show any mutation in the *RB1* gene.

Conclusions: The findings proved that the mutations detected in DNA extracted from blood of patients were constitutional. The identified mutations were *de novo* mutations as both parents of respective patients lacked the abovementioned mutation in their *RB1* gene. The experiment results were consistent with past reports that patients with bilateral retinoblastoma usually harbour germ-line mutations in the *RB1* gene. Based on the study result, exon 23 and CGA codons are apparently more frequent mutational targets and should be initially screened in Malaysian patients with retinoblastoma though no mutation hot spots have been reported in *RB1* gene thus far. However, further studies with extended sample size would be needed to evaluate and establish a protocol for routine *RB1* mutation analysis.

ABSTRAK

Latar belakang: Retinoblastoma (*RB1*; OMIM#180200) adalah tumor intraokular yang paling kerap berlaku di peringkat awal kanak-kanak. Kanser ini menjejaskan terutamanya kanak-kanak di bawah umur lima tahun, dengan kelaziman 1:15 000 hingga 1:20 000. *RB1* atau gen kecenderungan retinoblastoma adalah gen yang dikaitkan dengan retinoblastoma dan terletak pada kromosom 13q14.2. Seluruh rantau pengekodan *RB1* mempunyai 27 ekson. Gen ini mengekod sejenis penindas tumor iaitu protein retinoblastoma (pRB). Retinoblastoma terjadi apabila kedua-dua alel gen penindas tumor dijadikan tidak aktif oleh mutasi hilang-fungsi. Predisposisi terhadap penyakit ini adalah melalui keturunan yang diakibatkan oleh mutasi titisan germa di *RB1*. Oleh sebab *RB1* merupakan sejenis gen penindas tumor, pesakit yang terselamat daripada retinoblastoma keturunan juga mempunyai risiko tinggi menghidap kanser bukan okular dalam kehidupan berikutnya. Identifikasi mutasi titisan germa onkogenik di dalam gen *RB1* pesakit retinoblastoma adalah penting untuk kaunseling genetik. Setakat ini, amat kurang laporan mengenai mutasi *RB1* yang ditemui pada pesakit retinoblastoma di Malaysia walaupun beberapa kajian telah diterbitkan berkaitan kelaziman dan ciri-ciri klinikal penyakit ini.

Matlamat: Ini adalah satu kajian retrospektif yang berasaskan hospital untuk menyaring seluruh rantau pengekodan *RB1* bagi mutasi titisan germa baharu di kalangan pesakit yang ada atau tidak mempunyai sejarah keluarga retinoblastoma.

Kaedah: Kajian ini telah melibatkan sebuah kohort yang terdiri daripada enam kes retinoblastoma sporadik yang tiada kaitan antara satu sama lain, dengan kesisian penyakit yang berbeza: tiga kes yang melibatkan satu sisi mata dan tiga kes yang melibatkan kedua-dua sisi mata. DNA genom telah diekstrak daripada darah periferi

kontrol sihat dan pesakit retinoblastoma. Reaksi rantai polimerase (PCR) telah dijalankan untuk mengamplifikasi 27 ekson *RB1*. Seterusnya, amplikon PCR tulen telah dilalui penjujukan DNA dwiarah. Data jujukan telah dibandingkan dengan urutan DNA *RB1* dua kontrol sihat dari etnisiti Melayu dan Cina dan jujukan rujukan *RB1* (no. induk GenBank L11910.1) untuk mengesan mutasi. Analisis mutasi ekson demi ekson ini telah dijalankan menggunakan perisian komputer.

Keputusan: Analisis DNA daripada leukosit darah mendedahkan mutasi-mutasi heterozigus dan yang diwarisi di dalam gen *RB1* dua pesakit retinoblastoma dua sisi mata. Mutasi-mutasi yang dikenal pasti merupakan penggantian bes tunggal yang serupa dan telah menyebabkan suatu transisi, iaitu C kepada T: g.162237C> T. Mutasi tak bererti itu ditemui di ekson 23 dan didapati mengubah kodon 787 daripada jenis liar CGA kepada mutan TGA (p.Arg787X), mengakibatkan suatu kodon terminasi pra-matang. Ketiga-tiga pesakit retinoblastoma satu sisi mata tidak menunjukkan sebarang mutasi di dalam gen *RB1*.

Kesimpulan: Penemuan membuktikan bahawa mutasi yang dikesan di dalam DNA yang diekstrak daripada darah pesakit adalah konstituen. Mutasi yang ditemui adalah mutasi baru kerana kedua-dua ibu bapa pesakit berkenaan tidak menunjukkan sebarang mutasi di dalam gen *RB1*. Keputusan eksperimen adalah konsisten dengan laporan sebelumnya bahawa pesakit retinoblastoma dua sisi mata biasanya mempunyai mutasi titisan germa di dalam gen *RB1*. Berdasarkan hasil kajian ini, ekson 23 dan kodon-kodon CGA merupakan sasaran mutasi dan perlu disaring dahulu pada pesakit retinoblastoma walaupun tiada kawasan khas mutasi telah dilaporkan di dalam gen *RB1* setakat ini. Walau bagaimanapun, kajian lanjut dengan saiz sampel yang lebih besar diperlukan untuk menilai dan mewujudkan protokol untuk analisis mutasi yang rutin di dalam *RB1*.

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→	to
A	absorbance
as	antisense
°C	degree Celsius
ADA	adenosine deaminase
AMD	amplification mismatch detection
ATF	activating transcription factor
Arg	arginine
BC	before Christ
bp	base pair(s)
BRCA1	breast cancer 1, early onset
CA	California
cDNA	complementary DNA
C-terminal	carboxyl-terminal
cdk	cyclin-dependent kinase
CpG	– C – phosphate – G –
CTS	computer tomography scan
ddH_2O	double-distilled water

DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DGGE	denaturant gradient gel electrophoresis
dNTPs	deoxynucleotide triphosphates
EBRT	external beam radiation
EDTA	ethylenediaminetetraacetic acid
et al.	and others
E1A	adenovirus early region 1A
g	gram
G1 phase	Gap 1 phase
HKL	Hospital Kuala Lumpur
HUKM	Hospital Universiti Kebangsaan Malaysia
i.e.	id est (Latin) / in example
Inc.	Incorporated
IPS	Institute of Postgraduate Studies
kb	kilobase
kDa	kilodaltons
LDL	low density lipoprotein

LOH	loss of heterozygosity
M	molar
MA	Massachusetts
MEN	multiple endocrine neoplasia
min	minute
mA	milliampere
mPCR	multiplex polymerase chain reaction
mg	milligram
ml	millilitre
mM	millimolar
mPCR	multiplex polymerase chain reaction
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MO	Missouri
n.d.	no date
ng	nanogram
nm	nanometer
N-terminal	amino-terminal
NY	New York

NaCl	natrium chloride
OMIM	Online Mendelian Inheritance in Man
ORF	open reading frame
PCR	polymerase chain reaction
p53	protein 53
PB	Peripheral blood
pRb/ pRB	*RB1*- encoded protein
PTC	premature termination codon
PTT	protein truncation test
q	long arm of chromosome
13q14.2	region 1, band 4, sub-band 2 on long arm of chromosome 13
Rb / RB	retinoblastoma
RB / RB1	retinoblastoma / retinoblastoma 1 gene
RBC	red blood cell
RetCam	retinal camera
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute

RT	reverse transcriptase
SDS	sodium dodecyl sulphate
se	sense
secs	seconds
SSC	saline-sodium citrate
SSCP	single strand conformation polymorphism
S phase	synthesis phase
Sp1	specificity protein 1
TBE	tris borate EDTA
TE	tris-EDTA
TP53	tumor protein 53
µl	microliter
µg	microgram
µM	micro molar
UMMC	University of Malaya Medical Centre
uPCR	uniplex polymerase chain reaction
USA	United Stated of America
UV	ultraviolet
V	volt

VHL	Von Hippel-Lindau
W	watt
Xp	short arm of the X chromosome
yr	year