ANALYSIS OF P27 AND CYCLIN D1 GENES IN GLIOMAS AND MENINGIOMAS USING MOLECULAR GENETIC, IMMUNOHISTOCHEMICAL AND IMMUNOGOLD ELECTRON MICROSCOPIC TECHNIQUES

FARIZAN BINTI AHMAD

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by

FARIZAN BINTI AHMAD

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Dedication

In the name of Allah, The Most Gracious, The Most Merciful...

My heartfelt appreciation specially goes to my utmost beloved mom and dad (Wan Zainab Abu Bakar and Ahmad W. Nik), my lovely brothers and sisters, and my dearest fiancé (Zul Faizuddin Osman). I am grateful for their continuous enriching love, understanding and encouragement. Thank you for always be there for me.
I love you all.

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LIST OF ABBREVIATIONS

AA215Gly	Aspartic acid to Glycine at codon 215
AA217Gly	Aspartic acid to Glycine at codon 217
bp	Base pair
BPH	Benign Prostatic Hyperplasia
C104T	C to T base substitution at codon 104
C223T	C to T base substitution at codon 223
CDK	Cyclin-dependent kinase
CDKI	Cyclin-dependent kinase inhibitor
CNS	Central Nervous System
c-onc	c-oncogene
CSGE	Confirmation Sensitive Gel Electrophoresis
Cyl-1	Cylicin-1
DHPLC	Denaturing High Performance Liquid Chromatography
DNA	Deoxyribonucleic Acid
dNTP	Deoxy Nucleotide Phosphatase
dsDNA	Double strand deoxyribonucleic acid
EDTA	Ethylenediaminotetra-acetate
EDTA	Ethylene Diamine Tetraacetic Acid
EGFR	Epidermal Growth Factor Receptor
G142A	G to A base substitution at codon 142
G242A	Guanine to Adenine at codon 242
GSK-3ß	Glycogen synthase-3beta
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HER	Herstatin
HIF-1α	Hypoxia Inducible Factor 1 alpha
HRP	Horse Radish Peroxidase
kb	Kilo base
LOH	Loss of Heterozygoosity
Lys223Lys	Lysine to Lysine at codon 223
M	Mitosis phase of cell cycle
M	Molar
$MgCl_2$	Magnesium Cloride
ml	Milliliter
N	Normal
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NES	Nuclear Export Signal
nm	Nanometer
p	Significant value
PBS	Phosphate buffered saline
PCR	Polimerase Chain Reaction
PRAD	proline-rich attachment domain
pRb	Retinoblatoma protein???
Pro214Arg	Proline to Arginine at codon 214
RNA	Ribonucleic acid
rpm	Revolutions per minute
SNP	Single nucleotide polymorphism

SSCP	Single Strand Confirmation Polymorphism
ssDNA	Single strand deoxyribonucleic acid
STR	Short tandom repeat
T109G	T to G base substitution at codon 109
T119C	T to C base substitution at codon 119
T215C	T to C base substitution at codon 215
TBE	Tris Boric EDTA
TBS	Tris buffered saline
TDGS	Two-Dimensional Gene Scanning
TEAA	Triethyammonium acetate
TEM	Transmission Electron Microscope
UV	Ultraviolet
WHO	World Health Organization

ANALISIS GEN P27 DAN CYCLIN D1 DALAM GLIOMA DAN MENINGIOMA DENGAN MENGGUNAKAN TEKNIK GENETIK MOLEKUL, IMMUNOHISTOKIMIA DAN MIKROSKOPI ELEKTRON IMMUNOGOLD

ABSTRAK

Meningioma dan glioma merupakan dua jenis tumor otak yang paling kerap di laporkan di seluruh dunia. Kedua-dua jenis kanser ini mungkin berlaku akibat daripada gangguan pada kitaran sel yang normal yang dikawal rapi oleh gen p27 dan cyclin D1. Kajian ini dijalankan untuk menentukan status mutasi gen p27 dan cyclin D1, tahap pengeksresan protein dan lokasi kedua-dua protein tersebut melalui beberapa analisis termasuklah analisis genetik molekul, immunohistokimia, mikroskopi elektron immunogold. Analisis genetik molekul menunjukkan mutasi berlaku pada ekson 4 gen cyclin D1 tetapi tiada mutasi dilaporkan pada ekson 5 gen cyclin D1, dan ekson 1 dan 2 gen p27 yang turut dikaji. Lima mutasi yang berlainan telah dikesan dalam 2 sample glioma (8.0%) dan 3 sampel meningioma (11.5%). Penjujukan DNA yang dilakukan pada dua sampel glioma tersebut menunjukkan kehadiran mutasi tidak bererti akibat perubahan nukleotida C kepada T pada kodon 223 (Lys223Lys). Di dalam sample glioma yang pertama, kami juga menjumpai delesi nukleotida G pada kodon 214 yang mengakibatkan mutasi anjakan rangka (Pro214Arg). Selain itu, kami juga menjumpai mutasi salah erti pada sampel glioma yang kedua. Perubahan nukleotida T kepada C telah dijumpai pada kodon 215 dan 217 yang telah menyebabkan perubahan asid aspartik kepada Glysin pada dua lokasi yang berlainan. Penskrinan mutasi dalam kes meningioma telah menemui 3 mutasi tidak bererti dan 3 mutasi salah erti dalam 3 sampel yang berasingan. Dalam ketiga-tiga sampel, kami menjumpai 3 kes perubahan nukleotida C kepada T yang mengakibatkan mutasi tidak bererti pada kodon 223 (Lys223Lys). Dalam sampel kedua dan ketiga, kami menjumpai perubahan nukleotida T kepada C pada kodon 215 yang mengakibatkan mutasi salah erti (Asp215Gly). Satu lagi

mutasi salah erti telah dijumpai pada kodon 217 yang menyebabkan perubahan nukleotida T kepada C dalam sampel meningioma yang ketiga. Analisis imunohistokimia pada kumpulan sampel yang sama menunjukkan protein p27 mengalami peningkatan dalam pengekspresannya dalam semua kes termasuklah meningioma (82.6%), glioma gred rendah (80.0%) dan glioma gred tinggi (84.6%). Kami kemudiannya menjumpai peningkatan dalam pengekspresan protein cyclin D1 dalam kes meningioma (70.8%) dan pengurangan dalam kes glioma peringkat tinggi (76.9% kes adalah mengalami pengekspresan protein cyclin D1 yang rendah). Kami juga menjumpai kadar pengekspresan yang sama dalam kes glioma peringkat rendah di mana 50% daripada kesnya mengalami pengekspresan protein yang rendah dan 50% mengalami pengekspresan protein yang tinggi). Analisis mikroskopi elektron immunogold pada protein p27 dan cyclin D1 menunjukkan kehadiran kedua-dua protein pada sitoplasma dan nukleus sel. Analisis statistik tidak menunjukkan sebarang hubungkait yang signifikan antara kehadiran mutasi pada gen cyclin D1 dengan pengurangan dalam tahap pengekspresan proteinnya dala kedua-dua kes meningioma (p=0.616) dan glioma (p=0.905).

Kata kunci: meningioma; glioma; p27; cyclin D1; genetik molekul; imunohistokimia; mikroskopi elektron imunogold

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ABSTRACT

Meningiomas and gliomas are two most commonly reported brain tumor cases worldwide. These types of tumors might occur due to the disruption of the normal cell cycle which is highly controlled by p27 and cyclin D1 genes. This study was performed to determine the mutational status of p27 and cyclin D1, level of both protein expression and the localization of both proteins at ultrastructural level via analyses of molecular genetic, immunohistochemistry and immunogold electron microscopy respectively. The molecular genetic analysis revealed mutations in exon 4 of cyclin D1 gene but none was detected in other studied regions of exon 5 of cyclin D1 gene and exon 1 and 2 of p27 gene. Five different mutations were detected in 2 glioma (8.0%) and 3 meningioma (11.5%) samples. DNA sequencing for the two gliomas samples revealed the presence of non-sense mutation which resulted to the change of C to T nucleotide at codon 223 (Lys223Lys). In the first glioma sample, we also detected a G base deletion at codon 214 which caused a frameshift mutation (Pro214Arg). In addition to that, we also found two other missense mutations in the second glioma sample. T to C nucleotide changes were detected at codon 215 and codon 217 which caused aspartic acid to Glycine changes in two different loci. Screening of mutations in meningiomas cases revealed 3 cases of non-sense mutations and 3 cases of missense mutations in a total of 3 samples. In all 3 samples, we found 3 cases of C to T nucleotide change which resulted to non-sense mutations at codon 223 (Lys223Lys). In the second and third meningioma samples, we found an additional of T to C nucleotide changes at codon 215 which caused missense mutations (Asp215Gly). Another missense mutation was found at codon 217 which showed T to C nucleotide change in the third meningioma sample.

Immunohistochemistry analysis of the same group of samples revealed p27 protein overexpression in all cases including meningiomas (82.6%), low grades gliomas (80.0%) and high grades gliomas (84.6%). We subsequently found high level of cyclin D1 expression in meningiomas (70.8%), equal expression of cyclin D1 in low grades of gliomas (50% are low expressors and 50% are high expressors), and downregulation of the protein in higher grades of gliomas (76.9% were low expressors). Immunogold electron microscopy analysis of cyclin D1 and p27 proteins showed that both proteins were found to be localized at cytoplasm and nucleus of the cells. Our statistical analysis gave no significant correlation between the presence of cyclin D1 mutations with the downregulation of the protein in both meningiomas (p=0.616) and gliomas (p=0.905).

Keywords: meningiomas; gliomas; p27; cyclin D1; molecular genetics; immunohistochemistry; immunogold electron microscopy

CHAPTER I

LITERATURE REVIEW

1.1 INTRODUCTION

Cancer is a complex and unpredictable genetic disease which is referred to as an abnormal growth of cells. It is a multifaceted disease, which has long been regarded as a genetic disease (Cornelisse, 2003b). In 1914, the famous biologist Theodor Boveri postulated that abnormal distribution of chromosome could be the cause of cancer. Furthermore, he suggested that tumor could arise from a single abnormal cell (monoclonal origin), and predicted that specific chromosomal changes and genetic instability are important for tumor development (Cornelisse, 2003a). His opinion was extensively supported by many researchers, postulating that the original cause of cancer is by the accumulation of genetic alterations and consequently gene expression pattern changes (Evan and Vousden, 2001, Fingleton and Coussens, 2005, Garnis *et al.*, 2004, Zingde, 2001) which could allow them to grow outside their normal growth restraints (Garnis *et al.*, 2004). The accumulation of genetic aberrations are thought to drive the progression of normal cells through hyperplastic and dysplastic stages to invasive cancer and, finally, metastatic disease (Garnis *et al.*, 2004). Cancer cells posses the ability to invade and metastasize (Zingde, 2001), as well as to induce vascularization of the tumour in order to receive oxygen and nutrients (angiogenesis), and to suppress programmed cell death (apoptosis) (Garrett, 2001, Zingde, 2001).

Several essential steps are compulsory for normal cells to become cancer cells. Researches on experimental carcinogenesis in animals have shown that cancer