

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

FARIZAN BINTI AHMAD

UNIVERSITI SAINS MALAYSIA

2008

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

by

FARIZAN BINTI AHMAD

**Thesis submitted in fulfillment of the requirement for the degree of Master of
Science**

2008

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.

Farizan, 2008.

ACKNOWLEDGEMENTS

This study was conducted at the Department of Neurosciences, Human Genome Center and Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia. I would like to take this opportunity to acknowledge numerous people who have been involved in this study.

I would like to dedicate my deepest appreciation to my main supervisor, Professor Dr. Jafri Malin Datuk Hj. Abdullah and my co-supervisor, Associate Professor Dr. Hasnan Jaafar for giving me the opportunity to work in their research group. I am deeply thankful for their continuous support, professional guidance and encouragement throughout my study.

My utmost gratitude is extended to all staffs and students of Human Genome Centers, Department of Pathology and Department of Neurosciences who have been directly or indirectly involved in this study. Your generosity, help and valuable time are highly appreciated. Not forgetting, many thanks to all my friends, for giving me continuous support, help and encouragement.

I wish to acknowledge the surgeons and nurses from Department of Neurosciences and Department of Surgery for their co-operation and assistance in tissue collection procedures. Last but not least, I owe my sincere thanks to Ministry of Science, Technology and Innovation (MOSTI) for the National Science Fellowship (NSF) award for my MSc study. Highest appreciation also goes to Majlis Kanser Nasional (MAKNA) for their support through USM-UPM-MAKNA.

LIST OF CONTENTS

CONTENTS	PAGE
TITLE	i
DEDICATION	ii
ACKNOWLEDGEMENT	iii
LIST OF CONTENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	x
ABBREVIATIONS	xvii
ABSTRAK	xix
ABSTRACT	xxi
CHAPTER 1 LITERATURE REVIEW	
1.1 Introduction	1
1.1.1 Definition and category of brain tumors	4
1.2 Central Nervous System Tumors	6
1.2.1 Central Nervous System Tumors Classification according to World Health Organization (WHO)	6
1.2.2 Types of Central Nervous System Tumors	9
1.2.2.1 Gliomas	9
1.2.2.1.1 Astrocytic (glial) tumors	9
1.2.2.1.2 Oligodendroglial tumors	10
1.2.2.1.3 Ependymal tumor	10
1.2.2.2 Medulloblastoma	10
1.2.2.3 Meningiomas	11
1.2.2.4 Other types of CNS tumors	11
1.3 Cell cycle	13
1.3.1 p27 gene	21
1.3.1.1 Regulation of p27 expression	23
1.3.1.2 p27 and cancer	24
1.3.2 cyclin D1 gene	26
1.3.2.1 Regulation of cyclin D1 expression	27
1.3.2.2 cyclin D1 and cancer	29
1.4 Detection of genetic alterations	31

1.4.1 Principles of DHPLC	34
1.5 Objectives of study	37
1.6 Research design	37

CHAPTER II MATERIALS AND METHODS

2.1 Materials	40
2.1.1 Mutation detection analysis	40
2.1.1.1 Tissue and blood DNA extraction kit	40
2.1.1.2 Reagents for Polymerase Chain Reaction, PCR	40
2.1.1.3 Electrophoresis buffer solution	41
2.1.1.3.1 0.5M EDTA (pH 8.0) preparation	41
2.1.1.3.2 10X TBE (Tris Boric EDTA) solution preparation	41
2.1.1.3.3 1X TBE (Tris Boric EDTA) preparation	41
2.1.1.4 Electrophoresis medium	41
2.1.1.5 Loading buffer and DNA marker	42
2.1.1.5.1 Blue/orange 6X loading buffer	42
2.1.1.5.2 DNA marker	42
2.1.1.6 DNA staining material	42
2.1.1.7 Thermocycler	43
2.1.1.8 Horizontal gel electrophoresis system	43
2.1.2 Immunohistochemistry analysis	43
2.1.2.1 Immunohistochemistry staining kit	43
2.1.2.2 Primary antibody for immunohistochemistry analysis	44
2.1.2.3 Preparing 3% hydrogen peroxide	45
2.1.2.4 Preparing 0.01M citrate buffer, pH 6.0	45
2.1.2.5 Preparing 1mM EDTA buffer, pH 8.0	45
2.1.2.6 Preparing 50mM Tris buffered saline, TBS pH 7.6	45
2.1.2.7 Reagents for immunohistochemistry staining	46
2.1.3 Immunogold electron microscopy analysis	46
2.1.3.1 Fixative solution (4% paraformaldehyde 3% glutaraldehyde)	46
2.1.3.2 TBS-Tween (0.05M BS, 0.05% Tween 20, pH 7.6)	46
2.1.3.3 Sucrose solution, 0.2M	47
2.1.3.4 LR White resin	47
2.1.3.5 Blocking buffer	47
2.1.3.6 Uranyl acetate solution, 5%	47
2.1.3.7 1N NaOH solution	48
2.1.3.8 Reynold's Lead Citrate solution	48
2.1.3.9 Primary antibodies	48
2.1.3.10 Secondary antibodies	49
2.1.3.11 Phosphate buffered saline (PBS), 10mM, pH 7.2-7.4	49
2.1.3.12 Toluidine blue 1%	49

2.1.3.13 Preparing formvar-coated grids	49
2.2 Methodology	51
2.2.1 Molecular genetic analyses	51
2.2.1.1 Specimens	51
2.2.1.2 DNA extractions from tumor tissue	51
2.2.1.3 DNA extractions from peripheral blood	52
2.2.1.4 Concentration and purity measurements of extracted DNA	54
2.2.1.5 Polymerase Chain Reaction, PCR	55
2.2.1.5.1 Oligonucleotide primers	55
2.2.1.5.2 Master mix preparation	55
2.2.1.6 Gel electrophoresis	56
2.2.1.6.1 1% agarose gel preparation	57
2.2.1.6.2 Agarose gel electrophoresis	60
2.2.1.6.3 Product visualization	60
2.2.1.7 Denaturing High Performance Liquid Chromatography, DHPLC	61
2.2.1.8 DNA sequencing analysis	62
2.2.1.8.1 DNA purification	62
2.2.1.8.2 DNA sequencing	63
2.2.2 Immunohistochemistry analysis	64
2.2.2.1 Tissue samples	64
2.2.2.2 Tissue sectioning	64
2.2.2.3 Immunohistochemistry	64
2.2.2.4 cyclin D1 and p27 scoring	67
2.2.3 Immunogold electron microscopy analysis	68
2.2.3.1 Tissue/samples fixation	68
2.2.3.2 Dehydration and infiltration	68
2.2.3.3 Polymerization	68
2.2.3.4 Sectioning (semithin and ultrathin)	69
2.2.3.5 Immunogold labeling/staining	69
2.2.3.6 Viewing	71
2.2.4 Statistical analysis	71
CHAPTER III RESULTS	
3.1 Genomic DNA isolation	72
3.2 p27 and cyclin D1 genes amplification through Polymerase Chain Reaction, PCR	72
3.3 Mutation screening of p27 and cyclin D1 genes using Denaturing High Performance Liquid Chromatography, DHPLC	72
3.3.1 Temperature mapping	73
3.3.2 The frequency of p27 gene mutation in brain tumors	73
3.3.3 The frequency of cyclin D1 gene mutations in brain tumors	73

3.4 DNA sequencing	85
3.5 Immunohistochemistry analysis	89
3.6 Immunogold electron microscopy analysis	100
3.7 Statistical analysis	108
CHAPTER IV DISCUSSION	117
4.1 Mutational screening of cyclin D1 and p27 in meningiomas and gliomas	117
4.1.1 Genetic alterations of cyclin D1 gene	117
4.1.2 Genetic alterations of p27 gene	120
4.2 cyclin D1 and p27 protein expression and localization	121
4.2.1 cyclin D1 translocalization	127
4.2.2 p27 translocalization	131
4.3 Combination of risk factors of brain tumors	135
4.4 Strength and limitations of study	135
CHAPTER V CONCLUSION	138
BIBLIOGRAPHY	139
LIST OF PRESENTATION	147

LIST OF TABLES

		PAGE
Table 1.1	Classification of Tumors of the Nervous System	7
Table 2.1	Nucleotide sequence for oligonucleotide primers used to amplify both p27 and cyclin D1 genes	58
Table 2.2	Master mix preparation for polymerase chain reaction analysis	59
Table 3.1	List of cyclin D1 (exon 4) gene mutations found in meningioma and glioma patients	86
Table 3.2	Immunohistochemistry results of p27 protein expression in meningiomas and gliomas.	91
Table 3.3	Immunohistochemistry results of cyclin D1 protein expression in meningiomas and gliomas.	91
Table 3.4	Statistical analysis of Chi-Square for glioma samples.	109
Table 3.5	Statistical analysis of Chi-Square for meningioma samples.	109
Table 3.6	Accumulative data for mutational screening and immunohistochemistry analysis of p27 and cyclin D1 in meningiomas.	110
Table 3.7	Accumulative data for mutational screening and	114

immunohistochemistry analysis of p27 and cyclin D1 in low grade gliomas.

Table 3.8 Accumulative data for mutational screening and 115
immunohistochemistry analysis of p27 and cyclin D1 in high
grade gliomas.

LIST OF FIGURES

	PAGE
Figure 1.1: Checkpoints and cell cycle	14
Figure 1.2 A schematic diagram showing the temporal relationship between different cyclin/CDK complexes and cell cycle phases	17
Figure 1.3 Schematic representation of pRb phosphorylation by cyclin/CDK complexes and E2F release during G1 to S progression.	18
Figure 1.4 Cyclins, Cdk's and the cell cycle.	20
Figure 1.5 Schematic model of p27 ubiquitination. Phosphorylated p27 on T187 is recognised by Skp2 and ubiquitinated by the concerted actions of E1, E2 and SCFSkp2. Cks1 is an accessory protein that enhances binding of phosphorylated p27 to Skp2.	26
Figure 1.6 Structure of cyclin D1. Schematic representation of genomic structures of Cyclin D1 gene (A), functional domains of cyclin D1 protein (B), and alternative splicing of cyclin D1 (C). Cyclin D1 sequence derived from intron 4 is shown in <i>blue</i> .	28
Figure 1.7 Principle of mutation detection by DHPLC	37

Figure 1.8	Flow-chart shows the methodology involved in the molecular genetic analysis and histological analysis	39
Figure 3.1	Analysis of PCR product for exon 1a of p27 gene using electrophoresis 1.5% agarose gel	75
Figure 3.2	Analysis of PCR product for exon 1b of p27 gene using electrophoresis 1.5% agarose gel	76
Figure 3.3	Analysis of PCR product for exon 2 of p27 gene using electrophoresis 2% agarose gel	77
Figure 3.4	Analysis of PCR product for exon 4 of cyclin D1 gene using electrophoresis 3% agarose gel.	78
Figure 3.5	Analysis of PCR product for exon 5 of cyclin D1 gene using electrophoresis 3% agarose gel	79
Figure 3.6	An example of temperature mapping result for exon 1a of p27 gene.	80
Figure 3.7	Example of dHPLC analysis for exon 1a and exon 1b of p27 gene. All samples revealed same homoduplex peak profiles in gliomas and meningiomas samples.	81
Figure 3.8	Example of dHPLC analysis for exon 2 of p27 gene. All samples revealed same homoduplex peak profiles in gliomas and meningiomas samples.	82
Figure 3.9	Example of dHPLC analysis for exon 4 of cyclin D1 gene.	83

Figure A (Astrocytoma Grade III) and B (Meningioma Transitional Type) represent homoduplex peak profiles while Figure C (Meningioma) and D (Astrocytoma Grade III) represent heteroduplex peak profiles for brain tumor samples. The analysis was carried out under temperature of 62⁰C for all samples

- Figure 3.10** Example of dHPLC analysis for exon 5 of cyclin D1 gene. 84
Figure A-D represents homoduplex peak profiles for brain tumor samples
- Figure 3.11** Base substitution of C to T at codon 223. The T base 87
substitution caused non-sense mutation for producing Lysine (Lys223Lys)
- Figure 3.12** Base substitution of T to C at codon 217. The C base 87
substitution caused missense mutation for producing Glycine instead of Aspartic Acid (Gly217Asp)
- Figure 3.13** Base substitution of T to C at codon 215. The C base 88
substitution caused missense mutation for producing Glycine instead of Aspartic Acid (Gly215Asp)
- Figure 3.14** G base deletion at codon 219. The G base deletion caused 88
frameshift mutation for producing Proline instead of Arginine (Pro219Arg)
- Figure 3.15** Immunohistochemical determination of cyclin D1 in Breast 92

carcinoma tissue (positive control) (Magnification x100)

- Figure 3.16** Immunohistochemistry result of cyclin D1 in meningioma tissue. Immunohistochemistry demonstrated high expression of cyclin D1 in meningioma cancer cells. Arrows indicate cytoplasmic localization of the protein. (Magnification x100) 93
- Figure 3.17** Immunohistochemistry result of cyclin D1 in ependymoma (WHO Grade II) tissue. Immunohistochemistry demonstrated low expression of cyclin D1 in ependymoma cancer cells. (Magnification x100) 94
- Figure 3.18** Immunohistochemistry result of cyclin D1 in Glioblastoma Multiforme (WHO Grade IV). Immunohistochemistry demonstrated decrement of cyclin D1 protein expression in GBM compared to lower grades tumors. Arrows indicate cytoplasmic localization of the protein. (Magnification x100) 95
- Figure 3.19** Immunohistochemical determination of p27 in benign prostatic hyperplasia tissue (positive control) (Magnification x100) 96
- Figure 3.20** Immunohistochemistry result of p27 in meningioma tissue. Immunohistochemistry demonstrated high expression of p27 protein in meningioma cancer cells. Arrows indicate 97

cytoplasmic localization of the protein. (Magnification x100)

- Figure 3.21** Immunohistochemistry result of p27 in Oligodendroglioma (WHO Grade II) tissue. Immunohistochemistry demonstrated high expression of p27 protein oligodendroglioma cancer cells. Arrows indicate cytoplasmic localization of the protein. (Magnification x100) 98
- Figure 3.22** Immunohistochemistry result of p27 in Glioblastoma Multiforme (WHO Grade IV). Immunohistochemistry demonstrated increment of p27 protein expression in GBM compared to lower grades tumors. Arrows indicate cytoplasmic localization of the protein. (Magnification x100) 99
- Figure 3.23** p27 immunogold staining of Benign Prostatic Hyperplasia (BPH) tissue (positive control). Immunogold labeling is represented by discrete black spots scattered in the nucleus and cytoplasm of the cell. **N**, Nucleus; **C**, Cytoplasm. (Magnification x12 432) 102
- Figure 3.24** cyclin D1 immunogold staining of Breast carcinoma tissue (positive control). Immunogold labeling is represented by discrete black spots scattered in the nucleus and cytoplasm of the cell. **N**, Nucleus; **C**, Cytoplasm. (Magnification x7827) 103
- Figure 3.25** p27 immunogold staining of meningioma tissue. Immunogold labeling is represented by discrete black spots scattered in the nucleus and cytoplasm of the cell. The spots 104

were seen at the dense and loose area of nucleus chromatin and along cytoplasmic area. **N**, Nucleus; **C**, Cytoplasm. (Magnification x5893)

Figure 3.26 p27 immunogold staining of astrocytoma tissue. 105

Immunogold labeling is represented by discrete black spots scattered in the nucleus and cytoplasm of the cell. The spots were seen at the dense and loose area of nucleus chromatin and along cytoplasmic area. **N**, Nucleus; **C**, Cytoplasm. (Magnification x16 000)

Figure 3.27 cyclin D1 immunogold staining of meningoma tissue. 106

Immunogold labeling is represented by discrete black spots scattered in the nucleus and cytoplasm of the cell. The spots were seen at the dense and loose area of nucleus chromatin and along cytoplasmic area. Only a few black spots were detected in the samples, due to downregulation of the protein expression. **N**, Nucleus; **C**, Cytoplasm. (Magnification x7873)

Figure 3.28 cyclin D1 immunogold staining of astrocytoma tissue. 107

Immunogold labeling is represented by discrete black spots scattered in the nucleus and cytoplasm of the cell. The spots were seen at the dense and loose area of nucleus chromatin and along cytoplasmic area. The spot was also observed at the nucleus membrane of the cell. **N**, Nucleus; **C**, Cytoplasm. (Magnification x5464)

- Figure 4.1** Mechanism of cyclin D1 protein translocation from nucleus to cytoplasm via the nuclear pores. 130
- Figure 4.2** Mechanism of p27 protein translocation from nucleus to cytoplasm via the nuclear pores. 134

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	Ethylenediaminetetra-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-3β	Glycogen synthase-3beta
H₂O	Water
H₂O₂	Hydrogen peroxide
HCl	Hydrochloric acid
HER	Herstatin
HIF-1α	Hypoxia Inducible Factor 1 alpha
HRP	Horse Radish Peroxidase
kb	Kilo base
LOH	Loss of Heterozygosity
Lys223Lys	Lysine to Lysine at codon 223
M	Mitosis phase of cell cycle
M	Molar
MgCl₂	Magnesium Chloride
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 tandem 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	Triethylammonium 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 dalam kedua-dua kes meningioma ($p=0.616$) dan glioma ($p=0.905$).

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

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

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 possess 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