THE EVALUATION OF *O6-METHYLGUANINE-DNA-METHYLTRANSFERASE* (*MGMT*) GENE METHYLATION STATUS AMONG HOSPITAL UNIVERSITI SAINS MALAYSIA GLIOMA PATIENTS

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By

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PENILAIAN KE ATAS STATUS METILASI *O6-METHYLGUANINE-DNA-METHYLTRANSFERASE* (*MGMT*) GEN DI KALANGAN PESAKIT GLIOMA HOSPITAL UNIVERSITI SAINS MALAYSIA

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LIST OF ABBREVIATIONS

WHO	:	World Health Organization
HUSM	:	Hospital Universiti Sains Malaysia
LGGs	:	low-grade gliomas
HGGs	:	high-grade gliomas
MGMT	:	O6-methylguanine DNA methyltransferase
NCI	:	National Cancer Institute
CNS	:	Central Nervous System
GBM	:	Glioblastoma multiforme
H&E	:	Hematoxylin and Eosin
CBTRUS	:	Central Brain Tumour Registry of the United States
IDH 1	:	Isocitric Dehydrogenase 1
TMZ	:	Temozolomide
CCNU	:	chloroethyl-cyclohexyl nitrosourea
DNA	:	deoxyribonucleic acid
DNMTs	:	DNA methyltransferases
EORTC	:	European Organization for Research and Treatment of Cancer
NCIC	:	National Cancer Institute of Canada
MSP	:	Methylation-specific PCR
FFPE	:	formalin fixed paraffin-embedded

C	:	Cytosine
U	:	Uracil
mC	:	modified Cytosine
PCR	:	Polymerase Chain Reaction
TBE	:	Tris base, boric acid and EDTA
RNA	:	Ribonucleic acid
ddH2O	:	double-distilled water
bp	:	base pair
SPSS	:	Statistical Package for the Social Sciences
UM	:	Unmethylated
M	:	Methylated
PC	:	Positive control
NC	:	Negative control
$MgCl2,$	:	Magnesium chloride
dNTPs	:	Deoxyribonucleotide triphosphates

LIST OF SYMBOLS

%	:	Percentage
n	:	Sample size
p	:	Prevalence
Z	:	*Z* statistics for a level of confidence
Δ	:	Precision
μm	:	micrometre
mL	:	*millilitre*
rpm	:	Revolutions per minute
°C	:	Degree Celcius
uL	:	microlitre
ng	:	nanogram
ug	:	Microgram
mM	:	millimolar
L	:	Litre
g	:	Gram
V	:	Voltage
p	:	P value
SD	:	Standard deviation

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ABSTRAK

Banyak kajian telah membuktikan bahawa memahami peranan bio-penanda telah meningkatkan persepsi semasa berkenaan genesis glioma, penilaian ramalan dan perancangan rawatan untuk pesakit. Sebagai contoh, pengenalpastian status metilasi promoter gen *O6-methylguanine-DNA-methyltransferase* (*MGMT*) yang mengekod MGMT, iaitu sejenis protein yang terlibat dalam aktiviti pembaikan DNA, dapat meningkatkan keberkesanan penjagaan piawai semasa glioma, apabila baru-baru ini, status metilasi telah diperkenalkan sebagai bio-penanda untuk stratifikasi rancangan rawatan. Bagi lebih memahami peranan *MGMT*, kami telah menilai status metilasi promoter *MGMT* pesakit glioma di Hospital USM. Dalam kajian ini, 41 sampel tisu glioma yang yang disimpan secara parafin-terbenam (FFPE) telah diperolehi berdasarkan penggredan dari Gred II (n = 11), III (n = 10) dan IV (n = 20). Selepas itu, pengekstrakan DNA dan status metilasi telah disahkan melalui kaedah tindak balas berantai polimerase metilasi-spesifik *(MSP)* dengan menggunakan dua pasang primer khusus mensasarkan *unmethylated* (*UM*) dan *methylated* (*M*) di dalam gen *MGMT*, masing-masing. Keputusan *MSP* dikenalpasti mengandungi *intratumoral heterogeneity* yang tinggi dalam semua gred tumor. 9.1% daripada Gred II glioma adalah *M* manakala selebihnya 90.9% adalah kombinasi *UM* dan *M*. Lebih-lebih lagi, Gred III glioma menunjukkan kedua-duanya adalah 50% *M* dan kombinasi 50% masing-masing *UM* dan *M*. Gred IV glioma menunjukkan 15% *UM*, 30% *M* dan 55%

kombinasi *UM* dan *M*. Walau bagaimanapun, analisis menggunakan *Fisher's exact test* mendapati tiada kaitan statistik yang signifikan antara status metilasi *MGMT* dengan mana-mana parameter klinikopatologi yang diuji seperti gred tumor, umur, jantina dan bangsa pesakit ($p > 0.05$). Kesimpulannya, kajian ini mendapati metilasi *MGMT* berlaku dengan kadar yang kerap di dalam pesakit glioma di Hospital USM, tanpa mengira gred tumor, umur, jantina dan bangsa. Lebih-lebih lagi, penemuan status kombinasi *MGMT* dalam semua gred glioma mencadangkan kehadiran *intratumoral heterogeneity* di dalam tumor tersebut. Selain menunjukkan *intratumoral diversity*, ia juga mungkin menggambarkan cabaran yang mungkin dihadapi dalam usaha menentukan rawatan secara peribadi berdasarkan status epigenetik *MGMT*.

Kata kunci: glioma, *MGMT*, metilasi, tindak balas berantai polimerase metilasi-spesifik (*MSP*)

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ABSTRACT

Growing evidences demonstrate the understanding of the biomarkers and it significantly increased our current perception of gliomagenesis, prognostic evaluation, and treatment planning for the patients. For instance, identification of the promoter methylation status of *O6-methylguanine-DNA-methyltransferase* (*MGMT*) gene encodes MGMT, a protein with DNA repair activity may improve the efficacy of current standard care in glioma as the methylation status has been recently introduced to be a predictive biomarker for stratification of the treatment plan. To further understand the roles of *MGMT*, we aimed to evaluate the *MGMT* promoter methylation status of glioma patients in Hospital USM. In this study, 41 samples of paraffin-embedded glioma tissue (FFPE) were obtained based on their grading from Grade II (n = 11), III (n = 10) and IV (n = 20). Subsequently, DNA extraction and methylation status was validated by methylation-specific PCR (MSP) method using two pairs of primers specifically targeting the unmethylated (UM) and methylated (M) regions of the *MGMT* gene, respectively. MSP results identified high intratumoral heterogeneity of the samples in all grades of the tumours. In Grade II glioma, 9.1% were M and 90.9% were combination of UM and M. Moreover, Grade III glioma indicated 50% M and combination of UM and M, respectively. Besides, Grade IV glioma exhibited 15% were UM, 30% were M and 55% were both UM and M. Nevertheless, analysis using Fisher's exact test found no statistical association of the *MGMT* methylation status

with any of the tested clinicopathological parameters such as tumour grade, age, gender, and race of the patients ($p > 0.05$). In conclusion, this study found frequent *MGMT* methylation, regardless of the tumour grade, age, gender and race of the glioma patients at Hospital USM. Moreover, the occurrence of *MGMT* combination status in all grades of gliomas suggested intratumoral heterogeneity of the tumour. Besides imparting intratumoral diversity, it may also indicate possible challenges in defining personalized treatment based on the epigenetic status of *MGMT*.

Keywords: glioma, *MGMT*, methylation, methylation-specific PCR (MSP)

CHAPTER 1

INTRODUCTION

Glioma is a type of brain tumour that begins in glial cells within the brain or spinal cord. It is the most predominant type primary brain tumours which can be found in adults which comprises of 30% to 40% of all intracranial tumours where its utmost prevalence are between the ages of 40 and 65 (Schneider et al., 2010). According to World Health Organization (WHO) 2016 classification, gliomas were stratified from Grade I to Grade IV which can be grouped into two categories such as low-grade and high-grade gliomas (Louis et al., 2016). Additionally, in relation to the classification, Grade I and II, and also Grade III and IV are termed as low-grade gliomas (LGGs) and high-grade gliomas (HGGs) respectively.

Gliomas with the tendency of growing and infiltrating normal brain tissue characteristics often complicates the surgical procedures and sometimes makes it impossible to remove the tumour. As such, low-grade gliomas which often occur in young or healthy patients have a slow-moving course with extended survival rate compared to high-grade gliomas. Clinical manifestation chiefly holds responsible for prognosis information for treatment plans. For example, seizures are common symptom that makes up to 80% of patients, also changes of behaviour, focal neurological loss, increasing intracranial pressure like headache or papilledema. However, it was also reported that some patients being asymptomatic adds on issues for treatment schemes (Forst et al., 2014; Pouratian et al., 2007). Although the

histopathologic investigation of tissues was set to be gold standard for diagnosis and grading of LGG with imaging methods, more new advanced imaging techniques needed (Forst et al., 2014).

On top of that, HGGs are the recurrent unit which accounts over 50% of the primary malignant brain tumours mainly in older ages. Among the concerns in HGGs, the same treatment plans or even research procedures applied for both Grade III and IV which reports as one of the drawback despite of the understanding of general molecular machinery of HGGs, surgeries, radiation or chemotherapy as well as imaging techniques that has been applied till date. Yet, the overall prediction especially for treatment plans still remains a challenge (Brown et al., 2006; Mirimanoff, 2014).

Hence, it is high-priority to find a new therapeutic approach where it can be potentially used as new modality as the failure to identify the specific molecular identity of the tumour can be resulting in an ineffective treatment and may worsen the prognosis (Ray et al., 2014). From a clinical point of view, a biomarker should be highly sensitive and specific in providing information relevant for diagnosis, prognosis or therapy of a disease. Concerning about the approaches, *MGMT* gene has been shown to have association with improved outcome in glioma and can be a predictive marker of sensitivity to alkylating agents. The methylation status of the *MGMT* promoter has been identified as a strong and independent predictive factor of favourable survival in glioblastoma patients undergoing chemotherapy with alkylating agents where it shows 35%–45% of malignant gliomas (WHO grades III and IV) and in about 80% of WHO grade II gliomas (Hegi et al., 2005a; Ahluwalia, 2011). The range of median survival

of the patients with methylated *MGMT* promoter was 21.7 months compared with 12.7 months for patients without (Christians et al., 2012a).

Furthermore, *MGMT* methylation status also provides insight as a molecular biomarker in which it aids in understanding of glioma development. It increases the patients' prognostic profiling and help for a possible personalised treatment plan which helps in clinical relevance (Thon et al., 2013). Therefore, to address this issue, evaluation of *MGMT* methylation status and its association with the clinicopathological data of the glioma patients at Hospital Universiti Sains Malaysia was carried out in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Brain tumours

2.1.1 Classification of brain tumours

Brain tumours are defined as an abnormal cell growth in the brain. It can be classified as benign or malignant type, as stated by the National Cancer Institute (NCI). American Brain Tumour Association reported that benign tumour occurs in people of all ages while malignant types are more prevalent among adults compared to children. Apart from that, they can also be stratified as primary brain tumour which originates and stays in the brain while secondary brain tumour referred as the cancer that begins somewhere else in the body and spreads to the brain.

Based on histogenesis concept, the classification of brain tumours has been made where it was classified according to their microscopic similarities with different putative cells of origin and their recognised levels of differentiation for the past century (Louis et al., 2007). The histological characterisation was based on light microscopic features in hematoxylin and eosin-stained sections, immunohistochemical expression of lineage-associated proteins and ultrastructural characterisation. For instance, the tumours of an astrocytic phenotype distinguished separately from oligodendroglial phenotype although they were clinically similar or unrelated as per classified by World

Health Organization (WHO) Classification of Tumours of the Central Nervous System 2007 (2007 CNS WHO). Some studies clarified that the classification of tumours were based on tumourigenesis at genetic level as well as with some unusual brain tumour entities. However, this information could not be used to define specific entities but conventional histology has proven the prognostic data within the diagnostic categories (Louis et al., 2007).

Recently, WHO classifies the CNS tumours by using molecular parameters in addition to histology to define many tumour entities, thus formulating a concept for how CNS tumour diagnoses should be structured in this molecular era as shown in Figure 2.1. As such, the 2016 CNS WHO demonstrates the classification which includes the evaluation of both molecular features and its histology (Louis et al., 2016).

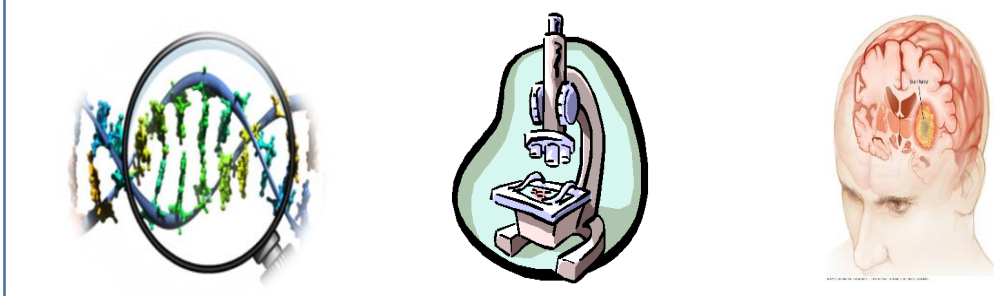


Figure 2.1 Classification approach in brain tumours (Adapted and modified from Louis et al., 2016)

Table 2.1 The 2016 World Health Organization (WHO) Classification of Tumours of the Central Nervous System (CNS) (Adapted from (Louis et al., 2016)

Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	II
Anaplastic astrocytoma, IDH-mutant	III
Glioblastoma, IDH-wildtype	IV
Glioblastoma, IDH-mutant	IV
Diffuse midline glioma, H3 K27M-mutant	IV
Oligodendroglioma, IDH-mutant and 1p/19q codeleted	II
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q codeleted	III
Other astrocytic tumours	
Pilocytic astrocytoma	I
Subependymal giant cell astrocytoma	I
Pleomorphic xanthoastrocytoma	II
Anaplastic pleomorphic xanthoastrocytoma	III
Ependymal tumours	
Subependymoma`	I
Myxopapillary ependymoma	I
Ependymoma	II
Ependymoma, *RELA* fusion-positive	II or III
Anaplastic ependymoma	III
Other gliomas	
Angiocentric glioma	I
Chordoid glioma of third ventricle	II
Choroid plexus tumours	
Choroid plexus papilloma	I
Atypical choroid plexus papilloma	II
Choroid plexus carcinoma	III
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	I
Gangliocytoma	I
Ganglioglioma	I
Anaplastic ganglioglioma	III
Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos)	I
Desmoplastic infantile astrocytoma and ganglioglioma	I
Papillary glioneuronal tumour	I
Rosette-forming glioneuronal tumour	I
Central neurocytoma	II
Extraventricular neurocytoma	II
Cerebellar liponeurocytoma	II

Table 2.1 Continued…

Tumours of pineal region	
Pineocytoma	I
Pineal parenchymal tumour of intermediate differentiation	II or III
Pineoblastoma	IV
Papillary tumour of pineal region	II or III
Embryonal tumours	
Medulloblastoma (all subtypes)	IV
Embryonal tumour with multi-layered rosettes, C 19MC-altered	IV
Medulloepithelioma	IV
CNS embryonal tumour, NOS	IV
Atypical teratoid/rhabdoid tumour	IV
CNS embryonal tumour with rhabdoid features	IV
Tumours of the cranial and paraspinal nerves	
Schwannoma	I
Neurofibroma	I
Perineurioma	I
Malignant peripheral nerve sheath tumour (MPNST)	II, III or IV
Meningiomas	
Meningioma	I
Atypical meningioma	II
Anaplastic (malignant) meningioma	III
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour/haemangiopericytoma	I, II or III
Haemangioblastoma	I
Tumours of the sellar region	
Craniopharyngioma	I
Granular cell tumour	I
Pituicytoma	I
Spindle cell oncocytoma	I

As for tumour grading that has been classified by WHO CNS 2016, there are four types of gliomas which comprises of grade I, II, III and IV.

Table 2.2 WHO Brain Tumour Grades (Adapted from Louis et al., 2016)

	Grade	Characteristics	Tumour Types
Low Grade	WHO Grade I	• Least malignant (benign) • Possibly curable via surgery alone • Non-infiltrative • Long-term survival • Slow growing	• Pilocytic astrocytoma • Craniopharyngioma • Gangliocytoma • Ganglioglioma
	WHO Grade II	• Relatively slow growing • Somewhat infiltrative • May recur as higher grade	• Diffuse astrocytoma • Pineocytoma • Pure oligodendroglioma
High Grade	WHO Grade III	• Malignant • Infiltrative • Tend to recur as higher grade	• Anaplastic astrocytoma • Anaplastic ependymoma • Anaplastic oligodendroglioma
	WHO Grade IV	• Most malignant • Rapid growth, aggressive • Widely infiltrative • Rapid recurrence • Necrosis prone	• Glioblastoma multiforme (GBM) • Pineoblastoma • Medulloblastoma • Ependymoblastoma

As denoted in the table above, low-grade gliomas which includes grade I and II are non-malignant while grade III and IV are malignant ones also known as high-grade gliomas. Grade I are commonly correlated with long-term survival, as their nature of growing steadily and normal appearance via microscope makes the surgery alone as an effective treatment whereas the form of grade II tumours are faintly abnormal under the microscopic view. Similarly, some spreads as higher grade tumour to nearby normal healthy tissues and reoccur although they grow slowly. As for grade III which are known to be malignant, often reoccur as grade IV, and are actively replicating anomalous cells which can be grown neighbouring the normal tissues. Grade IV tumours are the most malignant tumours that proliferates fast into normal healthy brain tissues. They have an unusual, peculiar appearance, and have deceased cells in their core, also forms new blood vessels which aids in their rapid growth. The most common example of grade IV tumour is the glioblastoma multiforme.

In brief, molecular guidelines have been used to lay the foundations of brain tumour diagnosis for the first time as the 2016 CNS WHO represents useful step forward which has been descended from 2007. The 2016 CNS WHO incorporates the objective molecular data in classifications as an intermediate stage which allows the betterment of patient therapy for clinical trials, epidemiological as well as experimental studies.

2.1.2 Symptoms of brain tumour

In general, the commonly first noticed symptom of brain tumour is an increased intracranial pressure. Also, tumours with extensive peritumoral swelling which is also known as oedema naturally leads to an elevated intracranial pressure which rendered into headaches, vomiting (with or without nausea), dilation of pupil on the lesion side (anisocoria), altered state of consciousness, for example, coma and somnolence, papilledema are to be noticed as primary symptoms (Davies and Clarke, 2004). An increase of an intracranial pressure may result in brain herniation of certain parts of the brain. For instance, cerebellar tonsils or temporal uncus which results in lethal brainstem compression while it causes an increase in the diameter of the skull and bulging of fontanelles in very young children.

Salander classified the symptoms as 'trigger' symptoms which an early phase symptoms faced by the patients will be referred to the physicians for consultations. Hence, he grouped them into four categories such as headache, seizure, motor or sensorial dysfunction and mental dysfunction (Salander et al., 1999).

A. Headache

   It was reported to be more persistent and more intense than a normal headache where some accompanied by vomiting. The mean duration of this symptom was 7 months (range 0-2 years).

B. Seizure

5 months which is in range of 0.5 to 2 years was the mean duration of this symptom.

C. Motor or sensorial dysfunction

These symptoms are less important in this group of patients compared to headache and seizure group. The mean duration of this symptom was less than a month (range 0-5 months).

D. Mental dysfunction

Some patients were reported to have this symptom who later turned out to be psychotic as well.

Moreover, the clinical signs and symptoms of brain tumours can be general or focal which means general symptoms such as headache and seizure. This is due to an increased intracranial pressure while focal symptoms are like unilateral weakness or personality changes due to pressure or impairment in specialised regions where the initial symptoms of brain tumours are often focal. In additional to that, it usually progresses to generalised symptoms as the tumour increases in size and spreads which happened to be neurological signs as shown in Table 2.3 below (Perkins and Liu, 2016).

Table 2.3 Indications of brain tumour by locations with neurological symptoms

TUMOUR LOCATION	NEUROLOGICAL SIGN
Frontal lobe	Dementia, changes in personality, gait disturbances, generalised/focal seizures, expressive aphasia
Parietal lobe	Sensory loss, receptive aphasia, hemianopia, spatial disorientation
Temporal lobe	Behavioural changes, quadrantanopia, complex partial/generalised seizures
Occipital lobe	Contralateral hemianopia
Thalamus	Behavioural changes, contralateral sensory loss, language disorder
Cerebellum	Ataxia, dysmetria, nystagmus
Brain stem	Ataxia, nystagmus, hemiparesis, papillary abnormalities, cranial nerve dysfunction, autonomic dysfunction

Another study by Chandana (2008) revealed that headache accompanied by nausea and vomiting usually associated with tumour where some patients with brain tumour often report a bifrontal, tension-type headache. Additionally, there was a cause of an evaluation for brain tumour with a chronic, persistent headache with nausea, vomiting, seizures, alteration in headache pattern, neurologic symptoms or even with worsening of locations (Perkins and Liu, 2016). On top of that, it was also reported that cognitive dysfunction which includes language, attention and executive functioning is one of the incidental symptoms of brain tumour (Taphoorn and Klein, 2004).

In psychiatric view of brain tumour, it may present together with mood symptoms, psychosis, memory issues, changes in personality, anxiety or anorexia which may complicates the clinical image where the signs generally do not have any localizing values, hence, it suggested a work up with neuroimaging prior to diagnosis (Madhusoodanan et al., 2015). According to Keschner (1938), he reported that 78% of 530 patients in his study displayed psychiatric symptoms but only 18% of them presented as the first clinical indication of brain tumour. Besides that, a lesion in one region the brain expresses a profusion of symptoms which also depends on the activity of the repressed neuronal foci due to the neuronal connection of the brain which as well makes the brain tumour symptoms not consistent (Madhusoodanan et al., 2007).

2.1.3 Risk factors of brain tumour

Risk factor in another word is the components that affect the chances of getting diseases. Thus, different diseases have different risk factors. Epidemiologic studies offered a whole understanding of any diseases including prevention and treatment of the diseases. However, epidemiologic studies for brain tumour with respect to risk factors have been mostly inconclusive due to various factors (Florian et al., 2013).

a) Age and race

   It was reported that more Caucasians are affected than African or Asian descendants and this has been more accurate for all ages (Ohgaki and Kleihues, 2005). As for age, the developed countries have higher rate as such it was reported that age between 75 and 84 years has peak occurrence of brain tumour (Ostrom et al., 2015).

b) Gender

   Gender is now concurred in most of the studies as the fact that females showed less incidence of brain tumour compared with males. This was supported by the data from CBTRUS where brain tumour showed a higher rate in males than female (7.17/100,000 person-years vs. 5.07/100,000 person-years) (Ostrom et al., 2015). In general, females retain longer survival rate in most types of tumour. This fact was proved with an experimental research where female rats were transplanted with the brain tumours, as a result, the female rats possessed a slow growth rate and a longer survival rate (Florian et al., 2013).

c) Family history and genetic factors

Family history accounts a small proportion of brain tumours which also reflects genetic factors (Crump et al., 2015). A study showed that the existence of family member with brain tumour with genetic factors involved proclaimed to have higher incidence of brain tumours (Florian et al., 2013; Malmer et al., 2003). However, brain tumours with genetic factors are yet to be clarified (Bondy et al., 2008). The above statement assisted where the study showed non-significant increased rate of brain tumour with epidemiologic factors as it is not well defined and the rate of those genetically transmitted diseases associated with brain tumours like neurofibromatosis and familial cancer syndromes (e.g., Li-Fraumeni) (Ommi, 2015).

2.2 Gliomas

According to the cell origins as well as histopathological indication, where it aids in the prediction of the tumour behaviour, central nervous system (CNS) tumours are defined. Gliomas as such are neuroepithelial tumours which originated from glial cells which is a supporting cells of CNS (Louis et al., 2007). WHO categorises the tumour classification system accordingly from Grade I (lowest grade) to Grade IV (highest grade). On the other hand, gliomas are also classified in two main groups known as low grade gliomas (LGGs) and high grade gliomas (HGGs). LGGs which includes Grade I and II while HGGs includes Grade III and IV. They are generally classified based on their histopathological characteristics such as anaplasia,

cytological atypia, activity of mitosis, microvascular proliferation and necrosis (Louis et al., 2016).

2.2.1 Clinical and pathological characteristics

LGGs often occur in young patients which are also a distinctive group of primary brain tumours. In Grade I tumours, they contain none of the aforesaid histologic attributes while in Grade II tumours, they are specified by the existence of cytological atypia alone. The histopathology characteristics were determined by using Hematoxylin and Eosin (H&E) staining. For instance, diffuse astrocytomas have well-altered fibrillary or gemistocytic neoplastic astrocytes on a loose matrix, while oligoastrocytomas consist of dispersed infiltrating tumours along with the concoction of oligodendroglial and astrocytic type of cells and oligodendrogliomas which contains cells with even-looking nuclei and perinuclei are infiltrating tumours which are often called as “fried egg” appearance (Forst et al., 2014; Louis et al., 2016). Into the bargain, Grade I has low proliferative rate compared to Grade II which also has low proliferative rate despite reoccur often is infiltrative in nature generally. Furthermore, the tendency of Grade II of progressing to higher grades supports the evident that Grade II does recur. This can be seen when low-grade diffuse astrocytomas change to anaplastic astrocytomas and glioblastoma. Similarly, it happens in oligodendrogliomas and oligoastrocytomas (Louis et al., 2007).

HGGs are the gliomas that grow and spread rapidly which usually found in adults and 8-12% in paediatrics (Bondy et al., 2008). HGGs which generally occur from LGGs are aggressive in nature. Grade III often represent as an intermediate stage in the development from diffuse astrocytomas to glioblastomas in terms of both histological and molecular characteristics. The most common Grade III type is anaplastic astrocytomas. Besides that, Grade IV which is highly proliferative does infiltrate the neighbouring tissues. The aggressiveness characteristic of Grade IV also depends on high frequency of abnormality, nuclear hyperchromatosis and necrotic areas (Urbańska et al., 2014). The common clinical characteristic that can be found in gliomas are headaches, ataxia, visual interference and seizures (Schneider et al., 2010; Urbańska et al., 2014).

2.2.2 Epidemiology

Gliomas have significant impact on the patients as the patients with gliomas have a poor prognosis, in which LGGs are often curable with surgery while HGGs are the complex ones with low survival rate and high mortality. It was reported that there is a limited clinical epidemiological data of glioma patients due to the high mortality, low incidence and heterogeneous infection with variety of tumour subtypes (Krogh Rasmussen et al., 2017). The Central Brain Tumour Registry of the United States (CBTRUS) proclaimed that gliomas report for 32% of primary CNS tumours in which 17% of them are astrocytic tumours and 28% of them are glioblastomas in young adults with age in the range of 20-34 years old (Ostrom et al., 2015). However, the incident

rate in LGGs is lower compared to HGGs. The reason for this inconsistency is uncertain.

The incident that was reported by the distribution of glioma by specific histology showed that glioblastoma comprises the major percentage of gliomas compared to others (Forst et al., 2014; Ostrom et al., 2015) as shown below in Figure 2.2.

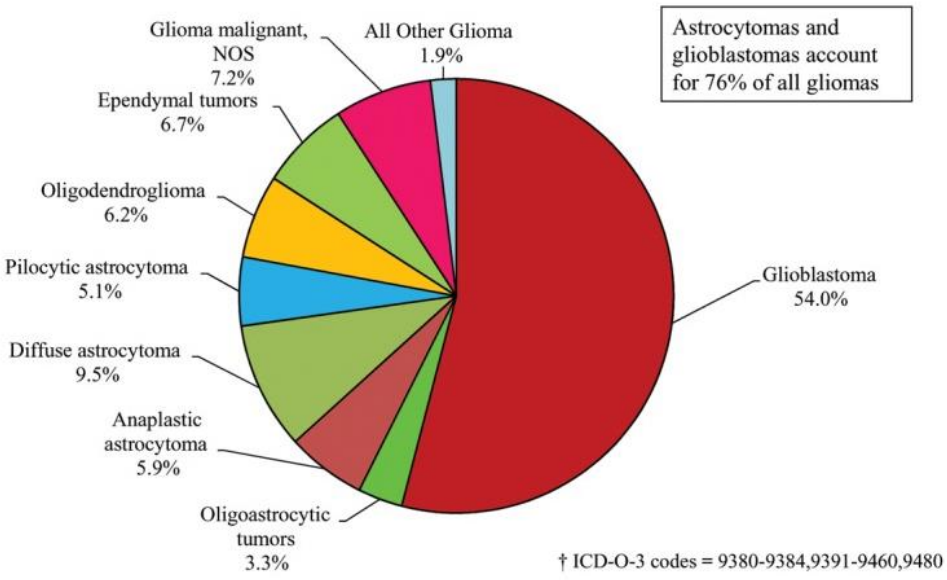
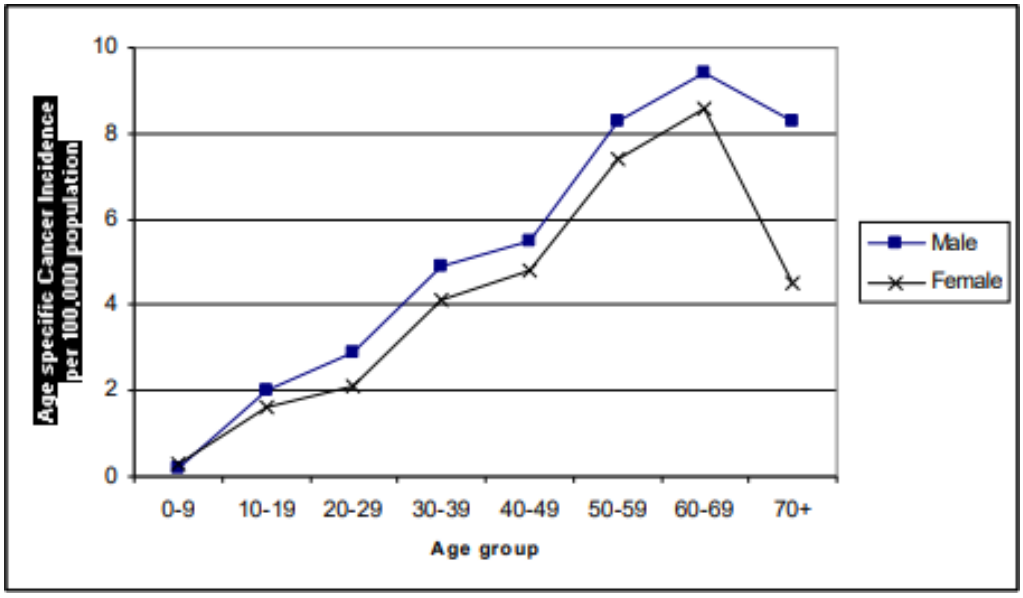


Figure 2.2 Distribution of CNS gliomas by Histology Subtypes (Adapted from Ostrom et al., 2015)

The incident rate of gliomas is generally higher in men compared to women in Denmark population (Ohgaki and Kleihues, 2005; Rasmussen et al., 2017). Moreover, it was reported by Malaysian Cancer Statistics-Data and Figure Peninsular Malaysia

2006 that the incident rate of gliomas by age per 100,000 population was between 60-69 years old as shown in Table 2.4.

Table 2.4 Brain tumour Age specific Cancer Incidence per 100,000 population (Malaysian Cancer Statistics 2006)



2.2.3 Current Treatment Strategies

Treatment approaches for glioma patients usually will be carried out depending on the type, size grade and location of the tumour. The initial stage of treatment was surgical or resection of tumour tissues. Before attaining total resection, biopsy was done to obtain pathological diagnosis (Davis, 2016). Besides that, LGGs primarily affect young adults while HGGs affect adults and older patients. In comparison with grade III and IV gliomas, LGGs usually have more favourable prognosis. In LGGs,

surgeries were conducted to confirm histology of lesion's nature, improving the neurological condition of the patients, minimize the tumour growth risks, preventing the malignant transformation as well as seizures control (Van den Bent, M. J et al., 2012). Most of the studies demonstrated that size of LGGs is an independent prognostic factor and has contrary association between the resection and lesion size.

For instance, the poor defined deep lesions usually are not considered for resection, hence, because of this various growth pattern of gliomas, a molecular background study is anticipated (Pignatti F et al., 2002) such as *MGMT* promoter methylation, IDH 1 mutations, 1p/19q co-deletion. Other than that, radiation therapy was also used as a treatment for LGGs where it showed some improvement in progression free survival from 3.4 to 5.3 years. The study also suggested that the neurological function and seizure has been improved after the exposure of the radiation (Shaw E et al., 2002). As for chemotherapy role in LGGs, it is still incompletely understood but radiation and chemotherapy found to have benefit at initial stage of diagnosis. Surgeries, radiation and chemotherapy are still considered as an initial treatment as well as treatment after recurrence in HGGs. It is believed that the combination with radiation and surgery, chemotherapy may revamp the survival rate in the patients with high grade of gliomas. Unlike for paediatrics, surgeries and radiation are regular approach and a better prognosis for children compared to adults. Moreover, the common and widely used drug is Temozolomide (TMZ) in clinical studies. Besides TMZ, chloroethyl-cyclohexyl nitrosourea (CCNU or lomustine), vincristine, and prednisone used during postsurgical and been dominant to the standard surgical treatment and radiotherapy (MacDonald et al., 2011).

In recent days, due to the advanced scientific knowledge as well as research of molecular genetics, it has led to the evolution of targeted drug therapy in gliomas. For instance, bevacizumab (Avastin) has been given intravenously to stop the new blood vessels formation which eventually cuts off the blood supply to the tumour and kills the tumour. It was also supported by the evident from angiogenesis inhibition with bevacizumab in combination with irinotecan which had showed positive results at initial stage (Schneider et al., 2010).

Another application of therapeutic approach that was carried out recently is the antisense oligonucleotides directed against TGF-β2, a highly immunosuppressive cytokine, through a catheter that is surgically inserted into the tumour (Hau et al., 2007).

Another study by Chandramohan and his colleagues (2012) demonstrated that usage of virus like oncolytic viruses also has been used as one of the treatment at the pre-clinical and clinical stages. This study proves that the concept of targeting cancer cells using an antibody-toxin which was investigated in 1970 by Moolten and Cooperband 1970. This was also supported by (Todo et al.,2012) which has demonstrated the same concept by using exotoxins. Immunotherapies involving vaccines showed long term survival in both adults and children with newly diagnosed as well as recurrent gliomas (Okada et al., 2007).

2.3 DNA Methylation

DNA methylation is a hereditary epigenetic mark which involves the transfer of methyl group to the C-5 position of the cytosine ring of DNA by DNA methyltransferases (DNMTs) (Jin et al., 2011). DNA methylation often occurs at cytosines in any condition of the genome in mammals (Lister et al., 2009). Besides that, DNA methylation contributes to decoding of human epigenome (Kulis and Esteller, 2010). It is also serves as a biomarker for patients' prognosis (Shinawi T et al., 2013). DNA methylation is important in normal development in mammals but it causes diseases in methylation defects. Histones which was wrapped by double-stranded DNA forms chromatin in which the state of chromatin can be either "active" or "silent" based on the interaction between promoters or enhancers of the genes and transcriptional factors. The mechanism is not clear by which the gain of methyl groups in CpG sites knocks down the gene expression (Cheung et al., 2009). Figure 2.3 below shows the mechanism of DNA methylation in normal and cancer cell.

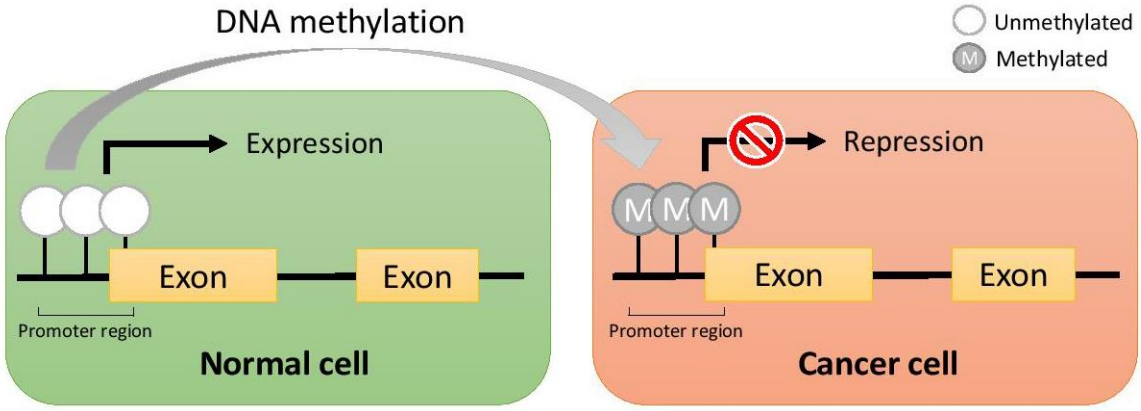


Figure 2.3 Mechanism of DNA methylation

2.3.1 Molecular basis of DNA methylation

Generally, DNA methylation acts as a gene expression regulator in which it becomes decisive for development of cancer, mainly in higher eukaryotes. To date, three types of DNA methyltransferase have been pinpointed such as DNMT1, DNMT3A and DNMT3B. All those three DNA (cytosine-5)-methyltransferases helps in the establishment of DNA methylation pattern. Each genes plays a critical role in initiation and maintenance of DNA methylation. DNMT1 plays a role for the conservation of cytosine methylation. Moreover, DNMT3A involves in parental imprinting (Kimura and Shiota, 2003). All the above genes transfer methyl group from S-adenosyl-L-methionine to cytosines in CpG dinucleotides (Hermann et al., 2004; McCabe et al., 2009).

2.3.2 Types of DNA methylation

DNA methylation can be divided into hypomethylation and hypermethylation. Hypomethylation was reported to be the first to describe the changes of epigenetic in human cancer (m^5C residues replaced by unmethylated C residues) in 1983 where they have observed the alterations of DNA methylation throughout the genome in many different cancers against the wide variety of normal tissues (Ehrlich M et al., 1983). For instance, a study has well described the hypomethylation of few cancer-irrelevant gene regions in colon adenocarcinomas versus normal colonic epithelium where they have reported that metastases are more susceptible to cancer-linked DNA than in control tumours (Feinberg and Vogelstein, 1983).

As for hypermethylation, it is known to be contrast of methylation change where it is also one of the most stable aspect of genome of cancer (Rauch et al., 2009). Figure 2.3 best describes the similarity and differences of hypomethylation and hypermethylation of DNA.

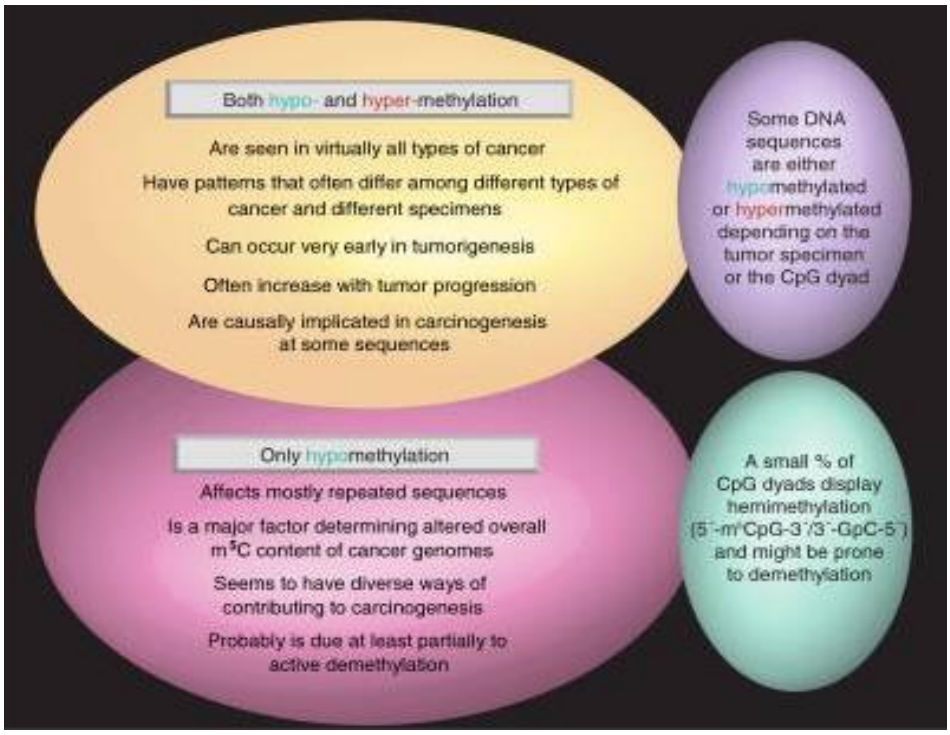


Figure 2.4 Similarities and differences in cancer-associated hypo- and hyper-methylation of DNA (Adapted from Ehrlich, 2009)

Figure 2.4 showed that hypomethylation comprises almost half of the genome. It is also highly associated with tumour progression or malignancy degree (Ehrlich, 2006).