

## REVIEW ARTICLE

# WHAT WE KNOW ABOUT THE MOLECULAR GENETICS OF CENTRAL NERVOUS SYSTEM (CNS) TUMOURS IN MALAYSIA

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The new millennium has been regarded as a genomic era. A lot of researchers and pathologists are beginning to understand the scientific basis of molecular genetics and relates with the progression of the diseases. Central nervous system (CNS) tumours are among the most rapidly fatal of all cancers. It has been proposed that the progression of malignant tumours may result from multi-step of genetic alterations, including activation of oncogenes, inactivation of tumour suppressor genes and also the presence of certain molecular marker such as telomerase activity. In this paper, we review some recent data from the literature, including our own studies, on the molecular genetics analysis in CNS tumours. Our studies have shown that two types of tumour suppressor genes, p53 and PTEN were involved in the development of these tumours but not in p16 gene among the patients from Hospital Universiti Sains Malaysia (HUSM). Telomerase activity also has been detected in various types of CNS tumours. Thus, it is important to assemble all data which related to this study and may provide as a vital information in a new approach to neuro-oncology studies in Malaysia.

*Key words : molecular genetics, CNS tumours, Malaysia*

## Introduction

Brain and spinal cord are the components of central nervous system (CNS) and any tumour located near vital brain structures or sensitive spinal cord nerves can seriously threaten health. However, spinal cord tumours are less common than brain tumours (1). Primary CNS tumours such as gliomas and meningiomas are named by the types of cells they contain, their location, or both. Among the tumours of the CNS, gliomas are the biggest group. Astrocytomas, the most common category of gliomas, are typically graded on a scale of I to IV based on how quickly the cells are reproducing, as well as their potential to invade nearby tissue. Grade I and II astrocytomas are the slowest growing tumours, and are also called low-grade astrocytomas. Most meningiomas are slowly growing benign tumours that histologically correspond to grade I of the World Health Organization's classification of central nervous system tumours.

Over the last decade, a lot of ideas have been emerged about the genetic alterations that occur in human cancers and how they contribute to the tumorigenesis (2). Laboratory studies may play a vital role to investigate changes occurring on a cellular level that will lead to better understanding of the biological mechanisms involved in brain tumor formation. Several specific tumour markers have been studied to determine their importance in cancer research. Tumour suppressor genes are one of the cancer-related genes that involved in malignant transformation (3). Many tumour suppressor genes have been identified for example p53, PTEN, p16, RB1, NF2 and APC, and inactivation of these genes have been reported in association with carcinogenesis in human cancers. The ability to detect mutations in tumour suppressor genes plays an important role in cancer diagnosis and prognosis (4). Presence of certain markers for cancer has also been proposed to be involved in

human tumorigenesis such as telomerase enzyme. According to the previous report, telomerase activity is detected in 61.7% of neuroepithelial brain tumours; it is present in 0-20% of Grade I and II astrocytomas, 40% of anaplastic astrocytomas and 72-100% of anaplastic glioblastoma multiforme (5). Molecular staging using markers such as telomerase activity in combination with other molecular markers may be particularly useful for early detection of cancer (6). Basic scientists and clinicians must work together to prove this strategy that can be applied in patient management and future treatment of cancer.

### ***Mutations in p53 gene***

The best-studied molecular alteration in malignant (non-pilocytic) astrocytic tumors is the inactivation of the tumor suppressor p53 gene. The p53 gene is located on the short arm of chromosome 17 (17p13.1), encodes a protein of 53 kD in molecular weight (393 amino-acid nuclear phosphoprotein). It was first identified as a cellular protein in 1979 because it formed a tight complex with the SV 40 large T antigen (7). Since then, p53 has been believed to influence multiple aspects of cellular functions, including progression through the cell control (8), DNA repair after radiation damage (9), genomic stability (10), and the induction of programmed cell death, apoptosis (11). p53 acts as a transcription factor to induce or repress the transcription of multiple genes whose regulatory region through sequence-specific interaction with DNA.

p53 is frequently found to be mutated in many types of human cancer, including brain tumors (12,13). p53 mutations in adult astrocytoma were first described in 1989 (10) and were followed by more extensive analyses of gene mutations (14-20) and protein alteration (17,19,21-24). In studies that have examined both p53 gene mutations and loss of heterozygosity (LOH) on chromosome 17p, 70% of tumors with p53 mutation have corresponding loss of chromosome 17p (15-18,20,25). Loss of an allele from a chromosome is called LOH, and is thought to be important in the process of human tumorigenesis. Although p53 can be inactivated at either the gene or the protein level, gene inactivation appears to be most common mechanism in astrocytoma formation (17). In astrocytoma, p53 gene mutation, with or without loss of the corresponding normal 17p allele, is by far the most common mechanism for p53 dysfunction. p53

mutations have been reported in approximately 40% of astrocytic tumors of all grades (26).

Genetic alterations of the p53 gene such as point mutations, deletions or insertion occur in four domains which are highly conserved among different species (27). Missense mutations in the p53 gene are the most common (13,28). Single amino acid changes result in a mutant p53 protein with a longer half-life allowing its accumulation to high levels with the cell nucleus (29). In contrast, normal or wild-type p53 protein has a very short half-life (less than 30 minutes) and therefore is present in small amounts in normal cells (30).

The prognostic implication of p53 mutations has not yet been defined clearly. The authors of numerous studies have attempted to address whether the absence or presence of p53 mutations has a prognostic role in patients with glioma. Therefore, at present, the prognostic implication of either a p53 genetic mutation or protein expression in gliomas is uncertain.

Rapid methods currently used for the detection of p53 alterations have been developed including serological test, genetic analysis and immunostaining (31,32). The most widely used has been single-strand conformation polymorphism (SSCP) evaluation (33). Most studies of the p53 gene in human astrocytomas and glioblastomas to date have used SSCP analysis to detect mutations (18,34-39).

Recently, we have reported the frequency of the p53 gene mutations in our Malay patients with gliomas using single strand conformation polymorphism (SSCP) analysis and direct DNA sequencing (40-41). We have improved this technique by developing a procedure of non-isotopic PCR-cold SSCP using the Dcode Universal Mutation Detection System, making it rapid and suitable for screening numerous DNA samples (40).

### ***Loss of Heterozygosity of 10q, 9p, 17p and 13q and PTEN gene***

Loss of heterozygosity (LOH) on several loci and mutations on PTEN tumor suppressor gene (10q23.3) occur frequently in sporadic gliomas. Polymerase chain reaction (PCR)-LOH analysis using microsatellite markers and single-stranded conformational polymorphism (SSCP) analysis were performed to determine the incidence of allelic losses on chromosome 10q, 9p, 17p and 13q and mutations of exons 5, 6 and 8 of the PTEN gene in malignant gliomas. The rationale of analyzing allelic loss is to

determine the involvement of putative tumor suppressor genes which might contribute to the transformation of malignant glioma.

12 of 23 (52.2%) malignant glioma cases showed allelic losses. The highest number of cases with LOH were detected on chromosome 10q23.3 harboring the PTEN gene. This region was reported to be frequently deleted in LOH studies involving the q arm of chromosome 10 and fine mapping studies of the entire chromosome (42-45). LOH was also detected on the regions 10q25.1, 10q22, 9p21 and 13q12.3. These regions have been documented to contain genes or tumor suppressor genes involved in several carcinomas, including malignant gliomas (42,46,47-49). Less frequent LOH was detected on chromosomes 17p13.1 whereas no allelic loss was detected on the region 13q12.1, suggesting that these regions are not typically involved in the progression of malignant gliomas in Malay patients.

7 of 23 (30.4%) samples showed aberrant band patterns and mutations of the PTEN gene. Most of PTEN gene mutations detected in the present study were anaplastic astrocytoma and glioblastoma. Five codons containing CpG dinucleotides were found mutated on exons 5 and 6 of the PTEN gene, which involved entirely missense and nonsense mutations. The codon most frequently affected or mutated was codon 173 which is conserved in tensin, auxilin and bacterial phosphatase (50). The mutations of this gene which lead to amino acid substitutions may generally affect conserved residues or structurally conserved features of the protein (44). It is believed that the N-terminal half of PTEN is functionally more significant for tumor suppression because of homology to tensin, auxilin and phosphatase, regions that may control cell cycle, invasion and metastasis (51). These findings suggest that mutations of PTEN are concentrated to the N-terminal phosphatase domain with cluster of mutations in the region 5' to the core phosphatase motif and the 5'-end of exon 6.

4 of the tumors containing PTEN mutations also showed loss of heterozygosity in the chromosome 10q23 region flanking the PTEN gene. The pattern of allelic losses on 10q23 as well as mutations of the gene itself appear to be associated with the progression of glioma (52,53) and indicated complete loss of the wild-type PTEN gene (54). These findings also suggest that PTEN gene might be inactivated by point mutations or small deletions (52) and that both alleles of the PTEN gene were inactivated by a classical two-hit mechanism (55), therefore confirming the previous idea that PTEN

acts as a tumor suppressor gene.

### ***Absence of p16 gene alterations in CNS tumours***

The p16 gene is a tumour suppressor gene located at chromosome 9p21. It is also known as Major Tumor Suppressor 1 (MTS1), Inhibitor of Cyclin-dependent 4a (INK4a) and Cyclin-dependent Kinase Inhibitor 2a (CDKN2A) which consists of 3 exons and 2 introns which encodes 156 amino acids, 15.8 kD protein (56,57). This protein acts as a negative protein regulator and loss of its function may lead to cancer progression by allowing unregulated cellular proliferation (56). Alterations of p16 gene can occur via different mechanism such as homozygous deletion, point mutation and hypermethylation of this gene in promoter region (58).

High frequent mutations and deletions of this gene in human cancer cell lines first suggested an important role in the occurrence of many types of cancer. Germ-line mutations have been reported in melanomas as reported by previous author (59). Somatic mutations also have been detected in various types of cancer such as pancreas, esophagus, lung and brain (60-63).

Molecular analysis of p16 gene was carried out using 50 cases of CNS tumours from Malaysian patients. We performed PCR-SSCP technique to screen p16 gene mutation and confirmed with DNA sequencing analysis. Homozygous deletion of this gene has been detected using multiplex-PCR method as previously described (59). Our study indicated that there was no alteration of p16 gene via homozygous deletion and point mutation at exon 1 and 2 in CNS tumours from Malaysian patients and this gene might not play a major role in tumorigenesis mainly of malignant gliomas (64).

It has been reported that deletion of p16 gene was more frequent in high grade of gliomas (48). In most studies, investigators also have revealed that deletion of this gene was less common in primary tumours than in cell lines (65,66). In accordance to previous study by Jen and colleagues in 1994, the p16 gene was often homozygously deleted in glioblastoma multiformes (GBM) but not in medulloblastomas or ependymomas (65). It has been shown that this gene was often mutated in GBM, but the frequency of p16 mutations was still low. Other study reported that p16 gene mutations was a rare mechanism in astrocytic tumours (67). It is possible that there is another mechanism of p16 inactivation involved in the development of CNS

tumours in Malaysian patients such as hypermethylation of this gene in the promoter region. Further studies that involved more samples of high-grade tumours, however, should be undertaken to determine the importance of the role of p16 in tumourigenesis.

### **Telomerase activity in CNS tumours**

Telomerase activity has been proposed to be valuable in the diagnosis and prognosis of malignant tumors since this unique ribonucleoprotein enzyme is detected in most cancer cells but do not in almost normal somatic tissues. With every cell division, the length of the telomeres decreases due to the end replication problem. However, this problem can be prevented by maintaining telomeric length by telomerase. Both telomerase and telomere have been identified as targets for anticancer therapy since there were an evidence of a strong correlation between telomerase reactivation, cellular immortalization and cancer (68).

In our study, telomerase activity was detected in 6 out of 23 cases of CNS tumours (26.1%) including oligodendroglioma, GBM, paraganglioma and meduloblastoma. We also revealed that there was a significance association between telomerase activity status with tumour grade ( $p < 0.05$ ). Detection of this enzyme in analyzed tumours supports the fact that activation of telomerase may associate with tumour progression. Our results also showed similar findings with other studies reported that telomerase activity was detected in GBM, oligodendroglioma and meduloblastoma (70,71). In contrast, telomerase activity was not detected in all schwannoma and meningioma samples. Similarly it was reported by the authors of the previous studies (69,71-73). According to previous review, 758 of 895 (85%) of malignant tumours (higher grade tumours), but none of 70 normal somatic tissues, expressed telomerase activity (74). This strong association of telomerase activity with malignant tissue is good evidence that telomerase can be an important marker for diagnosing cancer (74).

Telomerase activity was determined based on the PCR-Telomeric Repeat Amplification Protocol (TRAP) assay using TRAP<sub>EZE</sub> Telomerase Detection Kit (Intergen Co., USA) which derived from an improved version of the original method as previously described (75). We accomplished in detecting this telomerase activity using a non-isotopic TRAP-silver staining method. According to the previous studies, TRAP assay combined with

silver staining method could be a common practice for the routine laboratory because it has been considered to be a quick, easy, safe and cost-effective staining protocol (76-78). It has been suggested that TRAP-silver staining assay of telomerase activity may be used as a routine diagnostic method for brain tumour detection in the future (79).

### **Conclusions**

These new approaches will undoubtedly contribute to a better understanding of carcinogenesis. Further investigations should be lead to generate other tumour markers which can possibly be used in the future screening, early diagnosis, staging and surveillance of cancer. An important aspect of this review is to provide an insight into central nervous system tumours etiology important for the development of prevention strategies.

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