CASE REPORT

A Dual Genetic Alteration (Mitochondrial and Nuclear DNA): First Case in Malaysia Detected in Glioblastoma Multiforme

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ABSTRACT

Although the precise etiology of Glioblastoma multiforme (GBM, WHO grade IV) remains unknown, its progression is believed to be driven by the accumulation of multiple genetic alterations. Here, we report a case of a patient who developed GBM, and associated with dual alterations, particularly 4977-bp deletion in mtDNA (mtDNA^{4977}) and p.Arg132His (R132H) mutation in IDH1. A 35-year old Malaysian woman patient who primary diagnosed with astrocytoma WHO grade I and subsequently after four years developed a GBM, was detected with a mtDNA^{4977}. This deletion appears to be a sporadic mutation. Additionally, analysis of patient's tumor tissue also found to harbor a heterozygous IDH1 R132H mutation. This represents the first case report of coexisting mtDNA^{4977} together with IDH1 R132H mutation in a Malaysian patient of GBM. The findings of dual alterations could be of therapeutic benefit if these alterations were justified to be contributing to GBM growth and aggressiveness.

Keywords: Glioblastoma multiforme, 4977-bp mtDNA deletion, IDH1 (R132H) mutation, Double alteration

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INTRODUCTION

Glioblastoma multiforme (GBM) is one of the most aggressive of adult brain malignancies and histopathologically classified into the highest grade IV astrocytoma and carries a worse prognosis.

The progression of GBM involves the accumulation of various genetic alterations in proto-oncogenes and/or tumor suppressor genes that are encoded by the nuclear DNA (nDNA) (1). So far, the involvement of our second genome which is the mitochondrial genome (mtDNA) in GBM has not yet been fully elucidated and knowledge about its role on genetic pathways of GBM tumorigenesis is still limited.

mtDNA is sensitive to oxidative stress-linked damage. Impaired mitochondrial stability caused by mtDNA alterations is expected to lead to a loss of mitochondrial energy production efficiency and an enhanced production of ROS. These processes may promote to uncontrolled cell growth, proliferation and eventually cancer.

Various type of mtDNA alterations are suspected to be associated with human carcinogenesis (2). mtDNA alteration such as the 4977-bp large-scale deletion (mtDNA^{4977}), recognized as "common deletion", has been detected in various types of cancers (3). To date, mtDNA^{4977} in GBM has never been reported.

In the present study, we describe a case of the coexistence of mtDNA^{4977} and IDH1 R132H mutation in a Malaysian GBM patient. Informed consent form was signed by patient, and research protocol was approved by the institutional ethical committee (IRB Reg. No: 00004494).

CASE REPORT

We report a case of a 35-year-old lady (patient B5) who presented with late onset epilepsy with intermittent right parietal headaches. Examination reported no neurological deficits. MRI brain revealed a large solid mass in the right frontal parasagittal region (2.9cm x 5.8cm x 5cm) with perilesional edema. The mass was hypointense on T1WI and hyperintense on T2WI and had no contrast enhancement but shows small cystic areas within it. She then underwent a tumor biopsy and the histopathology revealed astrocytoma Grade I (Figure 1A). She was subsequently managed conservatively with follow-up MRI.

However, four years following surgery, she developed progressive lower limb and upper limb weakness. She also demonstrated personality changes with emotional lability, memory impairment and speech disturbances.

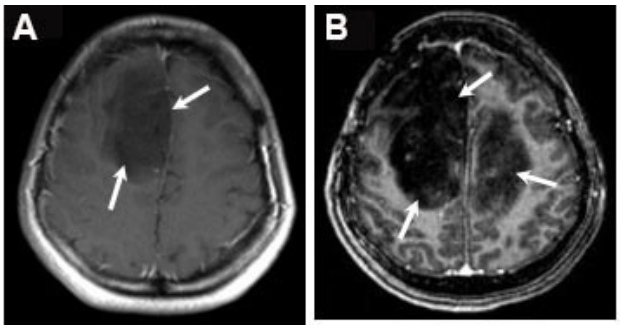

Figure 1: (A) MRI finding on first presentation before tumoral biopsy was made. The histopathology reported as astrocytoma grade I (white arrows). **(B): Contrasted MRI brain done four years later in which the histopathology disclosed as a GBM grade IV (white arrows).** MRI=magnetic resonance imaging.

She also suffered from recurring seizure attacks over the last 4 years. Examination at this juncture revealed reduced word output with monotonous speech, impaired recent memory and signs of upper motor neuron deficits in the lower limbs. She was then subjected to a repeat MRI study which revealed a solid-cystic lesion over right parasagittal region with extension to the left involving body of corpus callosum, right caudate nucleus, right thalamus and right internal capsule measuring 6.9cm x 6.8cm x 4.5cm in the largest diameter. She was then subjected to a bifrontal craniotomy and debulking of the tumor; the histopathology of the tumor was reported as GBM (Grade IV) (Figure 1B).

Detection of mtDNA^{4977}

Genomic DNA was extracted from tumor tissue and patient-matched blood. PCR primers used were as follows: L8150-F, 5′-CCGGGGGTATACTACGG-3′ and H13650-R, 5′-GGGGAAGCGAGGTTGACCTG-3′. PCR was performed for 35 cycles of 94°C for 30s, 58°C for 30s and 72°C for 1min with a final extension at 72°C, 5min. In the presence of mtDNA^{4977}, the PCR amplification product was 524-bp in size (Figure 2A). In the absence of the deletion or for the wild type mtDNA amplification, there was no yield of the product because the fragment is too large (>5 kb) to be generated with this PCR conditions. The primer pairs L8150 and H13650 allowed the 524-bp amplicon to be amplified from mtDNA^{4977}.

mtDNA^{4977} was validated by a second sets of PCR primers (Figure 2B). These primers consisted of two pairs (P1/P2 and P3/P4):
P1,5′-CTGAGCCTTTTACCACTCCAG-3′; P2,5′-GGTGATTGATACTCCTGATGCGA-3′; P3,5′-CCC ACTGTAAAGCTAACTTAGCATTAACCT-3′; P4,5′-GGTTTCGATGAT GTGGTCTTTGG-3′. PCR conditions were: 94°C 30s; then 30 cycles of (94°C 30s, 59°C 30s, 72°C 1min) with a final extension of 10min at 72°C. One primer pair (P1/P2) was designed to give a product (142-bp) only in wild-type, the other pair (P3/P4) allowed only a product (262-bp) in DNA with mtDNA^{4977}. The mtDNA^{4977} was identified in the patient tumor tissue whereas this deletion was not observed in the corresponding peripheral blood sample. The deletion was verified by repeated analyses including finally confirmed by DNA sequencing.

Detection of IDH1 R132H point mutation

In addition, we also examined for the hotspot of IDH1 R132H mutation on the patient sample using PCR-RFLP analysis. PCR primer sequences were: 5′-TGGGTAAAACCTATCATCATCGAT-3′ and 5′-TGTGTTGAGATGGACGCCTA-3′. PCR conditions

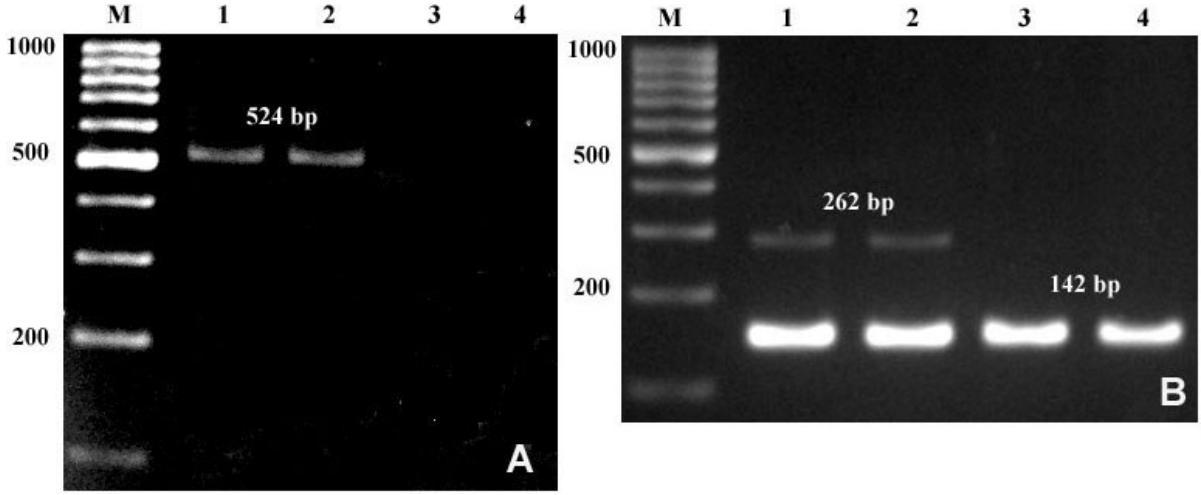

Figure 2: (A) mtDNA^{4977} in brain tumor. Using the specific primers, a 524-bp PCR product amplified from mtDNA^{4977} in GBM patient. (B) A second sets of PCR primers (2 pairs of primers, P1/P2 and P3/P4) were used to amplify and verify mtDNA^{4977}. One primer pair (P1/P2) was designed as an amplification control, provides a 142-bp product corresponding to wild-type mtDNA. To confirm loss of the mtDNA^{4977}, a second pair of primers (P3/P4) was used to anneal within the fragments flanking the deleted region and the amplicon size was a 262-bp band. Generation of both bands indicates the presence of heteroplasmy of mtDNA^{4977}. Lane M: 100bp DNA marker, Lane 1: Positive control (SNU-5 gastric cancer cell line that confirmed contains mtDNA^{4977}), Lane 2: Patient B5 (tumor tissue) - deletion, Lane 3: Patient B5 (blood sample) - no deletion, Lane 4: Patient X (tumor tissue obtained from other GBM patient as a negative control) - no deletion.

were: 98°C 30s; then 35 cycles of (98°C 30s, 55°C 30s, 72°C 30s) with a final extension of 5min at 72°C. The PCR amplification gave the expected size of 261-bp. The RFLP analysis led to generation of 237-bp and 261-bp fragments after PvuI digestion if there was IDH1 132R>H mutation present, and only one 237-bp fragment if IDH1 132R>H mutation was not present. Our PCR-RFLP analysis showed the presence of a heterozygous mutation of IDH1 R132H (Figure 3). This mutation was confirmed by direct sequencing (Figure 4).

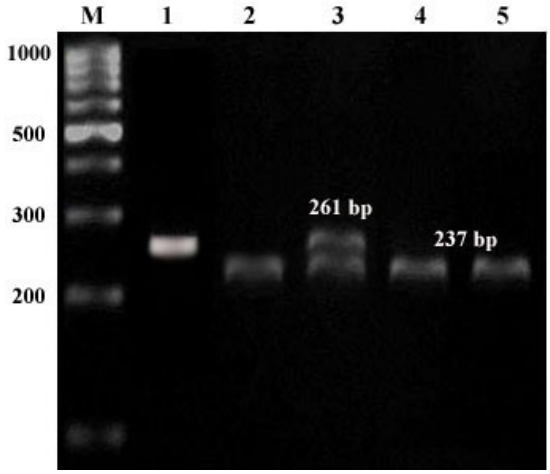

Figure 3: Heterozygous mutation of the IDH1 gene at c.395G>A (p.R132H). Heterozygous IDH1 mutant is represented by two bands at 261-bp and 237-bp by PCR-RFLP. Lane M: 100bp DNA marker, Lane 1: Patient B5 (Undigested PCR product) (261 bp), Lane 2: Patient B5 (blood sample), Lane 3: Patient B5 (tumor tissue) – heterozygous mutation, Lane 4: Patient X (blood sample obtained from other GBM patient as a negative control), Lane 5: Patient X (tumor tissue obtained from other GBM patient as a negative control). IDH1 = Isocitrate dehydrogenase enzyme isoform 1.

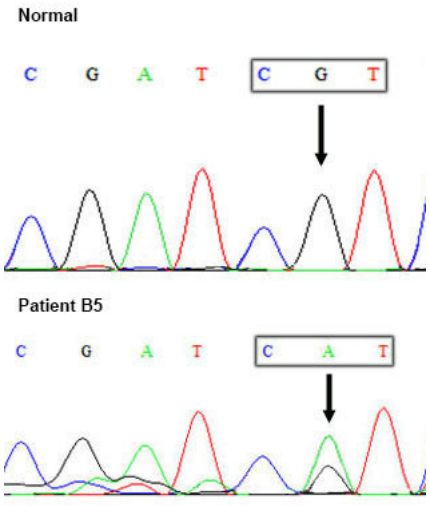

Figure 4: (Sequencing results of the c.395G>A (p.R132H) heterozygous mutation in the IDH1 gene. The transition G>A at position 395 of the coding region leading to an arginine to histidine change (R132H) and was identified in Patient B5 tumor tissue

DISCUSSION

High levels of oxidative stress can be potentially triggered by the hypoxic microenvironment in the tumor, promoting the accumulation of both nDNA and mtDNA damages (2, 3). mtDNA alterations are considered as early event in carcinogenesis and have been described in various neoplasms. Previously, we discovered that brain tumor tissues had a high frequency (51% were identified in 49 patients) of somatic mutations in the displacement-loop of mtDNA (4). Furthermore, we also revealed that 58% of all brain tumor patients harbored mitochondrial NADH-dehydrogenase subunit 3 (ND3) 10398A>G mutation (5).

Here, we report a mtDNA^{4977} case, which has yet to be reported in Malaysian brain tumor patients, was detected in a 35 year old Malaysian female diagnosed with GBM. The mtDNA^{4977} was detected in tumor tissue, but it was absent in the peripheral blood of patient. GBM cells are very fast-growing tumor and have the ability to invade and destroy surrounding normal brain tissue. In addition, this tumor is difficult to remove without excessive damage to nearby normal brain tissue. Due to these reasons, the non-tumor tissue adjacent to tumor tissue, was not used in this case. Alternatively, patient's peripheral blood served as a control.

Our analysis indicates that mtDNA^{4977} as a sporadic mutation. The mtDNA^{4977} has been identified in several cancer types (including breast cancer, colorectal cancer, lung cancer, etc.) (3) except there has been no report in brain tumor patients especially in GBM. This deletion involves between two 13-bp direct repeats (nucleotide positions 13447-13459 and 8470-8482). mtDNA^{4977} abolishes several tRNA and genes of the OXPHOS subunit (3). This deletion could severely affect ATP production and result in the mitochondrial function impairment.

Although there is a strong association between mtDNA alterations and cancers, our findings are still unclear whether mtDNA^{4977} is a causal factor for tumorigenesis or whether it directly derives from secondary effects of the neoplastic process. Further analyses on larger sample size are required to explore the role of mtDNA^{4977} on GBM progression.

Besides mtDNA^{4977}, our patient was also found to harbor the IDH1 R132H mutation. Mutations of the IDH1 were acknowledged their existence in 2008 as an early event in gliomagenesis. IDH1 mutations are mainly occurred in low-grade gliomas and secondary high-grade gliomas. Detection of an IDH1 mutation in secondary high-grade gliomas, it is believed that the tumor has grown from a lower grade precursor lesion. The IDH1 gene has been widely investigated for many years as a potential diagnostic biomarker for predicting longer survival and for the assessment of new targeted molecular drugs'

effectiveness. We found that our patient was first diagnosed with astrocytoma WHO grade I and later after four years developed a secondary GBM. The IDH mutant was reported in approximately 10% of secondary GBMs with malignant transformation from low grade glioma and was more frequent in patients younger than 45 years old (1). It has been used to differentiate primary from secondary GBM.

This case report postulates that mtDNA^{4977} and IDH1 mutation can together play a role in progression of GBM. The mtDNA^{4977} and IDH1 R132H mutation have been independently reported to be involved in tumorigenesis of various human cancer types but no previous reports of connection between both genomes alterations were found. The existence of dual alterations between mtDNA and nDNA could be a rare event in the common population, but it is not unusual in GBM cases. The accumulation of genetic alterations in GBM is considerably heterogenous, because the involvement of some mutations from both mitochondrial and nuclear genomes.

CONCLUSION

To our knowledge, this is the first report of a patient with GBM that showed the presence of a mtDNA^{4977} and at the same time carried a mutated IDH1 R132H. This observation indicates that the mtDNA^{4977} may have a role in the GBM's development in connection with mutation in nuclear gene and environmental factors. A more detailed analysis involving a large number of patients is needed in order to establish the exact role of this double genetic alteration in GBM.

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