The Effect of Mitochondrial DNA Mutations in Brain Tumors

Zübeyde Erbayraktar¹, Zeynep Haşimoğlu¹, R. Serhat Erbayraktar²

¹Dokuz Eylül University, Health Sciences Institute, Biochemistry, İzmir, Turkey
²Dokuz Eylül University Faculty of Medicine, Neurosurgery, İzmir, Turkey

Abstract

Purpose: Brain tumors are a group of diseases in which different genotypes result in different phenotypes at the molecular level. Although there have been a number of studies related to the role of alterations in nuclear genes, such as oncogenes and tumor suppressor genes, in the development of brain tumors, the effects of mitochondrial genes on tumorigenesis have not been well elucidated.

Methods: It is thought that mitochondrial DNA plays an important role in the tumorigenesis process due to the fact that it is more susceptible to mutations than the nuclear DNA and the repair mechanisms are weaker. Mitochondrial DNA mutations have been extensively studied for their use as biomarkers since they can reach high copy number in clonal characterization.

Results: MtDNA mutations play an important role in cancer developmental stages, but the mechanisms of cancer development and progression of these mutations are not fully explained.

Conclusion: The identification of mitochondrial DNA defects is anticipated earlier in the diagnosis of brain tumors, and more effective treatment protocols will be regulated.

Keywords: Brain tumor, mitochondrial DNA, tumorigenesis

INTRODUCTION

Brain tumors constitute about 85-90% of primary central nervous system tumors. Approximately 240,000 brain tumor patients are seen every year in the world (1). Especially, the number of cases in developed countries is increasing. Brain tumor is the second cause of death young adult male patients aged 20-39 years while the fifth in young adult female patients (2). It is well known that genetic factors as well as epigenetic and environmental factors play an important role in the development of brain tumors. Surgery, followed by radiotherapy and chemotherapy is used in the treatment of brain tumors. As the treatment of benign tumors usually results with cure, the treatment of malignant tumors requires a much more difficult process. Therefore, more effective new treatment approaches should be developed by increasing the research on better understanding of the molecular mechanisms involved in the formation of brain tumors. In recent years, researchers have focused on the molecular mechanisms involved in the formation of brain tumors, revealing differences with respect to oncogenes and tumor suppressor genes encoded by nuclear DNA. For instance, it has been reported that inactivation of the p53 tumor suppressor gene and activation of the receptor tyrosine kinase result with astrocyte tumor formation, whereas inactivation of the nf2 (merlin, schwannomin) tumor suppressor gene results with meningioma (2,3). On the other hand, interesting studies have also been reported regarding the effects of mitochondrial DNA on the formation of brain tumors (4,5). With this information, the treatment of brain tumors will be planned more effectively.

MITOCHONDRIAL GENOME

Mitochondria are a double membrane organelle that is responsible for oxidative phosphorylation and ATP (Adenosine triphosphate) production and is found in cell cytoplasm. Besides, being the energy center of the cell, mitochondria, which have important cell functions such as anabolic / catabolic reactions, metabolic regulation, signal transduction, calcium homeostasis,
ROS production, redox control and apoptosis, are both dynamic on their own and in working with other organelles (6,7). The number of mitochondria varies according to the energy needs of the cell, and mitochondria are found in more amounts in proportion to energy requirements in cells with high metabolic activity such as muscle and neuron cells (8).

Each mitochondrion contains 2 to 10 mtDNA molecules. The mitochondrial genome has its own genetic information system that can perform replication, transcription, and translation functions. Human mtDNA has a double-chain, closed circular structure and contains 16,569 base pairs [Figure 1]. MtDNA, which contains a total of 37 genes, including thirteen genes encoding proteins and 24 genes involved in the synthesis of these proteins (22 tRNA, 2 rRNA), plays a crucial role in providing cellular metabolic homeostasis (9). According to the nuclear DNA, mtDNA is more susceptible to oxidative damage and more susceptible to mutations due to the weaker repair mechanisms and the lack of histoprotective proteins (10). In conclusion, when mitochondrial dysfunction occurs, tumoral development is triggered by impairment of normal energy metabolism. The German scientist Otto Warburg described this interaction as “Warburg Influence” in 1956. Accordingly, the proliferative cell prefers the glycolysis pathway in 95% of the stroma for ATP production, even if there is oxygen, leaving the remaining 5% of energy production through the respiratory chain in the mitochondrial membrane (11). In this way, inactivation of mitochondria, as well as reduction of their number by changing their shape are accepted as symptomatic of tumoral progression.

**REACTIVE OXYGEN SPECIES AND TUMORIGENESIS**

The mitochondrial respiratory chain is one of the most important endogenous sources in the production of reactive oxygen species (ROS). During the oxidative phosphorylation, some electrons cannot be thrown out of the chain and bind to molecular oxygen to form ROS. [Figure 2]. It is believed that when ROS is low amount, it is thought to play a positive role in the expression of growth-related genes and signal transduction regulating cell proliferation. Excessive production of ROS; causing damage to cellular components such as nDNA, proteins, lipids, and mtDNA (12). Recent studies indicate that high levels of oxidants play a role in tumor development and progression (13). Non-repairable mtDNA damage constitutes permanent mutations and plays an accelerating role in tumorigenesis. In addition to energy metabolism, mitochondria also control the variability of oxidative stress and regulation of cell death. For this reason, the cancer...
is also responsible for the regulation of stem cell (CSC) activity (14). Progressive genetic alterations cause normal stem cells to transform CSC and thus develop malignant tumors. Although the mitochondrial functions in normal stem cells are well defined, the situation in CSC is different and not fully understood. In relation to this issue, Ye et al. reported that the amount of mtDNA, oxygen consumption, intracellular ATP and ROS levels are lower in lung CSCs where the mitochondrial membrane potential is higher (15). In another study regarding leukemic stem cells, lower ROS levels, decreased OXPHOS and anti-apoptotic Bcl-2 gene expression were found to be increased (16). Likely, cells isolated from a patient with epithelial over cancer, glucose uptake, OXPHOS and fatty acid β-oxidation genes were overexpressed (17).

Vlavi and colleagues have shown that glioma CSCs have a lower glycolytic capacity than glioma cells (18). Given these reports, the features of the mitochondrial genome for different types of cancer stem cells are different from each other. Therefore, understanding the properties of CSCs will be an important beginning in the development of therapeutic drugs for mitochondria, which play an important role in cancer formation.

MITOCHONDRIAL DNA MUTATIONS IN TUMOR

Nowadays, nuclear DNA mutations have been proven to be effective in tumor development and progression, but the relation between mtDNA and tumorigenesis has not been fully elucidated yet. Since there are studies suggesting that mtDNA mutations are effective in the tumorigenesis process (4), many researchers are investigating whether mtDNA may be a potential biomarker in different tissue types in the tumorigenesis process. Gasparre et al. reported that mtDNA mutations resulted in complex I deficiency in the mitochondrial respiratory chain in benign renal oncocytoma cells (19).

A wide variety of mutations in mtDNA have been observed in different types of cancer. The first somatic mutation of mtDNA was identified in human colonic cancer cells by Bert Vogelstein’s group fifteen years ago (20). After these initial findings, head and neck cancers prostate cancer (21) mtDNA mutations have been reported in many more cancers. Recent studies have shown that molecular abnormalities observed in mtDNA are genetic damages such as point mutations, deletions, insertions, microsatellite instability, polymorphisms, and changes in mtDNA copy number.

SOMATIC MITOCHONDRIAL DNA ALTERATIONS IN BRAIN TUMORS

Maternal mutations as well as somatic mutations can cause diseases. Mature tissues with high energy metabolism, such as brain and muscle tissue, are more susceptible to the accumulation of somatic mutations / deletions, particularly in the processes of aging and degeneration (22). There are numerous reports of brain tumors that have proven to be associated with the presence of mtDNA mutations. MtDNA database “Mitomap (http://www.mitomap.org)” reported that there are more than 30 mutations and sequential variations in brain tumors. It has been reported that the most common region of the mutation in the mitochondrial genome in brain tumors is the displacement (D-loop) region. In brain tumors; Numerous somatic mutations in mtDNA are observed, such as point mutations, deletions, insertions, MTMSI (mitochondrial microsatellite instability), and changes in the number of copies.

Point mutations

More than 260 point mutations and deletions are accused of causing diseases such as carcinoma and neurodegeneration (23). MtDNA, as well as mtDNA point mutations are transferred from the mother to all her children. It is estimated that there are thousands of mutations affecting approximately 1700 nDNA-encoded mitochondrial proteins (including OXPHOS structural proteins, metabolic enzymes, proteins involved in mtDNA repair and replication, protein translation, membrane integrity and dynamics) (24).

MtDNA point mutations have been detected in brain and other central nervous system tumors, such as the gliomas, astrocytomas, gliomatosis cerebri, medulloblastomas, menengiomas, schwannomas and neurofibromas (25,26). mtDNA somatic point mutations are most frequently observed in the D-loop region, especially in the repeated polycytosine (poly-C) mononucleotide sequences located between 303 and 315 nucleotides known as D310. This region has been identified as a problematic region for somatic mtDNA mutations in many tumors, including brain tumors. In 2005, Montanini and colleagues analyzed the D-loop region of mtDNA in 42 patients with malignant glioma and reported that there were sequence changes involving 16 somatic mutations in 36% of patients, mostly in the D310 region (27). In brain tumor patients, at least one somatic mutation in 40% of medulloblastoma patients (6/15) was found in one of the studies that completely excluded the mitochondrial genome except D-loop region. Again, the presence of mutations in CSF specimens of these patients has been confirmed. So that it has been suggested that somatic mtDNA mutations in CSF has been suggested to have a potential for being a biomarker of disease prognosis (28).

On the other hand, Lueth’s group supported previous findings on the frequency of somatic mtDNA mutations in medulloblastoma by showing the presence of somatic mtDNA mutations in 6 of 15 medulloblastoma patients (29). Prior to the investigations in medulloblastoma patients, they sequenced the mitochondrial genomic sequence of tumor tissues from 19 pilocytic astrocytomic patients and matched blood samples and found somatic mutations in 16 (84%) cases (30), too.

Kurtz et al. analyzed the state of neurofibromas in 37 patients with neurofibromatosis type 1 and found somatic mutations in 7 patients with cutaneous neurofibromas (37%) and in 9 patients with plexiform neurofibroma (50%). They reported that all mtDNA somatic mutations detected in that study were in the D-loop region (31). The reason why most genetic mutations occur in the
control region, which does not encode the mitochondrial genome, remains unknown. However, it is thought that mutations in the D-loop region affect the initiation of replication and promoter region, leading to impaired mitochondrial biogenesis and transcriptional and protein expression (32). Likely, glioblastoma (GBM) cells have mtDNA mutations in D-loop and protein coding regions, too. It has been reported that the D-loop region in these tumors contains not only nucleotide changes, but also new variants that constitute the instability in the regions (4,20).

Deletion
Among the large-scale deletions identified in the mitochondrial genome, the 4977-bp-wide deletion detected in various cancers such as breast cancer, cervical cancer, colorectal cancer, lung cancer and esophageal cancer is quite common. This deletion is referred to as “common deletion” and abolishes functions of the complexes required for the maintenance of normal mitochondrial OXPHOS function (33). Although there are numerous studies showing that the 4977-bp deletion in the mitochondrial genome plays a role in the tumorogenesis process, its effect on brain tumors has not been investigated yet. There is no study regarding the role of the deletion in brain tumors. However, Wallace’s group reported that 4977-bp deletion in the different regions of the cortex, putamen and cerebellum was greater in older individuals compared to those in younger individuals with normal brain and this finding was in accordance with the aging process (34). Thus, they suggested that this deletion of mtDNA might have an effect on aging-related neurological disorders. In another study, 4977-bp deletions were detected in the brain tissues of the patients suffering from bipolar disorder at the autopsy (35).

Mitochondrial microsatellite instability
Kirches et al. first identified high mtDNA sequence variants in 12 astrocytic brain tumors (36). Two years later, using laser microdissection and PCR technique, they examined 55 gliomas for variants in the poly-C unit of the mitochondrial D-loop and reported changes in 9% of the samples (4). In a study of gliomatosis cerebri in relation to mitochondrial genomic instability in 2003, it was also found that the poly-C unit as a clonal marker is hypervariable (25). Yeung et al. investigated the effect of GBM mitochondrial genomic variants and mtDNA variants in a series of GBM cell lines with using a combination of new generation sequencing and “high-resolution melt (HRM)” assays and reported that the highest frequency of mtDNA variants was at the beginning of D-loop and replication in non-coding regions. Furthermore, they found that the ND4 and ND6 genes, which encode the subunits in the complex I of the electron transport chain, were the most frequently affected genes in the coding region (5).

As a result, a number of studies have been carried out showing that the polymorphic structure of the mitochondrial genome has tumorigenesis inducer and promoter effects. In the study of Takibuchi et al. specific genes such as ND5 were evaluated for tumor promoter and promoter effects. In these and similar studies, mtDNA mutations have been associated with tumorigenesis (36), although this relationship has been rejected in some other studies concluding the lack of association between mtDNA mutations causing mitochondrial dysfunction and the development of any cancer form (37).

Copy number changes
In addition to mtDNA mutations and deletions, changes in mtDNA copy number were also investigated in gliomas. Liang et al. investigated changes in the number of mtDNA copies in 15 low-grade gliomas and reported an increase in the number of mtDNA copies compared to those in normal brain tissue. Likely, when low and high grade gliomas were examined, 39 (87%) of 45 patients were found to have a increase up to 25-fold in mtDNA copy numbers (38).

MtDNA copy number changes were first reported by Marucci et al. in oncotic GBM samples. In that study, it was observed that the number of mtDNA copies increased in 9 of 10 oncotic GBM cell lines (39).

In another study, oncotic GBM patients were compared with non-oncotic GB patients and longer survival was observed in oncotic GBM patients. That was an unexpected result for the oncotic phenotype previously associated with the mtDNA mutation (40). In 2013, Dickinson et al. also investigated the potential for differentiation of mtDNA copy number in GBM cell lines and found that the number of defective mtDNA copies decreased astrocytic differentiation in GBM cells compared to those in human neuronal stem cells (8).

MITOCHONDRIAL GENE EXPRESSION CHANGES
In 2005, Dmitrenko et. scanned cDNA libraries for human fetal glioblastomas and normal human brain samples and found that 80 different genes were expressed. They found that 30 of them correspond to mitochondrial (ATP6, COXII, COXIII, ND1, ND4 and 12S rRNA) genes. According to this finding, the mitochondrial transcripts were exposed at a lower level in glioblastomas when compared to those in the neighboring normal brain tissue (41).

ALTERATIONS IN THE OXIDATIVE PHOSPHORYLATION COMPLEXES
OXPHOS enzyme activities and protein levels were found to decrease in some tumors. In a series of 25 astrocytic tumors, OXPHOS complexes and citrate synthase activity levels were examined using immunohistochemical staining. Complex I-V and citrate synthase activities were reported to decrease by 56-92% when compared to those in normal brain tissue (42). Lloyd et al. have detected changes in the entire mitochondrial genome using a new generation sequencing analysis at 32 GBM and 10 cell lines. They identified more than 200 mtDNA mutations in complexes III and IV. In addition, mutations were analyzed using 3D structural mapping. Nine mutations were shown to have a significant functional effect on the level of protein conformational change, but the remaining 16 mutations were not functional (43).
PROGNOSTIC IMPACT OF MITOCHONDRIAL DNA ALTERATIONS IN BRAIN TUMOR

In a large number of studies, prognostic significance of changes in mtDNA mutations and/or mtDNA copy number of various cancer types has been investigated. Studies regarding cancers of breast, esophagus, colon, and rectum have shown that mtDNA changes were associated with poor prognosis (44). Lin et al. have shown that mtDNA D-loop somatic mutations correlate well with good prognosis in patients with oral squamous cell carcinoma (45). On the other hand, there are studies that do not show this correlation. For instance, Challen et al. reported that mtDNA D-loop somatic mutations were not common and did not have a predictor for prognosis in head and neck cancers (46). Likely, Lee et al. reported that clinicopathological features of gastric carcinoma and mtDNA changes were not related (47).

Today, there is not much information on the relationship between the prognosis of brain tumors and mtDNA changes. Studies in this area are very limited and the results are still to be discussed. Montanini et al., reported that mtDNA alterations had no effect on the prognosis of patients with glioma tumors and claimed that MtDNA mutations could not be used as an indicator for diagnostic or prognostic evaluations of gliomas (48). Similarly, Vidone et al. suggested that mtDNA genotyping in gliomas might not be an effective tool for predicting prognosis (49). As a result, discussion on the prognostic role of mtDNA changes in brain tumors still continues. Therefore, there is a need for large-scaled studies to explore the prognostic significance of mtDNA changes in brain tumors.

CONCLUSION

MtDNA mutations play an important role in cancer developmental stages, but the mechanisms of cancer development and progression of these mutations are not fully explained.

In the carcinogenetic process, the normal cell needs to be damaged at the genome level in order to transform into an abnormal cell form under the influence of genetic, epigenetic and environmental mutagens. The nDNA and mtDNA play the most important role in the disruption of the metabolic balance between the rate of formation of this damage and the rate of repair. In particular, mutations affecting the structure and function of proteins involved in energy metabolism of the cell are accelerating the process of tumorigenesis. In conclusion, in order to develop targeted therapeutic strategies in brain tumors, understanding the mechanisms leading to mtDNA mutations is crucial.

REFERENCES

17. Pastó A, Bellio C, Piloto G et al. Cancer stem cells from epithelial ovarian cancer patients privilege oxidative phosphorylation, and resist glucose deprivation. Oncotarget 2014; 5:4305-4319. [CrossRef]


33. Gashhi NG, Salehi Z, Madani AH, Dalivandan ST. 4977-bp mitochondrial DNA deletion in infertile patients with varicocele. Andrologia 2014; 46:258-262. [CrossRef]


35. Kato T, Stine OC, McMahon FJ, Crowe RR. Increased levels of a mitochondrial DNA deletion in the brain of patients with bipolar disorder. Biol Psychiatry 1997; 42:871-875. [CrossRef]


