MITOCHONDRIAL MICROSATELLITE GENOMIC INSTABILITY AND BRAF^{V600E} MUTATION IN CENTRAL NERVOUS SYSTEM TUMORS

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UNIVERSITI SAINS MALAYSIA

2020

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

August 2020

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious the Most Merciful, all praises to Him and thanks for His showers of blessings throughout my research work to complete the research successfully. I would like to express my deep and sincere gratitude to my research supervisor, Associate Prof. Dr. Abdul Aziz Mohamed Yusoff, for giving me the opportunity to do research and providing invaluable guidance throughout this research. It was a great privilege and honor to work and study under his guidance. My greatest appreciation goes to Dr. Farizan Ahmad, who has co-supervised me with her constructive comments and suggestions. My appreciation also extends to Prof. Dr. Zamzuri Idris for his advices and motivation.

I am extremely grateful to my parents and siblings for their love, prayers, caring and sacrifices for educating and preparing me for my future. I am extending my heartfelt thanks to my friends, Faten Anis Syairah, Fatin Hilyani and Adibah for their friendship, empathy, great sense of humor and for their constant encouragement throughout my study. Special thanks to my seniors, Zulaikha Nashwa, Wan Salihah, Iman, and other colleagues for their helps and support during my research work. Also, many thanks to all staff of Department of Neurosciences for their assistance and kindness during the completion of my study.

My thanks go to all the people who have supported me to complete the research work directly or indirectly. Finally, I thank the management of School of Medical Sciences, USM for providing all the necessities for me to complete the whole research project.

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LIST OF SYMBOLS

~	Approximately equal
°C	Degree Celsius
%	Percentage
<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to

LIST OF ABBREVIATIONS

AA	Anaplastic astrocytoma
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
D	Aspartic acid
DA	Diffuse astrocytoma
D-loop	Displacement loop
E	Glutamic acid
G	Glysine
GA	Gemistocytic astrocytoma
GBM	Glioblastoma
GPX	Glutathione peroxide
H	Histidine
HSP1	The promoters for transcription of the heavy-strand 1
HS2	The promoters for transcription of the heavy-strand 2
H_2O_2	Hydrogen peroxide
IMM	Inner mitochondrial membrane
IMS	Intermembrane space
K	Lysine
LSP	The promoters for transcription of the light-strand
MTND 1	Mitochondrial NADH dehydrogenase subunit 1
MTND 2	Mitochondrial NADH dehydrogenase subunit 2
MTND 5	Mitochondrial NADH dehydrogenase subunit 5
MTCO 1	Mitochondrially encoded cytochrome C oxidase
MTATP 6	Mitochondrially encoded ATP synthase membrane subunit 6
NA	Not available
OH	The origin of replication of the heavy strand
OL	The origin of light-strand replication
P	Proline
PA	Pilocytic astrocytoma
PXA	Pleomorphic xanthoastrocytomas
R	Arginine

RTK	Receptor tyrosine kinase
S	Serine
SGCA	Subependymal giant cell astrocytoma
T	Threonine
Y	Tyrosine
12S rRNA	12S ribosomal DNA

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KETIDAKSTABILAN GENOMIK MIKROSATELIT MITOKONDRIA DAN MUTASI BRAF^{V600E} DALAM TUMOR SISTEM SARAF PUSAT

ABSTRAK

Tumor sistem saraf pusat dikenali sebagai salah satu kanser merbahaya dan boleh membawa maut di seluruh dunia. Himpunan pelbagai perubahan genetik dalam genom nuklear dan mitokondria dipercayai terlibat dalam pembentukan tumor otak. Ketidakstabilan mikrosatelit mitokondria (mtMSI) adalah satu perubahan pada jujukan berulang dalam genom mitokondria yang sering berlaku dalam beberapa kanser manusia. Sementara itu, BRAF^{V600E} adalah onkogen nuklear yang bermutasi dan kerap dijumpai dalam pelbagai jenis kanser. Walau bagaimanapun, mutasi mtMSI dan BRAF^{V600E} dalam kes tumor otak belum dilaporkan di Malaysia setakat ini. Oleh itu, kajian ini bertujuan untuk menentukan status/tahap mtMSI dan mutasi BRAF^{V600E} dalam satu siri pesakit Melayu yang menghidapi tumor otak, seterusnya untuk mengenalpasti hubungan mereka dengan ciri-ciri klinikopatologi. Perubahan mtMSI dan mutasi BRAF^{V600E} dianalisa dalam 50 sampel tumor otak bersama-sama sampel darah pesakit. Status mtMSI dianalisa menggunakan primer-primer mtMSI yang spesifik dan keputusannya dibandingkan dengan data 'revised Cambridge Reference Sequences' (rCRS). Bagi analisis mutasi BRAF^{V600E}, ujian PCR-RLFP digunakan untuk mengenalpasti turutan variasi, diikuti dengan penjujukan langsung dan dibandingkan menggunakan BLAST dari pangkalan data NCBI. Keputusan menunjukkan lapan perubahan mtMSI dikesan pada D310 dan D16184 dari gelung anjakan (D-loop) (16%). Daripada jumlah ini, satu perubahan C_5TC_4> C_8TC pada D16184 belum pernah dilaporkan dalam pangkalan data MITOMAP yang dikenal pasti dalam kajian ini. Tiada hubungan ditemui antara status mtMSI dan data

klinikopatologi. Selain itu, mutasi $BRAF^{V600E}$ telah dikesan dalam 11 daripada 50 pesakit (22%). Bahkan juga, tiada kolerasi penting antara ciri klinikal dengan mutasi $BRAF^{V600E}$ yang diperhatikan dalam kajian ini. Seterusnya, kolerasi antara status mtMSI dan mutasi $BRAF^{V600E}$ juga dianalisa, namun, tiada hubungan penting dikenalpasti antara kedua-dua mutasi tersebut. Kajian ini memberi gambaran tentang ketidakstabilan genom mitokondria dan mutasi BRAF pesakit tumor otak. Oleh itu, analisa yang lebih terperinci melibatkan sejumlah besar pesakit diperlukan untuk menentukan peranan yang sebenar bagi kedua-dua perubahan genetik berkenaan di dalam kes tumor otak dalam populasi Melayu.

MITOCHONDRIAL MICROSATELLITE GENOMIC INSTABILITY AND BRAF^{V600E} MUTATION IN CENTRAL NERVOUS SYSTEM TUMORS

ABSTRACT

The central nervous system tumor is known as one of the fatal cancers worldwide. The accumulation of multiple genetic alterations of the nuclear and mitochondrial genome is believed to be engaged in brain tumorigenesis. Mitochondrial microsatellite instability (mtMSI) is a change in repetitive sequences of the mitochondrial genome, has been described as a high occurrence in several human cancers. Meanwhile, the BRAF^{V600E} is the most prevalent mutated nuclear oncogene that has been identified in multiple malignancies. Nevertheless, mtMSI and BRAF^{V600E} mutation in brain tumor cases have not been reported in Malaysia, so far. Therefore, this study aims to determine the mtMSI status and BRAF^{V600E} mutation in a series of Malay patients with brain tumors and to evaluate their association with clinicopathological features. The mtMSI alterations and BRAF^{V600E} mutations were examined in a total of 50 paired brain tumor tissues and blood samples. The mtMSI status was analysed using mtMSI specific primers and the results were compared with the revised Cambridge References Sequences (rCRS). For the analysis of the BRAF^{V600E} mutation, the PCR-RLFP assay was used for sequence variation, followed by direct sequencing and aligned using BLAST from the NCBI site. The results revealed eight mtMSI alterations were detected in D310 and D16184 of the displacement loop (D-loop) region (16%). Of these, one alteration C_5TC_4>C_8TC in the D16184 region has not been previously reported in the MITOMAP database identified in this study. No association was found between mtMSI status and clinicopathological data. Additionally, BRAF^{V600E} mutation has been detected in 11 out of 50 patients

(22%). Similarly, no significant association between clinical features with $BRAF^{V600E}$ mutation observed in this study. The correlation between mtMSI status and $BRAF^{V600E}$ mutation also was analysed, however, no association identified between both alterations in all screened patients. This study provides insights into mitochondrial genome instability and BRAF mutation of brain tumor patients. A more detailed analysis involving a large number of patients is needed to establish the exact role of these genetic alterations in brain tumor cases in the Malay population.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Central nervous system tumors (CNS) are relatively common and potentially life-threatening which its incidence is increasing annually in the world. CNS tumors consist of more than 120 distinct types which cover 15% to 20% of all malignancies occurring in childhood and adolescence (Johnson et al., 2017). Besides, it became the second most common cancer in children (11.4%) after leukemia (46.8%) between 2010 and 2012 in Malaysia (Rajagopal et al., 2017).

Nonetheless, molecular characterization of brain tumor is not fully understood at present. Brain tumors, like other solid tumors, are likely to develop due to multiple genetic alterations, including the oncogenes activation and the inactivation of functional tumor suppressor genes (Crespo et al., 2015; Mohamed Yusoff et al., 2015). The comprehension of genomic instability in tumorigenesis could provide a deeper level of understanding cancer as it is believed to contribute to the initiation and progression of tumors.

Microsatellite instability (MSI) is one of the major expressions of genomic instability. Most studies have determined the roles of nuclear MSI in many types of cancer, which are thought to have resulted from a defect during the replication process (Janavicius et al., 2010; Shokal and Sharma, 2012; Yamamoto and Imai, 2015). However, mitochondrial microsatellite instability (mtMSI) which is also considered as a subject to genetic alterations in cancer, is lacking in the understanding of its role in cancer progression. Studies in this field were relatively few, particularly in brain tumors (Kirches et al., 2001; Yeung et al., 2014). Therefore, the findings of this study

could provide a large quantity of data for future research whilst mtMSI could be developed as a useful molecular marker in clinical settings.

Apart from that, the activation of oncogenic mutations has been suggested in their role in brain neoplasms. Almost 80% of all genetic variations correlate to the hot spot T1799A trans-version which induces V600E mutation, consequently converts BRAF into oncogenic kinases (Wan et al., 2004; Cho et al., 2014). $BRAF^{V600E}$ mutation has been identified in several types of cancers including melanoma, thyroid and colorectal cancers (Tran et al., 2011; Hong et al., 2014; Maxwell et al., 2017). Recently, this oncogenic mutation was highly reported in a wide spectrum of brain tumors (Usubalieva et al., 2015; Behling et al., 2016; Bufalo et al., 2018). However, current targeted therapies in brain tumors are still exhausted, hence the understanding of molecular mechanisms based on the genesis and progression of this tumor is essential.

Identification of the molecular mechanisms involved in brain tumors formation with respect to the oncogenes encoded by the nuclear genome and defect in the mitochondrial genome is anticipated in the diagnosis of brain tumors. Thus, a better understanding of these molecular mechanisms is crucial to regulate more effective treatment protocols regarding brain tumor development.

1.2 Rationale of Study

Over the past few years, researches on mitochondrial alterations have been found to contribute in cancer development. Nevertheless, the current research on the analysis of microsatellite instability in the mitochondrial genome is still scarce compared to the nuclear genome. The instabilities of the mitochondrial genome in tumorigenesis need

to be understood clearly, as it could be developed as a reliable molecular marker for cancer development and have the potential for tracking tumor progression. Currently, MSI has been recognised as a useful screening tool for the identification of colorectal cancer. Therefore, the findings of this study were hoped to provide a large quantity of data of mtMSI in brain tumors for future research in order to provide a novel approaches and target in the development of anti-cancer therapeutics for brain tumors.

The development of more selective targeted therapies for $BRAF^{V600E}$ mutation and the design of future clinical trials for primary brain tumors is dependent on the understanding of the molecular genetic lesion that drives its pathogenesis. However, targeting $BRAF^{V600E}$ mutation has been proven to be beneficial for some cancers, but there are still restricted numbers of high-level evidence of the efficacy in primary brain tumors among Malay patients. Thus, this study may provide a better understanding of $BRAF^{V600E}$ mutation related to the pathogenesis of primary brain tumors that could be developed as a molecular indicator which can be used in clinical settings.

1.3 Hypotheses of the Study

In this study, it is hypothesized that:

i. There are occurrences of mtMSI and $BRAF^{V600E}$ mutation in brain tumor patients.
ii. There is an association between clinical parameters and mtMSI in brain tumor patients.
iii. There is an association between clinical parameters and $BRAF^{V600E}$ mutation in brain tumor patients.

1.4 Research Objectives

1.4.1 General objectives

The main aim of this study was to explore the instability changes of mitochondrial genome as well as the alteration of BRAF gene that might explain the aggressive nature of brain tumors.

1.4.2 Specific objectives

i. To evaluate the status of mitochondrial genome microsatellite instability (mtMSI) in patients with brain tumors

ii. To identify the prevalence of $BRAF^{V600E}$ mutation in brain tumor samples.

iii. To analyse the association between mtMSI and $BRAF^{V600E}$ with clinical findings of brain tumors in Malay patients.

iv. To assess the association between mtMSI and $BRAF^{V600E}$ mutation in brain tumor samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Central Nervous System Tumors

2.1.1 Classification of Brain Tumors

The central nervous system (CNS) tumor is referred to as neoplasm originating from the intracranial tissues and meninges caused by abnormal growth (McKinney, 2004). These tumors grow in the areas of the brain, spinal cord and meninges, but brain tumors are known as the largest group. CNS tumors could be fatal as even the benign (non-cancerous) tumor tends to transform into malignant (cancerous) tumors dependent on its location (Goh et al., 2014).

Brain tumors are classified based on World Health Organization (WHO) classification which was first published in the year 2000 assigning grade I to IV (Kleihues et al., 2002). This system is based on the similarity of the tumor cells to normal cells, tumor growth rate, the appearance of necrotic cells in the center of the tumor and the presence of definitive tumor margins as well as vascularity. Grade I and II are considered as slow-growing tumor (benign). Meanwhile, Grade III and IV are rapid-growing tumor (malignant) and actively invading adjacent tissue (Hill et al., 2002). Additionally, WHO classifications have been updated in 2007 (Louis et al., 2007). Classification regarding the distinct histology and clinical behavior is significant for specific clinical presentations, treatment and outcomes for each tumor subtypes (Ostrom et al., 2015).

Based on the comprehensive studies over the past two decades, clarification of the genetic principle of tumorigenesis in the common and several rarer types of brain

tumors create the prospect that may contribute to the brain tumor classification. Therefore, the WHO classification of CNS tumors system has formulated an approach of how the diagnoses of CNS tumor in the molecular stage should be structured. This classification is based on the molecular and histology parameters in many tumor entities (Louis et al., 2016). The classification of CNS tumors according to WHO is presented in Table 1.

Table 2.1 The 2016 WHO Classification of CNS tumors (adapted from Louis et al., 2016)

Types of tumors	Grade
Diffuse astrocytic and oligodendroglial tumors	
Diffuse astrocytoma, IDH-mutant	II
Gemistocytic astrocytoma, IDH-mutant	II
Anaplastic astrocytoma, IDH-mutant	III
Glioblastoma, IDH-wildtype	IV
Glioblastoma, IDH-mutant	IV
Glioblastoma, NOS	IV
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	II
Oligoastrocytoma, NOS	III
Other astrocytic tumors	
Pilocytic astrocytoma	I
Subependymal giant cell astrocytoma	I
Pleormorphic xanthoastrocytoma	II
Ependymal tumors	
Subependymoma	I
Myxopapillary ependymoma	I
Ependymoma	II
Ependymoma, RELA fusion-positive	II or III
Anaplastic ependymoma	III
Neuronal and mixed neuronal-glial tumors	
Dysembryoplastic neuroepithelial tumor	I
Gangliocytoma	I
Ganglioglioma	I
Anaplastic ganglioglioma	III
Embryonal tumors	
Medulloblastomas (all subtypes)	IV
Embryonal tumor with multilayered rosettes, C19MC-altered	IV
Medulloepithelioma	IV
Atypical teratoid/rhabdoid tumor	IV
Tumors of the cranial and paraspinal nerves	
Schwannoma	I
Neurofibroma	I
Perineurioma	I
Malignant peripheral nerve sheath tumor	II, III or IV
Meningiomas	
Meningioma	I
Atypical meningioma	II
Anaplastic (malignant) meningioma	III
Tumors of the sellar region	
Craniopharyngioma	I
Granular cell tumor of the sellar region	I

2.1.2 Epidemiology of Brain Tumors

Generally, the incidence of brain tumors is higher in the West countries compared to the East countries and most common in developed countries than in developing countries (Kalan-Farmanfarma et al., 2019). According to the Global Burden of Disease Study in 2016, the incidence of brain tumors was 330 000 cases and the deaths were 227 000 cases which occurred worldwide. The most top three countries that contribute to this number of cases were China, the United States, and India.

Brain and other CNS tumors are the tenth causes of cancer-related deaths in the United States (CBTRUS, 2018). According to CBTRUS 2018 report, it was estimated that 26,170 new cases of primary brain tumors were predicted to be diagnosed in the United States in the year of 2019 (CBTRUS, 2018). Nonetheless, the incidence of brain tumors from 2008-2012 was 356,858 cases including 117,023 of malignant and 239,835 of non-malignant tumors (Ostrom et al., 2015). Overall, the most frequently reported histology of brain tumors is meningioma, followed by tumors of the pituitary and glioblastoma (Ostrom et al., 2015). Besides, statistics had shown that the incidences of these particular tumors were diagnosed to be higher in males (139,608 cases) than in females (116,605 cases) in 2012 (Patel et al., 2019). The distribution of all primary CNS tumors from 2008-2012 in the United States shown in Figure 2.1.

In the United Kingdom, nearly 9000 patients are diagnosed with primary brain tumors each year. Furthermore, over 102,000 people are living with primary brain tumors, with only 14% of patients with primary brain tumors are alive after 10 years after diagnosis (Kurian et al., 2018). In Japan, the second major cause of deaths caused by malignant tumors at the age of 0-14 years was brain tumors. Nevertheless, brain tumors were the main factor in 2011 as the fatality rate of pediatric brain tumors

increased. Additionally, there were 84 deaths reported among children aged 0-14 years with brain tumors in 2013 (Nishi, 2014).

Among 2,589,448 populations in five cities in China, 636 people have been diagnosed with primary brain tumors which were found to be 31.16% of malignant and 68.94% of benign tumors between 2005 and 2006. The prevalence rate of primary brain tumors is higher in female compared to the male population. Additionally, glioma appeared to be common in the youngest age (0-19 years) while pituitary adenomas and glioma frequently occurred in patients age from 20 to 59 years (Jiang et al., 2011). However, the number of cases diagnosed in China increased between 96,980 to 119,885 cases in 2016 (Patel et al., 2019).

The prevalence of primary malignant brain tumors in East India occurred for both males and females with ratio of 2.3:1 and at the age of 20-60 years. The most common broad histological type is astrocytic tumors. Overall, the frequency of primary malignant brain tumors in East India cover up to 1% of all malignancies which involve mostly young and middle-age patients (Krishnatreya et al., 2014).

The incidence of brain tumors covers approximately 1.95% of all malignancies in Malaysia which shown an increasing trend every year among adults and children (Goh et al., 2014). Back in the year of 2016, it was detected that 598 incidence and nearly 431 deaths occurred in Malaysia (Patel et al., 2019). In Sarawak, it was calculated that the crude rate of brain tumors was 4.6 per 100,000 population between 2009 to 2012. The most common brain tumor was meningioma (32.3%) followed by astrocytoma (19.4%) (Goh et al., 2014). A cohort population-based study in the east coast of Malaysia revealed that the most prevalent primary brain tumor occurred in adult was meningioma tumor (32.7%) followed by glioblastoma (7.8%) (Md Dzali et

al., 2017). In that same study, meningioma has been recognized as the most frequent among elders.

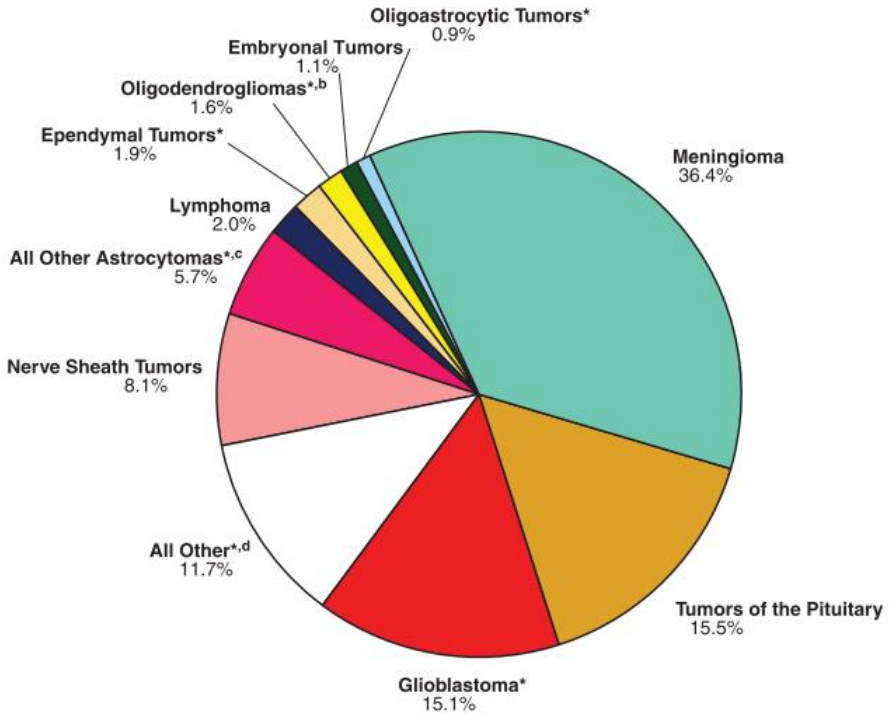

Figure 2.1 Distribution of primary brain and CNS Tumors by CBTRUS Histology Groupings and Histology according to CBTRUS Statistical Report: NPCR and SEER, 2008-2012. This figure adapted from (Patel et al., 2019).

2.2 Mitochondria and Tumorigenesis

2.2.1 Mitochondria

Mitochondria are ubiquitous intracellular organelles which can be found in most eukaryotic organisms that accounted for 25% of the cytoplasm volume. It has a variable length with a transverse diameter of 0.1 to 0.5 µM (Mohamed Yusoff et al., 2015). This organelle has an oblong or ovoid shape and its unique characteristics can be seen by electron microscopy (Medeiros, 2008). Generally, mitochondria have four compartments which are the outer membrane, inner membrane, intermediate space and matrix (Mohamed Yusoff et al., 2015).

The mitochondrial outer and inner membranes consist of a phospholipid bilayer and proteins. The outer membrane is highly permeable which allowing molecules <10,000 Da to diffuse through a special protein channel referred to as mitochondrial porin or voltage-dependent anion-selective channel (VDAC) (De Pinto and Palmieri, 1992; Mannella et al., 1992). In contrast, the inner membrane is impermeable to most ions and molecules. This membrane is essential in synthesis of ATP and electron transport chain. Enzymes, proteins, and peptides including chaperones, DNA polymerase, ribosomes, mtDNA, mRNAs, and tRNAs are located in the mitochondrial matrix (Mohamed Yusoff et al., 2015).

2.2.2 Mitochondrial Function

Mitochondria are best known as the powerhouse of cellular energy in the adenosine triphosphate (ATP) form via oxidative phosphorylation (OXPHOS) system (Lu et al., 2009). This ATP production is done by using the energy produced during the electron transport chain and occurs through chemiosmosis. OXPHOS system is carried out by five multi-subunit protein complexes which are embedded in the inner membrane of

mitochondria (Shen et al., 2010). Complex I – IV are involved in transferring electrons and oxygen molecules act as the final electron acceptor. By transferring the electron from one electron acceptor to another, some energy is released and is used in chemiosmosis to produce ATP from ADP and inorganic phosphate which catalysed by Complex V (ATP synthase).

Apart from that, mitochondria are a known contributor for reactive oxygen species (ROS) generation, metabolic homeostasis and responsible for initiation and execution of apoptosis (Turnbull et al., 2010; Indran et al., 2011; Sullivan and Chandel, 2014). Moreover, mitochondria also serve as a platform for producing biosynthesis building blocks (Zong et al., 2016). Thus, roles of mitochondria are critical in several cellular processes that are significant for cell metabolism, growth, and survival.

2.2.3 Human Mitochondrial Genome

Mitochondria contain their own genome with all the fundamental machinery needed for their expression (Schon et al., 2012). Human mitochondrial DNA (mtDNA) is maternally inherited and was first discovered in 1963 (Nass and Nass, 2004; Schon et al., 2012). The first sequence of the mitochondrial genome was available in 1981 and was subsequently revised in 1999 (Anderson et al., 1981; Andrews et al., 1999). mtDNA was presented in the form of close-double stranded circular DNA of 16,569 nucleotide pairs (Taanman, 1999).

The organization of human mtDNA is extremely compact with genes and some of the genes are overlapped as shown in Figure 2.2 (Mohamed Yusoff et al., 2015). The non-coding region is the control region of mtDNA including the displacement loop (D-loop) region that occupied 1122 bp in the mitochondrial genome (Clayton, 2000). Hypervariable 1 (HV1), hypervariable 2 (HV2) and hypervariable 3 (HV3) that

are rich in polymorphisms are also located in the control region (Tsutsumi et al., 2006). There are two distinct strands of mtDNA namely as a heavy strand (H-strand) and light strand (L-strand) according to their buoyant density in alkaline caesium chloride gradient (Montoya et al., 1982). D-loop region contains the origin for the replication of H-strand synthesis and two promoters for the transcription process. mtDNA consists of 37 genes coding for 2 rRNAs, 22 tRNAs and 13 polypeptides which are responsible for the OXPHOS system (Schon et al., 2012). The H-strand is rich in cytosine, consists of 2 rRNAs, 14 tRNAs, and 12 polypeptides whilst the L-strand is rich in guanine and composed of 8 tRNAs and only one polypeptide (Mohamed Yusoff et al., 2015).

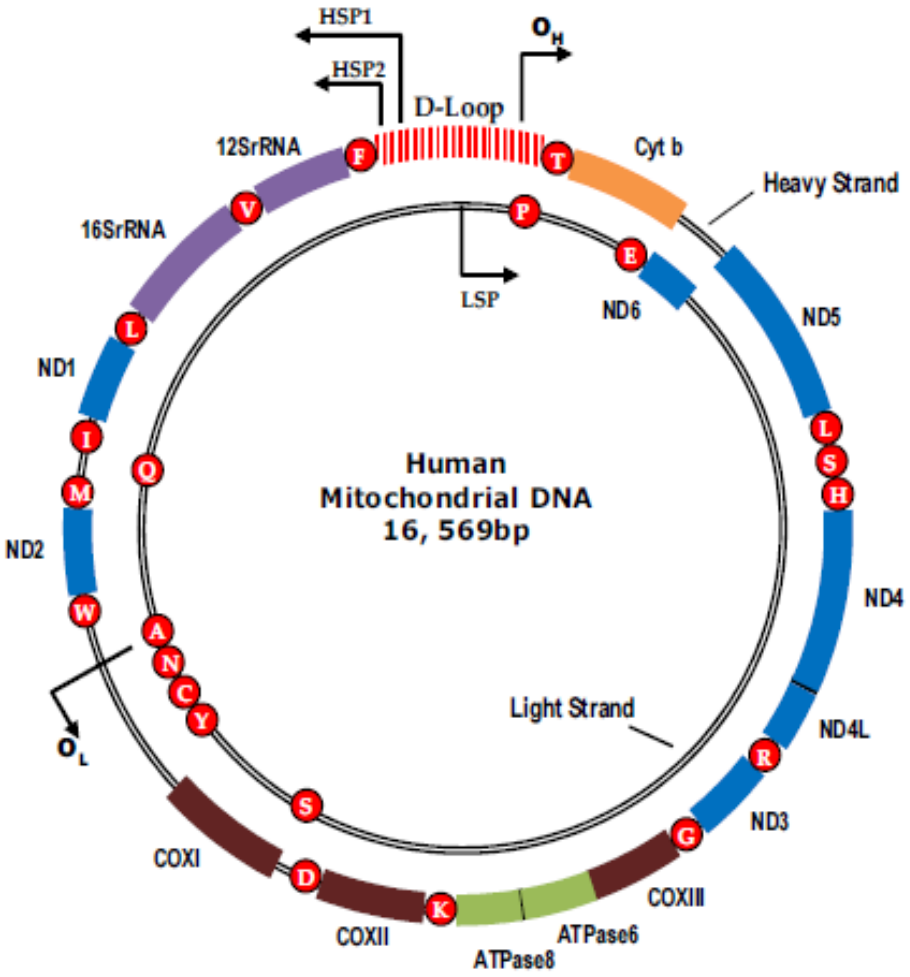

Figure 2.2 The human mitochondrial genome

Abbreviation: Y, Tyrosine; S, Serine; D, Aspartic acid; K, Lysine; G, Glycine; R, Arginine; H, Histidine; E, Glutamic acid; T, Threonine; P, Proline. The displacement loop (D-loop), or non-coding control region contains the promoters for transcription of the L (LSP) and H strands (HSP1 and HS2) and the origin of replication of the H strand (OH). OL, the origin of light-strand replication. This figure was adapted from (Mohamed Yusoff et al., 2015).

2.2.4 Alteration of Mitochondrial Functions and Tumorigenesis

Normal differentiated cells produce energy via the OXPHOS system that is essential for cellular processes (Hsu et al., 2016). Nonetheless, most cancer cells produce energy through glycolysis even in the existence of oxygen, termed as 'aerobic glycolysis'. This phenomenon has been observed by Otto Warburg in 1926 which encouraged him to propose that the alteration in mitochondrial respiration are the fundamental basis for aerobic glycolysis and tumor (Koppenol et al., 2011; Otto, 2016). Hence, researchers have begun to investigate the mitochondrial alterations in numerous cancers afterward. The previous study demonstrated that the restriction of the OXPHOS function through the cancer cells incubation with oligomycin led to a rapid increase of aerobic glycolysis, indicating that the impairment of mitochondrial bioenergetic function caused the tumor cells to become glycolytic (López-Armada et al., 2013). Due to this evidence, tumor cells have upregulated glycolysis as an adaptational mechanism to support their biosynthetic requirements rather than the mitochondrial respiratory system, suggesting the partial defect in mitochondria (Lu et al., 2009).

Reactive oxygen species (ROS) such as peroxide, superoxide, and hydrogen peroxide are the natural by-product of the mitochondrial respiratory chain when some of the transferring electrons are instead leaked out of the chain (Lu et al., 2009). Lower production of ROS is crucial in regulating cellular signaling, proliferation of normal cells, host defense and gene expression (Sullivan and Chandel, 2014; Nita and Grzybowski, 2016). Nevertheless, the uncontrolled ROS generation is capable of damaging cellular components including the DNA, proteins, and lipids, consequently caused the dysfunction of the cell (Lu et al., 2009; Sullivan and Chandel, 2014).

Furthermore, elevated levels of ROS production have long been recognised as a hallmark in many tumors and cancer cell lines (Szatrowski and Nathan, 1991). Based on the observation by Pelicano et al. (2009), the perturbation of the mitochondrial respiratory chain generates subclones of cells with an increase of ROS, active proliferation, increased cellular motility and invasive behaviours in vivo and in vitro of breast cancer cells. Therefore, it is suggested that stimulation of ROS is believed to contributes to genomic instability promoting tumorigenesis.

In addition, ROS can also lead to the degradation of the mitochondrial genome (Harman, 1988; Singh, 2006). This is due to the continuous exposure of ROS may induce oxidative stress which could lead to mtDNA alterations as the location of mtDNA is near the ROS production site (Figure 2.3). Particularly, mtDNA has a lack of introns and protective histones besides possessing a defective DNA repair system (Zong et al., 2016). Due to these reasons, mtDNA is susceptible to the high rate of mutations (Richter et al., 1988).

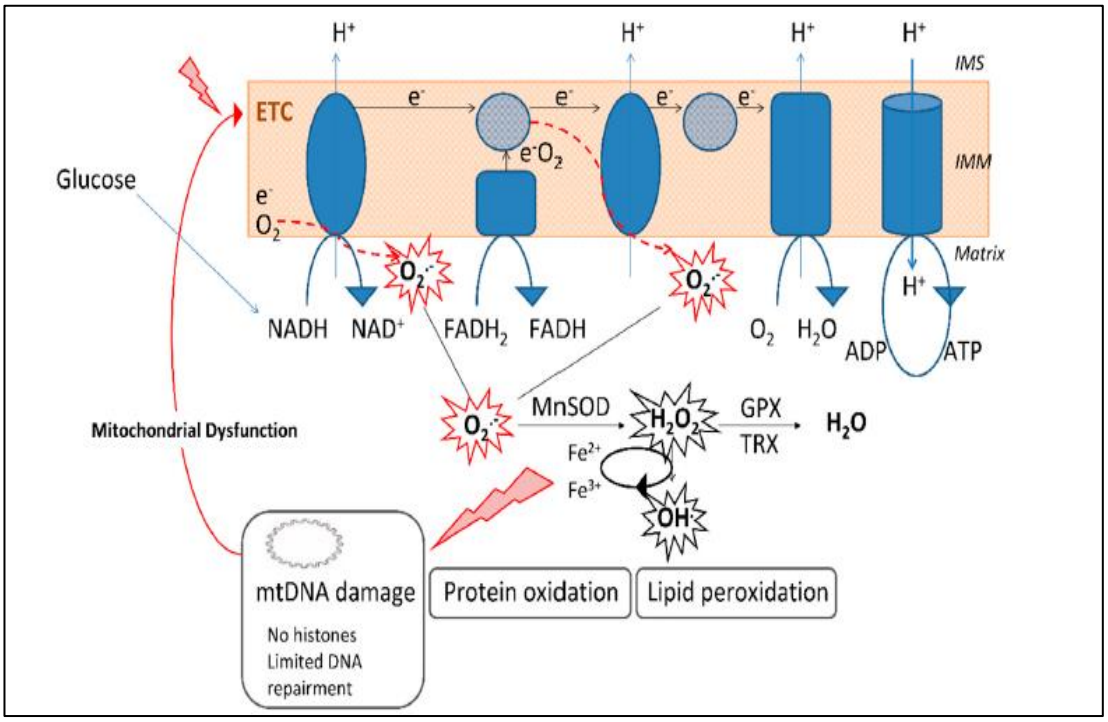

Figure 2.3 Mitochondrial superoxide (O_2•−) production by electron transport chain (ETC)

The elevated levels of O_2•− induce damage to macromolecules, including lipids, proteins, and nucleic acids, and promote mitochondrial dysfunction. Absence of histones in mitochondrial DNA (mtDNA) and limited DNA repair mechanisms make mitochondria highly susceptible to DNA damage induced by O_2•−.
Abbreviation: ADP, Adenoxine diphosphate; ATP, Adenosine triphosphate; GPX, Glutathione peroxidase; H_2O_2, Hydrogen peroxide; IMM, Inner mitochondrial membrane; IMS, Intermembrane space. This figure was adapted from (Burgos-Morón et al., 2019).

2.2.5 Mitochondrial DNA Mutations in Cancer

Notably, many solid tumors exhibit multiple alterations in the nuclear genome, but in recent years, researchers have now focused on the basis of mitochondrial DNA mutations because of its high frequency and wide distribution in carcinogenesis (Lee et al., 2010; Cormio et al., 2015)l. Previously, Larman and colleagues (2012) analysed 226 patients with five different cancer types; acute myeloid leukemia, glioblastoma, ovarian, colon, and rectal adenocarcinoma. From the findings, they found that the prevalence of somatic mtDNA mutations occur at 13% to 63% across the five cancer types, suggesting that mtDNA mutations as a standard mechanism for damaging metabolic pathways in tumorigenesis (Larman et al., 2012). Although the role and mechanism of mtDNA mutations in cancer progression is still controversial and unclear, there is still a possibility that mitochondria could be a potential biomarker in tumorigenesis (Parr et al., 2006; Cruz-Bermúdez et al., 2017).

Thus far, mitochondrial dysfunction caused by mtDNA mutations has been widely described in various cancer types such as lung, brain, thyroid, breast and colorectal cancers (Tong et al., 2003; Ma et al., 2010; Chen, 2012; Dai et al., 2013; Mohamed Yusoff, 2015; Gao et al., 2016). Despite that, studies of molecular alteration in the mitochondrial genome, for examples, large-scale deletions, point mutations, copy number changes, insertions, and microsatellite instability render a better understanding of mitochondrial dysfunction and tumorigenesis (Dai et al., 2013; Tipirisetti et al., 2013; Mohamed Yusoff et al., 2019). Over 30 mutations and sequential variations can be found in the mtDNA database; Mitomap (http://www.mitomap.org). These findings altogether indicate that mtDNA alterations have become a target in cancer research.

2.2.6 Mitochondrial Microsatellite Instability and Tumorigenesis

Microsatellites are short tandem repeats of DNA sequences ranging from one to six in length (Kelkar et al., 2010). These repeats are scattered throughout the genome in various length from one individual to another due to the variable number of tandem repeats at each locus. However, the alterations in these elements are defined as microsatellite instability (MSI) which occurs due to insertions or deletions (indels) during DNA replication and the inefficiency of the DNA mismatch repair system to amend these errors (Ashtiani et al., 2013; Geurts-Giele et al., 2015). MSI was first reported in the nuclear genome of patients with colorectal cancer and later discovered in other cancers (Thibodeau et al., 1993; Viana-Pereira et al., 2011; Lee, Lee, Kim & Hwang et al., 2015; Shahsiah et al., 2017). Nevertheless, the role of MSI in the mitochondrial genome (mtMSI) which also believes may contribute to carcinogenesis, is still scarce and insufficiently characterized.

An increase of mitochondrial instability has been found in various tumor types, hence, this event is considered as a key molecular step of mutations in cancer progression. The previous study by Kim et al. (2006) had identified the high frequency of mtMSI in colorectal cancer stroma among Korean patients. Another research based on primary and metastatic colorectal cancer tissues also reported that the mtMSI is shown to be greater in lymph node metastases compared to the primary tumor and distant metastases (Kleist et al., 2017).

In addition, the prevalence of mtMSI was previously reported to be ranging from 10.2% to 62% of gastric cancer patients (Lee and Kim, 2014). A report was done by Pavicic and colleagues (2009) also pointed out that mtMSI event had occurred in breast cancer tissues which showed the allele changes (CA) in 24 out of 94 cases.

In 2005, Wang and team examined the occurrence of mtMSI in different types of female cancers (cervical, endometrial, ovarian and breast) and a follow up study was done in 2006 (Wang et al., 2005, 2006). Based on their findings, mtMSI event has been proposed as a potential marker in cancer progression.

In brain tumor cases, only a few studies examined the occurrences of mtMSI alterations. The first study was conducted by Kirches et al. (1999) when they found a high frequency of mtDNA sequence variants in 12 astrocytic brain tumors. Two years later, they also reported a high prevalence of mtMSI in glioblastoma samples (15 out of 17) using laser microdissection and PCR technique (Kirches et al., 2001). In 2004, Vega and colleagues observed the instability in 27 (39.1%) of primary CNS tumors compared to the corresponding blood samples. Thus, it is proposed that mtMSI alterations may play a role in the development of brain neoplasms.

2.3 BRAF Gene

2.3.1 BRAF and MAPK/ERK Pathway

Mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway has emerged as a critical route for the regulation of inter- and intra-cellular communication including cell proliferation, differentiation, and survival (Cantwell-dorris et al., 2011). Specifically, the most dominant activator in MAPK/ERK signaling cascade is the v-RAF murine sarcoma viral oncogenes homolog B1 (BRAF) gene (Cantwell-dorris et al., 2011).

BRAF is one of the RAF family which encodes a serine/threonine-protein kinase and possessed the highest basal kinase (Behling et al., 2016). This gene located on chromosome 7 (7q34) consists of 18 exons. BRAF acts as a signal mediator in this pathway from the extracellular environment to the nucleus (Myung et al., 2012). The extracellular signals, for instance, cytokines, hormones, and other growth factors bind with their receptors, results in the activation of the RAS G-protein. This results in dimerized BRAF, subsequently activates MEK which, in turn, activates ERK and consequently activates downstream transcription factors to stimulate various biochemical processes (Cantwell-dorris et al., 2011).

The regulation of MAPK/ERK pathway is significant for homeostasis maintenance in response to extracellular signaling. It has been proposed that the hyperactivation of this pathway leads to cell-cycle arrest whereas aberrant signaling of the pathway may contribute to tumorigenesis (Davies et al., 2002). Also, alteration of MAPK/ERK pathway has been reported in ~30% of human cancers and previous data has revealed that BRAF mutation was found in 7% of cancers (Davies et al., 2002;

Garnett and Marais, 2004). Therefore, BRAF mutation has been identified as one of the important oncogenes involved in the alteration of MAPK/ERK pathway.

2.3.2 $BRAF^{V600E}$ Mutation and Tumorigenesis

In recent years, considerable efforts have been devoted to investigating the alterations of BRAF gene in various human cancers since the first investigation by Davies et al. (2002). They found that the high prevalence of BRAF mutation was frequent in many tumors (Davies et al., 2002). Since then, many researchers focused on the significance of BRAF alteration in cancer development and progression. The most common alteration observed in this oncogene is $BRAF^{V600E}$ mutation which substitutes the thymine to adenine at position 1799 of the gene (T1799A), subsequently, change of GTG to GAG at codon 600. This results in the exchange of the amino acid of valine to glutamic acid at position 600 (V600E) (Inumaru et al., 2014). Of this, $BRAF^{V600E}$ protein constitutively activates the serine/threonine tyrosine kinase and its downstream protein kinase signaling cascade consequently induce oncogenesis (Figure 2.4) (Cantwell-Dorris et al., 2011).

$BRAF^{V600E}$ mutation has been identified in several types of human cancers including melanoma, thyroid, colorectal, and brain (Davies et al., 2002; Tran et al., 2011; Myung et al., 2012; Hong et al., 2014). This oncogenic mutation is a well-characterized target in human melanoma, which has been detected up to 66% of primary cases (Davies et al., 2002). In 2014, Hong et al. (2014) reported an increase in BRAF-associated papillary thyroid cancers patients from 62.2% to 73.7% in the past two decades in Korea.

Moreover, researchers have extensively examined the occurrences of $BRAF^{V600E}$ mutation in brain neoplasms (Dougherty et al., 2010; Schindler et al., 2011;

Donson et al., 2014; Kieran, 2014; Behling et al., 2016; Schreck et al., 2018). According to Myung et al. (2012), $BRAF^{V600E}$ mutation was identified in 36 cases of CNS tumors including pleomorphic xanthoastrocytoma, gangliogliomas, pilocytic astrocytomas, malignant tumors, anaplastic astrocytomas, glioblastomas, and oligodendrogliomas. In epitheloid glioblastomas, the $BRAF^{V600E}$ mutation was found in 50% of the cases (Kleinschmidt-DeMasters et al., 2013).

Nevertheless, $BRAF^{V600E}$ was frequently detected in pediatric brain tumors compared to adults. This mutation is less common in adult gliomas with only 1-2% in glioblastomas and 2-5% in low-grade gliomas (Behling and Schittenhelm, 2019). Recently, a high frequency of $BRAF^{V600E}$ has been investigated in pediatric brain tumors including gangliogliomas, low-grade gliomas, epitheloid glioblastomas (Dougherty et al., 2010; Kleinschmidt-DeMasters et al., 2013; Donson et al., 2014). Donson et al. (2014) determined the high percentage of $BRAF^{V600E}$ mutation in pediatric gangliogliomas which occurred in five of 13 (38%) of the cases.

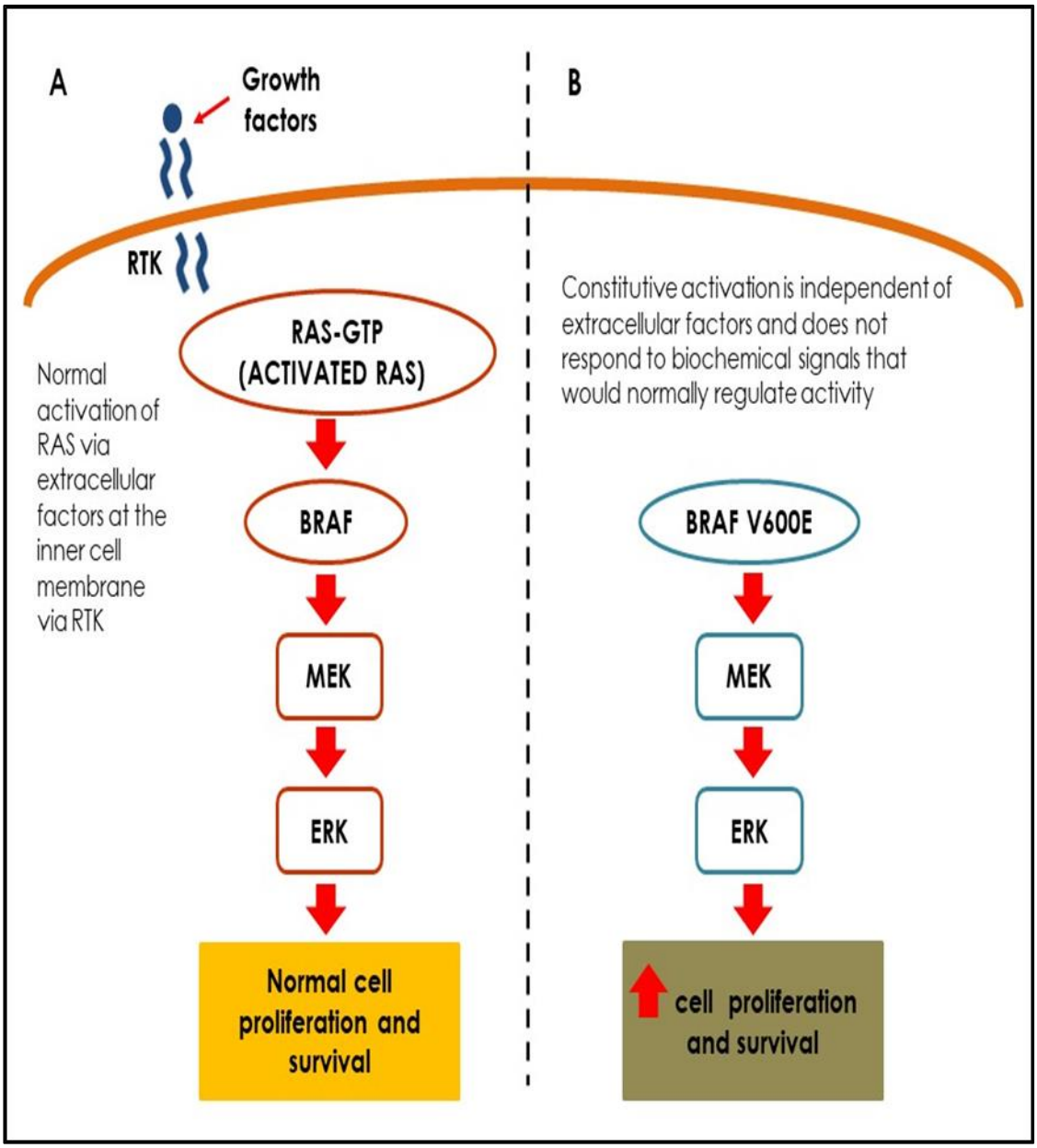

Figure 2.4 Oncogenic BRAF signaling pathway, A: MAPK/ERK pathway, B: Oncogenic $BRAF^{V600E}$ signaling
Abbreviation: RTK, receptor tyrosine kinase