UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

MITOCHONDRIAL DYSFUNCTION IN CENTRAL NERVOUS SYSTEM TUMORS: MICROSATELLITE GENOMIC INSTABILITY AND ALTERED RESPIRATORY CHIAN EXPRESSION

PENYELIDIK

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Progress Summary

Project Progress : 100.00%

Budget Used : 99.43%

Human Capital :100.00%

Current Outcome

Type	Number
Activities	5
Publication	2
Exhibition	0
Intellectual Property	0
Product	0

Milestone

No.	Description	Project Completion Contribution	Expected Completion Date	Completed Percentage	Actual Completion Date	Contributed Progress
1	MILESTONE 1 - i) DNA extraction, ii) mtDNA microsatellite instability by HRMA	20	30/06/2015	100	30/11/2016	20.00%
2	MILESTONE 2 - mtDNA copy number analysis by RT-PCR	20	31/12/2015	100	28/02/2017	20.00%
3	MILESTONE 3 - i) immuno-histochemistry	40	30/04/2016	100	25/02/2017	40.00%
4	MILESTONE 4 - i) statistics & data analysis & ii) final report writing	20	30/06/2016	100	20/12/2017	20.00%
	Overall Progress					100.00%

Research Abstract

Abstract

Background: Cancer progression involves the accumulation of various genetic alterations, which are present both in the nuclear as well as in the mitochondrial genomes. Microsatellite instability (MSI) is a sensitive indicator of genomic stability. Microsatellites are short tandem repeats of sequence motifs, usually ranging from one to five DNA bases. MSI is defined by changes of microsatellite length (due to either insertions or deletions of repetitive noncoding DNA sequences), within tumor DNA compared to that of normal tissue. Our previous study has identified a high prevalence of somatic mtDNA alterations in the mtDNA D-loop of mtDNA in the group of brain tumor patients. To extend these findings, we analyzed the occurrence of mitochondrial microsatellite instability (mtMSI) in other regions of mtDNA in brain tumor and determined the relationship between mtMSI and mtDNA copy number alterations in brain tumor

Method: Brain tumor tissues were collected from 45 patients along with the corresponding blood samples. The segments of mtDNA encompassing the candidate microsatellite regions were analyzed by polymerase chain reaction-High Resolution Melting analysis (PCR-HRMA) using mtDNA-specific primers and later were confirmed by direct DNA sequencing. Furthermore, the mtDNA content was analyzed by using a quantitative real time PCR method.

Results: The mtMSI were observed in 51.1% (23 out of 45) of our brain tumor patients. Moreover, we found that mtDNA copy number was significantly reduced in tumor tissues (13.49±9.32) compared to corresponding blood samples (36.65±9.32). Our study also revealed that 62.2% of our patients (28 out of 45) were detected to have the ND3 10398A>G mutation and no specific alterations of OXPHOS complexes were observed in all analyzed brain tumors samples.

Conclusion: We were able to trace the instability changes that occurred in mitochondrial genome in Malaysian brain tumor patients. Microsatellite instability mutation could be the important event in the progression of brain tumor, especially in the association with the reduced of mtDNA copy number. Therefore, we propose that mtMSI and reduced mtDNA content may play an essential role in the pathogenesis of brain tumors among Malaysian patients.

Summary of Research Findings

45 brain tumor samples have been screened for mitochondrial DNA microsatellite instability (mtMSI) at twelve repeat sites using the PCR-HRM method. We have detected mtMSI in 23 out of the 45 cases of brain tumor (51.1%). Among the 23 cases of brain tumor with mtMSI, 14 occurred in one locus and 9 in 2 loci. mtMSI affected mostly in the D-loop region. All these mtMSI are detected need to be verified by DNA sequencing. In addition, we found that mtDNA copy number was significantly reduced in tumor tissues (13.49±9.32) compared to corresponding blood samples (36.65±9.32).No specific alterations of OXPHOS complexes were observed in all analyzed brain tumors samples (from 45 samples) by immunohistochemical assay.

For 21 cases meningioma, 10 patients (47.6%) were detected to harbour a total of 27 somatic mtDNA D-loop mutations. Most of these mtDNA mutations were identified in the hypervariable segment II (HVII) (40.7%) with 33.3% were located mainly in the conserved sequence block II of the D310 sequence. Furthermore, 58 different germ line variations were observed at 21 nucleotide positions.

The ND3 10398A>G mutations were observed in 62.2% (28 of 45) of our patients, in which 23.8% of these mutations were heterozygous mutations and the rest were homozygous mutations. Presence of the ND3 10398A>G mutation did not show significantly correlation with any of the evaluated parameters such as patients age, gender, race and histological brain tumor types.

Problems/Constrains if Any

Time constrains due to broken-down of equipment (HRM apparatus)

Recommendation By Project Leader

Overview

Project Title

Mitochondrial Dysfunction in Central Nervous System Tumors: Microsatellite Genomic Instability and Altered Respiratory Chain Expression

Progress Report Details