UNIVERSITI SAINS MALAYSIA
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EFFECTS OF CHRONIC HYPOXIA ON SEMAPHORIN CLASS 3 EXPRESSIONS IN HUMAN HIPPOCAMPAL NEURONS AND ASTROCYTES

PENYELIDIK

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Progress Summary

Project Progress : 100.00%
Budget Used : 96.51%
Human Capital :33.33%

Current Outcome

Type	Number
Activities	6
Publication	0
Exhibition	0
Intellectual Property	0
Product	0

Milestone

No.	Description	Project Completion Contribution	Expected Completion Date	Completed Percentage	Actual Completion Date	Contributed Progress
1	To study the effects of chronic hypoxia on axonal and dendritic morphology	20	30/04/2014	100	30/04/2014	20.00%
2	Detection of morphological changes in astrocytes and neurons by Golgi staining	20	31/12/2014	100	31/12/2014	20.00%
3	Effect of hypoxia on proliferation of hippocampal and astrocytes cell lines	20	31/08/2015	100	31/08/2015	20.00%
4	Effect of hypoxia on expression of all Sema 3 class proteins (SemaA-G).	20	31/03/2016	100	31/03/2016	20.00%
5	Sema Class 3 family (Sema A to G) & Downstream effectors like Rho, RAC, GTPases, P53	10	31/10/2016	100	31/10/2016	10.00%
6	miRNA expression profiling in hypoxia-treated hippocampal and astrocytes cell lines	10	28/02/2017	100	28/02/2017	10.00%
	Overall Progress					100.00%

Research Abstract

Brain requires a continuous supply of oxygen to perform its normal function. Being the largest consumer of oxygen, it is especially sensitive to hypoxia, a condition in which brain receives reduced oxygen. Several studies have shown that injury to the brain due to loss of oxygen triggers memory loss and causes learning and memory deficits. Despite many animal studies reported that hypoxia caused neuronal damage in hippocampus which could deficits learning and memory, but the exact damage caused by chronic hypoxia on human hippocampal astrocyte has not been analysed yet. Our aim for this study is to understand the characterization of human hippocampal astrocyte following chronic hypoxia exposure and how the changes varied according to different concentration level of oxygen. For the laboratory work, we were using human hippocampal astrocytes cell line and also hypoxia chamber in mimicking the hypoxic condition. Based on the preliminary screening, almost 80% of cell death occurred after 20 min of hypoxia exposure at 3% Oxygen and 60% cell death occurred in 15 min of 3% Oxygen. From the data gained, 15 minutes was chosen as the time point and the cells were exposed to different oxygen percentage (15%, 10%, 5% and 3%). Analysis from Trypan blue viability assay showed about 15% of cells were dead in 15% oxygen, 25% dead cells in 10% oxygen, 48% dead cells in 5% oxygen and 65% dead cells in 3% oxygen. For the immunofluorescence Assay, a reliable marker GFAP was used in order to portray the architecture and morphology of astrocytes cells. Fluorescence scanning microscope revealed a filamentous and clear nuclear appearance in a control. In contrast, the rupture nuclei along with no rigid structure of cell were displayed in chronic hypoxia group, the 3% oxygen exposure. The control and hypoxia cells also were stained with the Annexin V FITC and then observed under a fluorescence microscope. Cultured astrocytes after hypoxia showed higher expression of nuclei but not in control. Merged between PI and FITC clearly showed the differences of nuclei expression between the control and hypoxia exposed group. Along with that, the HIF-1 staining was performed to confirm the cell death due to hypoxia exposure. Based on the fluorescence microscope viewed, there are dramatic expression of HIF-1 was displayed in exposed astrocyte cells compare to the control. For the molecular analysis, we chose several genes such as GAPDH, GFAP, HIF-1α and Bcl2 and ran RT-PCR. General view of human hippocampal astrocyte genomic response to hypoxia was obtained.
Key words: Hypoxia, human hippocampal astrocytes, oxygen percentage, cell viability, morphological changes, FITC Annexin V staining, GFAP marker, HIF-1α, GAPDH, Bcl2.

Summary of Research Findings

Experiments are going on.
We are optimizing cell lines maintaince.
We already set the chamber for maintaining hypoxia at 3%. We spent almost three months to optimize hypoxia chamber.
Now it is working in good condition.
Morphological staining procedures are being optimized now.
we observed SEMA receptor expression following hypoxia.

28/3/2017 (Progress)
1. Culturing human hippocampal astrocyte cells line in astrocyte media containing essential supplements for cells to growth. The cells start to accumulate and clump together.After several days, processes of cells start to form and keeps continue to develop more branches. The configuration of star-like cells start to appear after 10-14 days. The cells reach 90% confluent and ready for experiment.
2. The cells viability after expose cells to chronic hypoxia(3%oxygen) shows that the percentage changes in live hippocampal astrocytes cells decreasing as the time increasing.While for cell viability after expose cells to different oxygen level in 15minutes shows the higher percentage oxygen that has being expose to cells the higher the cell viability.
3. The percentage of cell death(FITC) in human hippocampal astrocytes increasing,as the oxygen level that has been expose to the cell decreasing.
4. Staining human hippocampal astrocyte cell line using the GFAP (Primary antibody) and Antigoat (Secondary antibody). Fluorescence scanning microscope revealed a filamentous and clear nuclear appearance in a control. In contrast, the rupture nuclei along with no rigid structure of cell were displayed in chronic hypoxia group, the 3% oxygen exposure. HIF staining human hippocampal astrocyte cell line. The green image stained cell showed the reactive astrocyte or hypoxia exposed astrocyte expressed higher intensity of HIF. Meanwhile, in control, the unexposed cell showed low intensity of HIF expression.

Problems/Constrains if Any

Recommendation By Project Leader

Results of experiment can proceed to new fundamental objectives that can look at more cascades associate with hypoxia especially in a human blood brain barrier model costing RM300,000.00