UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

MECHANISM OF HYPOXIC PRECONDITION IN REGULATING NEURAL STEM CELL MIGRATORY CAPACITY TO BRAIN TUMOR SITES IN VITRO

PENYELIDIK

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Progress Summary

Project Progress : 100.00%
Budget Used : 99.99%
Human Capital :100.00%

Current Outcome

Type	Number
Activities	4
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	Completion of methodologies optimization	16.67	31/05/2014	100	31/05/2014	16.67%
2	Completion of first part of the laboratory work - hypoxic cell culture and cell proliferation + viability test	16.67	30/11/2014	100	30/11/2014	16.67%
3	Completion of second part of the laboratory work - cell migration assay	16.67	30/11/2015	100	30/11/2015	16.67%
4	Completion of third part of the laboratory work – chracterization, differentiation and chromosomal stability assay	16.67	30/11/2016	100	30/11/2016	16.67%
5	Completion of data analysis	16.67	28/02/2017	100	28/02/2017	16.67%
6	Completion of report writing, presentation and publication	16.65	31/05/2017	100	30/05/2017	16.65%
	Overall Progress					100.00%

Research Abstract

Neural stem cells (NSCs) are primitive cells which are capable to self-renew or differentiate into various matured neuronal cells. These cells reside in subventricular zone of adult mammalian brain, a specialized niche that maintains the pluripotent stem cell characteristics. Recently, researchers reported that NSCs showed a preferential migration to brain tumor sites in vivo, opening the opportunity to use these cells as special delivery agent to deliver anti-cancer drugs to cancer sites directly to avoid unnecessary side effect on adjacent normal health cells. Here, we propose to evaluate the migration of NSC to glioma cells in vitro carrying anti-cancer drugs used in clinical (Tamoxiden and Temozolomide) and also the natural anti-cancer drugs extracted from medicinal plant - Quercus infectoria (QI). Besides, in this study, we also proposed to optimize the migration capacity via hypoxic preconditioning method. To do this, NSC line will be cultured under physiological oxygen levels (2% oxygen; termed hypoxia) and the resulting changes in NSC gene will be investigated using real time PCR and western blot and compared to the cells cultured under atmospheric oxygen (21% oxygen; termed normoxia). We found that hypoxic NSC showed increased HIF and CXCR4 expression. After that, QI was extracted using soxhlet technique with 100% (QI-100%) or 70% (QI-70%) methanol solvent. IC50 of QI-100% and QI-70% on human NSC line (H9-hNSC) and human glioma cell line (DBTRG-05MG) were determined using MTT assay. Both QI-100% and QI-70% showed anti-proliferative properties against DBTRG-05MG at IC50, but not on H9-hNSC. Free radicals scavenging activity (DPPH solution test) in the QI-100% and QI-70% were found to be 97.3±1.4% and 96.4±3.7%, respectively. Concentration of tannic and gallic acids measured using HPLC was 72.56 µg/ml and 43.66 µg/ml in QI-100% while in QI-70%, the concentrations were 72.41 µg/ml and 43.31 µg/ml, respectively. Taken together, both DPPH and HPLC data indicated that both QI extracts contained tannic and gallic acids which exhibit inherent antioxidant activity. QI-treated H9-hNSC was seeded in a modified Boyden chamber to investigate its migration capacity towards DBTRG-05MG. Result showed that H9-hNSC migrated towards DBTRG-05MG with 4-folds higher capacity compared to the control. However, there is no significant different between the normoxic and hypoxic NSC migration. In addition, the migration of QI-100% treated H9-hNSC successfully reduced the number of DBTRG-05MG cells, indicating the anti-GBM potential of these cells. In conclusion, we successfully demonstrated that the NSCs are able to migrate and deliver QI extracts towards glioma in vitro and reduces the glioma cell number.

Summary of Research Findings

This FRGS grant (Account No.: 203/PPSK/6171154) was approved starting from 01 December 2013 until 31 May 2017 (36 months + 6 months extension). Based on the Gantt Chart of Proposed Study as approved, all milestones had been achieved successfully.

In the first milestone (Dec 13 – May 14), the first three months of study period focused on procurement of research material and laboratory set up, and the next three months focused on optimization of methodologies proposed in this research plan, including: cell culture, RT-PCR, western blot, and cryopreservation techniques.

In the second milestone (June 14 – Nov 14), the first three months of study period focused on culturing and expanding the DBTRG-05MG Glioma Cells (human brain cancer cells), SVG-p12 Glial Cells (human brain normal glial cells), and Gibco H9-hNSC (human neural stem cells). We have successfully established stable and viable cell line resources (cryopreserved cell) for long term usage in our research group. In the next three months (Sept – Nov), we focused on the analysis of mRNA and protein expression in human glioma cells, glial cells, and neural stem cells using RT-PCR and western blot, respectively. We found that PPIA and RPL13A were the best reference genes to analyse RT-PCR data involve brain cancerous and normal cell types. We also found that neural stem cells expressed nestin and CD133 specially, and these markers were used a key markers to identify neural stem cells in our subsequent study. Next we did hypoxic culture and found out that cell proliferation was highest at 24 hours after hypoxic treatment.

In the third milestone (Dec 14 - May 15), the first three months focused on extraction of active compound from a natural plant known as Quercus infectoria using 70% and 100% methanol extraction method, respectively. This plant is more commonly known as Manjakani in Malaysia. It had been proved in our group research, previously, by Assoc. prof. Dr. Hasmah Abdullah, that it possessed anti-cancer property. In the following three months, we tested the cytotoxicity effect of these anti-cancer Quercus infectoria active compounds on the three cell types established since the second milestone. The IC50 value of Quercus infectoria for human glioma cells, glial cells and neural stem cells were determined as 0.206 µg/mL 0.715 µg/mL and 0.815 µg/mL, respectively. These result indicated that Quercus infectoria active compound was capable to kill can cancerous cells at low concentration and such low concentration had no cytotoxic effect on the glial cells and neural stem cells.

In the fourth milestone (June 15 – Nov 15), we focused evaluating the migration capability of human neural stem cells towards glioma cells. We found that the migration capacity of the stem cells is 4-fold higher than control cell (normal glila cells). We also tested the ability of these neural stem cells to carry the Quercus infectoria active compound along to kill glioma cells, and we found that the migration of neural stem cells with optimum concentrations of Q. infectoria methanol extracts and tamoxifen showed migration to glioma cells and it was able to reduce the number of these cells. However, we did not find any significant increase of migration capacity after hypoxic preconditioning.

In the fifth milestone (Dec 15 – May 16), the characterization of the neural stem cell before and after migration were investigated. We found that the expression of hypoxia-inducible factors (HIF) and its downstream genes such as VEGF and Nrf2 were increased after hypoxic treatment. Besides, the cardiac differentiation potential of neural stem cell also increased.