UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

MICRORNA REGULATION OF HUMAN CHOLINE KINASE GENE EXPRESSION

PENYELIDIK

PROFESOR MADYA DR. FEW LING LING

2019

FRGS FINAL REPORT

Title of Research : MicroRNA Regulation of Human Choline Kinase Gene Expression

Name of Research Leader : Assoc Prof Dr. Few Ling Ling (School of Health Sciences, USM)

Names of Co-Researcher : Assoc Prof Dr. Khoo Boon Yin (Institute for Research in Molecular Medicine, USM)

Dr. Nurhidanatasha Abu Bakar (School of Health Sciences, USM)

UNIVERSITI SAINS MALAYSIA

BORANG PENYERAHAN ASET / INVENTORI

==========

A. BUTIR PENYELIDIK

1. NAMA PENYELIDIK : PM DR. FEW LING LING
2. NO STAF :
3. PTJ : PUSAT PENGAJIAN SAINS KESIHATAN

5. TARIKH TAMAT PENYELIDIKAN : 31 MEI 2018

B. MAKLUMAT ASET / INVENTORI

BIL	KETERANGAN ASET	NO HARTA	NO. SIRI	HARGA (RM)
	Tiada Aset			

C. PERAKUAN PENYERAHAN

Saya dengan ini menyerahkan aset/ inventori seperti butiran B di atas kepada pihak Universiti:

Tarikh: 23 JULAI 2018

D. PERAKUAN PENERIMAAN

Saya telah memeriksa dan menyemak setiap alatan dan didapati :

☐ Lengkap
☐ Rosak
☐ Hilang : Nyatakan.....
☐ Lain-lain : Nyatakan .....

Diperakukan Oleh :

.....

Tandatangan
Pegawai Aset PTJ

Tarikh:

*Nota :
Satu salinan borang yang telah dilengkapkan perlulah dikemukakan kepada Unit Pengurusan Harta, Jabatan Bendari dan RCMO untuk rekod

FRGS FINAL REPORT

Title of Research: MicroRNA Regulation of Human Choline Kinase Gene Expression

Principal Investigator: Assoc. Prof. Dr. Few Ling Ling

Summary of Research & Findings

Choline kinase (CK) is the first enzyme in the CDP-choline pathway for biosynthesis of phosphatidylcholine. Increased activity of CK has been implicated in human carcinogenesis. MicroRNAs (miRNAs) are a large family of non-coding RNAs that regulate gene expression. Dysregulation of miRNA expression is common in many types of cancer and miRNA profiling has the potential applications for cancer diagnostic and prognostic. Despite the physiological and pathological importance of CK, the regulation of its expression by miRNAs has never been reported. We hypothesize that miRNAs regulate the expression of CK and affect cell cycle progression. This study aims to predict the miRNAs that bind CK mRNAs and determine the effect of the selected miRNAs on CK gene expression, cancer cell proliferation and morphology. MiRNAs binding was predicted by several online computer programs that utilize different algorithms. Potential miRNAs were selected for the synthesis of their mimics and transfected into cancer cell lines. The effect of the miRNAs on CK alpha mRNA and protein levels were determined by real-time PCR and Western detection. MTT assay was used to determine the effect of miRNAs on cancer cell viability. The effect of miRNAs on cell morphology was investigated by scanning electron microscopy.

Bioinformatic predictions of miRNAs that potentially downregulate human *ckα* gene expression have produced ten best miRNA candidates (out of initial 54 non-repeating miRNAs) based on the higher scores for minimum free energy for interaction, binding site features and sequence conservation among different species. Due to the relatively large number of potential miRNA candidates, this study has focused on miRNAs targeting *ckα* only although prediction for miRNAs targeting *ckβ* isoform was also conducted. Out of the ten shortlisted miRNAs potentially targeting *ckα*, three miRNAs namely miR-876-5p, miR-646 and miR-202-5p were selected for experimental validation based on their favorable complementarity with *ckα* gene 3'-untranslated region and binding energy.

To investigate the effect of these miRNAs on the expression of *ck* gene expression, the optimal conditions for miRNA transfection must be determined for the cell type used in this study. HepG2 cell line was used for the optimization of miRNA transfection. The cells were transfected with non-targeting (miRIDIAN microRNA Mimic Negative Control #1; Dharmacon) and GAPDH-targeting (Mimic Housekeeping Positive Control #2; Dharmacon) miRNAs at final concentrations of 25, 50 and 100 nM for a duration between 24 to 48 hours and 48 to 96 hours for mRNA and protein levels analyses, respectively. The results showed that transfection with 25 nM GAPDH-miRNA for 48 hours produced the strongest down-regulation of GAPDH mRNA and protein expression without apparent cytotoxic effect to HepG2 cells. The optimized transfection parameters determined in this study can be used as the general miRNA transfection protocol for HepG2 cell line. The GAPDH-miRNA is also suitable for experiments to validate potential miRNAs targeting *ckα* as it did not affect the expression of *ckα* for all the concentrations and transfection durations tested.

MiRNAs (miR-876-5p, miR-646 and miR-202) predicted to downregulate human *ckα* were transfected into HepG2 cells under the optimized conditions determined above. Real-time PCR results showed that miR-876-5p significantly reduced the expression of *ckα* by 30% compared to negative control. Both miR-646 and miR-202-5p did not show significant downregulation of *ckα* gene expression. The results also showed that *ckα2* was the predominant isoform in HepG2 cells since the levels of downregulation by miR-876-5p for total *ckα* and *ckα2* isoform were very similar. Similarly, Western blot detection also showed lower level of CKα protein in cells transfected with miR-876-5p compared to negative control.

Transfection of HepG2 cells with 25 nM miR-876-5p mimic for 48 hours reduced the cell viability by 25% compared to negative control and showed sign of apoptosis such as membrane blebbing under scanning electron microscope.

This study has identified potential miRNAs that modulate the levels of CK gene expression. Mir-876-5p has been shown to downregulate the expression of CK mRNA and protein levels in HepG2 cells. This miRNA also caused lower cell viability and apoptosis. This work has generated the first experimentally tested microRNA for potential regulation of CK gene expression. The involvement of miR-876-5p in cancer development and whether it could be applied in anticancer strategy that target choline kinase are some of the interesting questions

arise from this study that await further investigation. The findings also contributed to the fundamental understanding of miRNAs regulation of cellular phospholipid synthesis that could influence normal cell proliferation.

Asset Report

No equipment or accessories has been purchased under this grant.

Research Outcome

Publications

1. Chang, C.C., Few, L.L., Konrad, M. and See Too, W.C. (2016). Phosphorylation of Human Choline Kinase Beta by Protein Kinase A: Its Impact on Activity and Inhibition. PLoS ONE, 11(5): e0154702.

2. Sharzehan, M.A.K., S., Few, L.L. and See Too, W.C. (2016). Optimisation of miRNA transfection conditions and studying the effect of miRNA down-regulation on CKα gene expression in HepG2 cell line. Health and the Environment Journal, 7(1): 101-123.

3. Sharzehan, M.A.K., Few, L.L. and See Too, W.C. (2018). Downregulation of human choline kinase α gene expression by miR-876-5p. Molecular Medicine Reports, 17(5): 7442-7450.

Conference Presentations

1. Mohammad Shafiq, H., Few, L.L. and See Too, W.C. (2016). Development of ADP-GLO™ assay for Entamoeba histolytica choline kinase activity measurement. Malaysian Symposium of Biomedical Science, Universiti Putra Malaysia.

Sharzehan et al. 2016 | Pp 101-123
Health and the Environment Journal, 2016, Vol.7, No.1

Optimisation of miRNA transfection conditions and studying the effect of miRNA down-regulation on CKα gene expression in HepG2 cell line

Sharzehan Mohamad Ayub Khan, Few Ling Ling and See Too Wei Cun*

School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

ABSTRACT: MicroRNA (miRNA) is a small RNA molecule of 22 nucleotides long that regulates gene expression by binding to the 3'-untranslated region of a specific mRNA and subsequently leads to the degradation of the targeted mRNA. MiRNA plays vital role in human physiology and developmental processes. Thus, analysis of miRNA regulation of gene expression provides valuable information in the study of miRNA related diseases such as cancer and certain metabolic disorder. In order to study the effect of miRNA on the expression of a specific gene in mammalian cell culture, the transfection efficiency of miRNA mimics must be optimised for the specific cell line. This study aims to optimise the conditions for GAPDH-targeting miRNA transfection of HepG2 cell line and to investigate the effect of GAPDH-miRNA on the expression of choline kinase alpha (*ckα*) gene expression. The GAPDH-miRNA concentration and transfection duration were optimised for the strongest down-regulation of GAPDH mRNA and protein levels. MiRNA with the concentrations of 25, 50 and 100 nM and transfection durations of 24, 36, 48 and 72 hours were tested in triplicate experiments with non-targeting miRNA as negative control. After the transfection, GAPDH and *ckα* relative mRNA levels were quantified by real-time PCR and GAPDH protein was detected by Western blot detection. The results showed that 25 nM and 48 hour transfection duration resulted in the lowest GAPDH mRNA and protein levels without apparent cytotoxic effect to HepG2 cells. The optimised transfection parameters determined in this study can be used as the general miRNA transfection protocol for HepG2 cell line. The GAPDH-miRNA is also suitable for experiments to validate potential miRNAs targeting *ckα* as