INCREASING OF MICRORNA-137AS A BIOMARKER FOR ENCEPHALOPATHY

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ABSTRACT

Encephalopathy is an alteration of the mental status due to brain injury or metabolic derangement. The encephalopathy from the direct brain injury in several conditions increases the mortality rate and lead to dementia, seizures and coma. On the other hand, the encephalopathy from metabolic abnormality is reversible after the correction of the metabolic defect. Therefore, an indicator representing encephalopathy of the direct brain injury is necessary. MicroRNA-137 (miR-137) is a small RNA that specifically found in brain, and its function is to control brain development and cell proliferation. The objective is to investigate the association of miR-137 and encephalopathy. In this study, we selected 2 models associated with encephalopathy including sepsis and uremia, the encephalopathy of direct ischemic brain injury and from metabolic defect, respectively. C57BL/6 mice were separated in 3 groups; sham control (n=8), septic encephalopathy (cecal ligation and puncture model) (n=8) and uremia (bilateral nephrectomy model) (n=9). Sera were collected for miRNA isolation after 24-hour. The miR-137 expression was determined by real-time polymerase chain reaction (PCR). The results showed both of encephalopathy models have elevated of miR-137 expression compared to normal controls. In conclusion, the miR-137 expression might be a potential indicator for diagnosis encephalopathy-related disease, however, could not differentiate between direct brain injury and metabolic encephalopathy.

Keywords: microRNA, encephalopathy, sepsis-induced encephalopathy, uremic-induced encephalopathy, microRNA-137, indicator.

1. Introduction

Encephalopathy is an alteration of mental stage that associated with disease, damage, or malfunction of the brain. There are several causes which can induce encephalopathy; however; infection, liver damage, anoxia, and kidney failure are common causes of this condition. The symptom of encephalopathy is highly broad range from mild alteration in mental status (memory loss or subtle personality alteration) to severe (dementia) and fatal manifestations (seizures, coma, or death). Moreover, patients who have encephalopathy from any causes show the higher mortality rate than patient without encephalopathy (Eidelman et al., 1996; Shawcross et al., 2011). In addition, the patients with encephalopathy are trend to stay in Intensive Care Unit (ICU) longer than patient without encephalopathy and have a higher risk of infectious complications (Bleck et al., 1993). Currently, the diagnosis of

encephalopathy is usually made with clinical signs and symptoms with several tests; including mental status tests, memory tests, and coordination tests, which are time consuming and labor intensive. Thus, the biomarker indicating encephalopathy will be easier but is still lacking. Because of these, finding a biomarker to identify encephalopathy condition, use together with, and clinical examination tests is essential.

MicroRNAs (miRNAs) are small endogenous non-coding RNA with approximately 21-25 nucleotides in length. The function of miRNAs is to regulate many biological processes in a wide variety of organisms (plants, animal and virus) through binding the target mRNAs and cleavage or preventing protein synthesis (ChenandQin, 2015). Not only gene regulation, miRNAs play an important role in various cellular functions including organogenesis, cell proliferation, cell differentiation, apoptosis, and immune responses (Dai et al., 2016; Huang et al., 2015; Parkes et al., 2015). Recently, it is known that many miRNAs were useful biomarkers for the diagnosis of several diseases (Mall et al., 2013; Silva et al., 2015). The abnormality of miRNA expression represents cellular malfunctions in these conditions.

As such, miR-137 is a brain-enriched miRNAs that has significant regulatory roles on brain functions, including proliferation and differentiation (Sun et al., 2011) which is enriched during hippocampal formation (Wright et al., 2013). Besides, dysfunction of miR-137 is associated with mental illness and human cancer (neuroblastoma and glioblastoma multiform) (Silber et al., 2008; Whalley et al., 2012). A previous study found that the miR-137 also involved in controlling genes involved in psychosis (Steinberg et al., 2011). Hence, miR-137 is an interesting indicator for encephalopathy in this study.

2. Objectives of the study

To investigate the association between miR-137 and encephalopathy conditions.

3. Materials and methods

Animal model

C57BL/6 mice weight 20 – 25 g were used in this study. The mice were received standard mice chow and water during the entire experiment. In this report, we chose 2 models (sepsis and kidney failure) to represent encephalopathy. For sepsis-induced encephalopathy (n=8), we chose cecal ligation and puncture in this model. Mice were opened abdomen and tightly ligated the cecum with 6.0 silk then punctured with 21-gauge needle. After that, the cecum was returned to the peritoneal cavity, and the abdomen was closed with 6.0 silk. For uremic-induced encephalopathy (n=9), we used bilateral nephrectomy model. Both kidneys were removed through abdominal incision. Then, the abdomen was closed with 6.0 silk. For sham control (n=8), the abdomen were opened and closed by silk 6.0. Ibruprofen syrup was used as an analgesic drug post operatively. Animals were monitored thereafter, and sacrificed at 3, 6, and 24 hour after surgery for a sepsis-induced encephalopathy and uremic-induced encephalopathy.

Preparation of Plasma collection

When the mice were sacrificed at 3, 6, and 24hour of sepsis-induced and uremic-induced encephalopathy model, blood was collected in EDTA tube to prevent blood clotting via cardiac puncture, then centrifuged at 3,000 x g for 15 minutes at 4°c. Plasmas were collected at -80°c.

Isolation and expression of miRNA

Total RNAs of 200 µl of plasma were extracted by using QIAGEN serum/plasma miR-Neasy kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction. The microRNAs were converted to cDNA by RT-PCR according to TaqMan™ MicroRNA Assays kit (Applied Biosystems, Waltham, MA USA). Briefly, 2.5 µ of total RNA was reverse transcribed to cDNA using Multiscribe reverse transcriptase (Thermo Fisher Scientific, Waltham, MA USA) and the stem-loop RT primer (Thermo Fisher Scientific, Waltham, MA USA) in a scaled down (7.5 µl) RT reaction: Each reaction should be comprised of 1.405 µl H_2O, 0.5 µl 50X Reverse-Transcription Buffer, 0.095 µl RNase-Inhibitor (20 U/µl), 0.75 µl 10 mM dNTPs with dTTP, 0.5 µl Multiscribe Reverse Transcriptase (50U/µl), 1.5 µl RT primer. For the conditions of RT-PCR using 4 steps, first is temperature at 16° c for 30 minutes. Next, using temperature at 42° c for 30 minutes. Third step is temperature at 86° c for 5 minutes and hold on at 4° c. Primer sequence; UUAUUGCUUAAGAAUACGCGUAG (mmu-miR-137-5p, assay ID 001129) was purchased from Thermo Fisher Scientific. The cDNA samples were used for quantitative real-time PCR through TaqMan™ Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA USA). Relative expression was calculated using the $\Delta\Delta$CT method and normalized to the expression of cel-miR-39 (Applied Biosystems, Waltham, MA USA).

Statistical analysis

All data was analyzed by using SPSS 22.0 (SPSS Inc., IL, USA) and Graph Pad Prism version 7.0 software (La Jolla, CA, USA). The results were presented as mean ± standard deviation (S.D). The Mann-Whitney unpaired t-test was carried out to determine the differences in the expression of microRNA between groups of sepsis and control. $P < 0.05$ was considered as statistically significant

4. Results

Evaluation of microRNA-137 in encephalopathy model

Sepsis-induced encephalopathy

The result showed miR-137 expression was increase at 3, 6, and 24 hour after surgery (Mean = 113.5, 253.9, 293 respectively). At 3 hour after experiment, the expression of miR-137 was increase higher than 0 hour (Mean = 11.1) (fig. 1). Moreover, the miRNA at 6 hour after surgery were significantly higher expressed when compared with at the 0 hour time-point (**** $p < 0.0001$) and at 3 hour after surgery (ANOVA **** $p < 0.0001$, ** $p < 0.01$ respectively) as well as time-point 24 hour.

Uremic-induced encephalopathy

As see on fig. 2, the expression of miR-137 was increase after 24 hour of surgery (Mean = 548.6) but not at time-point 0, 3, 6 hour after surgery (Mean = 8.508, 57.42, 30.21 respectively). The high expression of miR-137 at 24 hour was significantly compare to other time-points (ANOVA *** $p < 0.001$).

Gene expression in encephalopathy model

After 24 hour of surgery in both of models, encephalopathy (sepsis-induced encephalopathy, n = 8, uremic-induced encephalopathy, n = 9) and control groups (n = 8), were measured gene expression by qualitative Real-Time PCR. The results showed both of sepsis- and uremic-induced encephalopathy had expression of miR-137 (Mean = 164.5, 438.9 respectively) higher than control group (Mean = 6.948). Uremic-induced encephalopathy were significantly higher than sepsis-induced encephalopathy (ANOVA * $p < 0.05$) and control (ANOVA *** $p < 0.001$) (fig. 3).

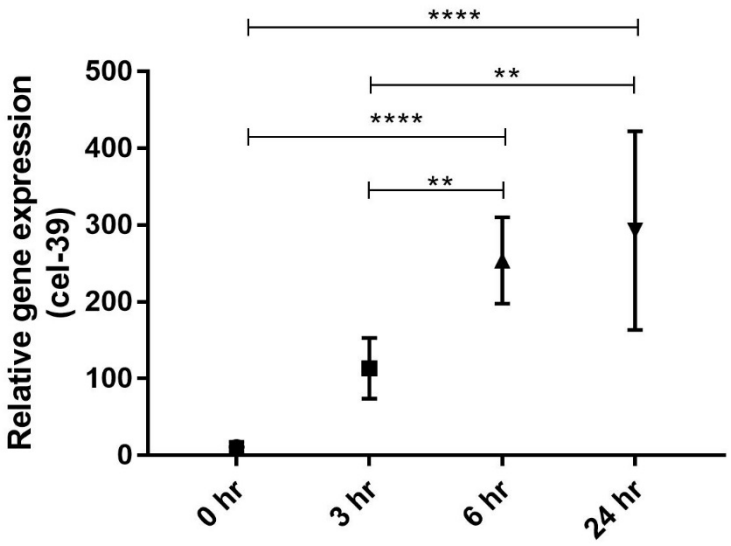

Figure 1. The expression levels of miR-137-5p in sepsis-induced encephalopathy were analyzed by qRT-PCR following 24 h after surgery. (** $P < 0.01$, **** $P < 0.0001$, n = 24).

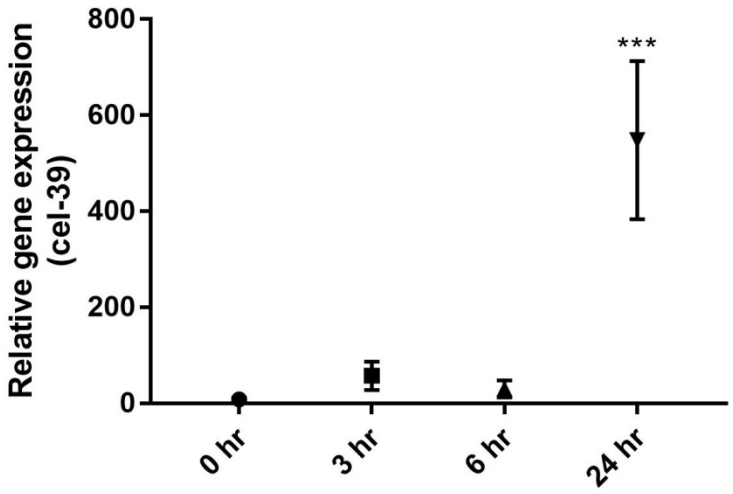

Figure 2 The expression levels of miR-137-5p in uremic-induced encephalopathy were analyzed by qRT-PCR following 24 h after surgery. (*** $P < 0.001$, $n = 22$).

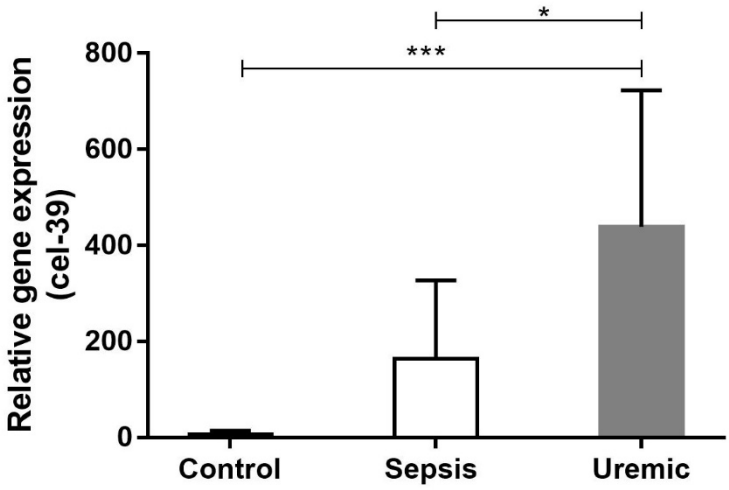

Figure 3 The expression levels of miR-137-5p in sepsis-induced, uremic-induced encephalopathy and control in mouse model were measured by qRT-PCR. (* $P < 0.05$, *** $P < 0.001$).

5. Discussion

In this study, we showed that the expression of miR-137 was associated with encephalopathy by two animal models. Our finding show mice with encephalopathy stage have increased expression of the miR-137 compare to normal mice.

Normally, patients will have a high mortality rate if patient have a sepsis or kidney failure (Case et al., 2013; Whittaker et al., 2015). Sepsis and kidney failure lead to encephalopathy through ischemic-brain and uremia,

respectively (du Moulin et al., 1985; Liu et al., 2008). In addition, encephalopathy in both conditions are resulting in the higher mortality (Sprung et al., 1990). Encephalopathy is a stage of mental change that correlates with brain damage or brain malfunction. In the same way, when brain has injury or damage, they response injury or damage by producing proteins that protect them from danger (Juul, 2002; Mattiasson et al., 2003). The production of such proteins is controlled, at least in part, by miRNA.

Indeed, miR-137 is a small RNA that can be found in brain and associate with brain functions (brain cell proliferation and brain development) (Silber et al., 2008). Moreover, some target genes such as ZNF804A are associated with psychosis (Steinberg et al., 2011). This gene is associated with control processes in brain such as neural migration, neurite outgrowth and synapse formation. Therefore, if the patients with sepsis or uremia in ICU have an expression of microRNA-137 it is possible that these patients developing encephalopathy.

6. Conclusion

This study shows the relationship between miR-137 and encephalopathy. Thus, this research can be the basic knowledge to learning about microRNA and encephalopathy. Additionally, we can use this miRNA as biomarker in the diagnosis of encephalopathy in adjunctive with other methods. The further study in patients is necessary.

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