Must be typed

（For JSPS Fellow）

Form B-5

Date（日付）
26/10/2015（Date/Month/Year:日/月/年）

Activity Report -Science Dialogue Program-
（サイエンス・ダイアログ事業　実施報告書）

- Fellow's name（講師氏名）: Chin-Yu Lin （ID No. P14406 ）

- Participating school（学校名）: Utsunomiya Girls' High School

- Date （実施日時）: 02/10/2015 （Date/Month/Year:日/月/年）

- Lecture title（講演題目）: （in English） **Polyplex Nanomicelles Assembled with Neprilysin mRNA Augmented Clearance of Amyloid-β in Mouse Brain**

（in Japanese） **自組裝 Neprilysin mRNA 奈米載體促進小鼠腦內 Amyloid Beta 分解**

- Lecture summary（講演概要）: Please summary your lecture 200-500 words.

Object: Gene therapy have received much attention because the genes can provide sufficient amount of therapeutic proteins in a sustained manner compared with direct administration of exogenous protein. Introducing plasmid DNA (pDNA) has been a standard approach to deliver genes, however, the safety issues regarding random integration of the pDNA into genome, that might cause insertional mutagenesis, have hampered its wide applications for clinical settings. mRNA is a promising alternative because mRNA has several advantages over pDNA; mRNA would be simply translate proteins in cytoplasm without any risk of genome integration. Since mRNA does not need to cross the nuclear membrane for protein expression, mRNA is likely to exert high efficiency in slowly- or non-dividing cells such as neural cells. In this study, we aim at applying mRNA administration for the treatment of neurological diseases in brain.

Materials and methods: We used neprilysin (NEP), a type II transmembrane protease contributing to mainly physiological clearance of amyloid-β (Aβ). As is commonly known, Aβ is the major component of neuritic plaques that often accumulate in the brains of Alzheimer's Disease (AD) patients and very toxic to brain neurons. As a proof-of-concept study to prove the feasibility of mRNA for treating AD, we constructed a mouse NEP-expressing mRNA and evaluated the capacity for degrading Aβ. For in vitro studies, the mRNA was transfected using Lipofectamine LTX to two cell lines that were mutated to overexpress human amyloid precursor protein (hAPP), N2A/PS/APP or 293/PS/APP (carrying presenilin 2 and hAPP Swedish K595N/M596L mutation). Furthermore, we evaluated in vivo activity of NEP-expressing mRNA by intraventricular injection of the mRNA using polyaspartamides nanomicelles prepared in our lab, that allows efficient mRNA introduction in vivo into neural tissues (Uchida et al., PLoS One 8: e56220, 2013) and other various organs (papers submitted or partly showed in other

presentations).

Results and Discussion: By evaluating hAPP production in the culture medium of N2A/PS/APP or 293/PS/APP at post-transfection, mRNA induced a significant decrease in hAPP concentration to a similar extent as the transfection of NEP-expressing pDNA. We also constructed a mRNA expressing chimeric NEP fused with green fluorescence protein (GFP) as a reporter. By transfection of the mRNA into cell lines or primary neurons, the expression of NEP-GFP was mainly found to be localized in the cell membrane. Thus, it is suggested that the NEP expressed on the cell surface as a membrane-bound protein effectively degraded the extracellular Aβ in the culture medium. From the ELISA evaluation of animal studies revealed that the mRNA injection significantly augmented the NEP level in the mouse brain and effectively reduced the total amount of Aβ in the brain, using a mouse model that had been pretreated with intracerebroventricular infusion of Aβ. Moreover, the mRNA transfection showed superior NEP expression and Aβ degradating capability than that of the pDNA transfection in primary neurons and mouse brain intraventricular injection. Collectively, mRNA administration is considered to be a new therapeutic approach for the intractable neurological-diseases such as AD.

- Language used（使用言語）: English

- Lecture format（講演形式）:

◆Lecture time（講演時間） 50 min（分）, Q&A time（質疑応答時間） 10 min（分）

◆Lecture style（ex.: used projector, conducted experiments）

（講演方法（例：プロジェクター使用による講演、実験・実習の有無など））

Used projector, video, audio to show the experiment, concept and introduction.

◆Interpretation（ex.: assistance by accompanied person, provided Japanese explanation by yourself）（通訳（例：同行者によるサポート、講師本人による日本語説明））

provide Japanese explantation by myself

◆Name and title of accompanied person（同行者 職・氏名）

None

◆Other note worthy information（その他特筆すべき事項）:

None

- Impressions and opinions from accompanied person（同行者の方から、本事業に対する意見・感想等がありましたら、お願いいたします。）: