CHAPTER 1

INTRODUCTION

1.1 Problem Background

1.1.1. Neurodegenerative Diseases

Neurodegenerative diseases (ND) are a major problem for human health. These age-dependent disorders will become a massive challenge in the future due to demographic changes worldwide (Heemels, 2016). Many people might acknowledge neurodegenerative diseases as conditions where elders start to lose their memory quality. However, it is much more than that. Neurodegenerative diseases affect neurons in human brain, which are the building blocks of nervous systems. When the neurons are damaged, they cannot be replaced by the body. Thus, it will cause problems and symptoms. Most common symptoms include apathy, rigid mucles, anxiety, speech changes, insomnia, even sexual problems, etc. (Levenson et al., 2014). The prevalence keeps on increasing, partly because of the lifespan extensions (Heemels, 2016). Alzheimer's disease is the best-known neurodegenerative disease, but there are still many other diseases. Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, frontotemporal dementia and the spinocerebellar ataxias are other examples of neurodegenerative diseases (Gitler et al., 2017). Each disease has unique characteristics and pathophysiology, with some cause problems with movement (ataxias), while others disturb mental functioning (dementia) (Ahmed et al., 2013). Currently, there is no available treatment can cure these diseases; available drugs only ameliorate some of the symptoms (Chen & Pan, 2015). A lot of research is going on trying to find the way to cure neurodegenerative diseases. Some research reported that neuritin is a therapeutic candidate and possible answer for neurodegenerative diseases (Shimada et al., 2013; An et al., 2014).

1.1.2. Neuritin

Neuritin is a protein encoded by NRN1 gene. It is an important neurothropin, family of proteins that induce the survival, development, and function of neurons (Huang & Reichardt, 2001). Currently, neuritin is still less studied. However, since its early reports in the 1980s, the research about neuritin has been focused and narrowed down into its activity against nervous damage. Primarily, neuritin is expressed in postmitotic-differentiating neurons in developing a nervous system and plasticity-associated neuronal structures in adult (Naeve et al., 1997). The expression of neuritin is also presence in postmitotic neurons along with other proteins such as attractin. Together those proteins induce neurite outgrowth. Shimada et al. in 2013 reported 3 functions of neuritin, which are: neuritogenesis, arborization, and axonal elongation. These findings based the idea of possible axonal regeneration by neuritin following nerve injury. Other study done by Karamoysoyli et al. in 2008 supported the important roles of neuritin in neurite outgrowth. They found that silencing neuritin gene expression inhibits axonal growth.

1.1.3. Neuritin Detection

Neuritin research relies heavily on its detection. Currently, there are two types of methods, which allow detection of single protein. The first one is spectrometry, which is based on the interaction matter and electromagnetic radiation. This method includes high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC/MS) (Tuli & Ressom, 2009).

The second method uses antibodies to detect and bind target proteins. These antibody dependent methods include enzyme-linked immunosorbent assay (ELISA), protein immunoprecipitation, immunoelectrophoresis, western blot, and protein immunostaining (Biochemical Science, 2000).

Besides the detection on the protein level, neuritin can also be detected in its RNA level. Based on central dogma, protein is a result from the translation of mRNA (Clancy & Brown, 2008). Hence, detecting neuritin mRNA expression is a way to predict its protein expression (Guo et al., 2008). Each current method has its advantages and disadvantages. Some of the major drawbacks of these methods are high costs, time-consuming, and complicated procedures. Therefore, it is necessary to find the most suitable neuritin expression detection to support future research.

SmartFlare™ Live Cell RNA Detection is an alternative to neuritin RNA detection. This technology offers promising advantages including, easy and practical, relatively quick, allows RNA analysis without needing cell lysis. However, this kit might produce different results when used in different kind of cells and with a different protocol. In this study, SmartFlare™ RNA Detection Probes will be used for neuritin RNA detection on PC12 cells as a study model.

1.2 Problem Formulation

SmartFlare™ RNA Detection Probes protocol requires testing and optimization to provide reliable information regarding the usage of the kit on PC12 cells.

1.3 Research Objectives

To optimize SmartFlare™ RNA Detection Probes protocol (concentration and incubation time) for neuritin RNA detection on PC12 cells.

1.4 Research Scope

- PC12 cells culture and maintenance
- Cell seeding, treatment, and lysis
- qPCR analysis
- Protein quantification

- Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
- Western blot
- Plate reader, fluorescent microscope, and flow cytometry