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MICRORNA REGULATION OF IGF-1

An Undergraduate Research Scholars Thesis

By

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DEDICATION

To my parents, they have always told me that anything is possible.

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ABSTRACT

MicroRNA Regulation of IGF. (May 2013)

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Stroke is the fourth leading cause of death worldwide. Low levels of IGF-1, a known neuroprotectant, correspond to aging and increased severity of stroke. We have previously demonstrated that there is an inverse relationship between the presence of anti-let7f or anti-miR-1 and levels of IGF-1. Further, the localization of let-7f was found to be within the microglial cell population. We hypothesize that let7f and miR-1 directly bind to the 3' UTR region of IGF-1. Microglial cells (EOC-20) will be transfected with an IGF-1 clone with a luciferase tag. IGF-1 synthesis following treatment with antagomirs to either miR-1 or let7f will be tested by a luciferase assay.

CHAPTER I

INTRODUCTION

On average, stroke is the leading cause of death worldwide (Beal 2010). In the United States each year on average 800,000 people have a stroke. There are many risk factors for stroke including environment, age and gender. Interestingly enough, post-menopausal women have a higher risk of stroke than men. It has also been shown that women are more functionally affected after a stroke than men (Wyller 1997). This striking statistic makes the need for a stroke therapy more prevalent. Currently, there is only one FDA approved treatment known as tissue Plasmogen Activator (Lisabeth 2006). Overall, women receive this treatment 30% less often than men (Reeves 2009). It has been speculated, that due to the disparity between symptoms and overall treatment, women are usually more severely disabled than men.

IGF-1 is a known neuroprotectant. There is known direct correlation between low levels of IGF-1 and increased severity of stroke, especially in women. This is seen throughout the female rat stroke model. A low level of IGF-1 is also associated with aging, a key factor in stroke severity. Thus identifying regulatory elements that increase IGF-1 levels will be crucial in potential treatment options for stroke. MiRNAs, a class of non-coding RNA, are key negative regulators of many pathways that can be exploited for stroke therapy.

MiRNAs are small noncoding RNAs which have been found to regulate many key pathways. MiRNAs have been found to be potential therapeutic targets in cancer, skeletal and muscle degenerative diseases and cardiovascular diseases (Sibley 2011, Dangwall 2012). There are several miRNAs that have been associated with stroke including, let-7f, miR-1, miR-126, miR-1259, miR-142-3p, -15b etc. (Tan 2009).

According to bioinformatics, there is a potential binding site of let-7f and/or miR-1 on the 3' UTR region of IGF-1. We have previously shown that anti-let7f and anti-miR1 treatment was able to mimic and even extend the neuroprotection due to IGF-1. Furthermore, anti-let7f treatment also increases the levels of microglial IGF-1. We hypothesize that miR-1 and Let7f directly binds to the 3' UTR region of IGF-1 to regulate expression of this growth factor. In order to confirm this relationship in vitro, we will transfect an IGF-1 construct into a microglial cell line. Antagomir to either miR-1 or let7f will be added to these cells and IGF-1 regulation will be determined by a luciferase reporter assay.

CHAPTER II

METHODS

Cell Culture

(LADMAC) were purchased from American Type Culture Collection (ATCC). Cells were grown in EMEM with 10% Fetal Bovine Serium (FBS) at 37°C. After two weeks, the conditioned media was filtered and stored at -20°C to be added to the EOC media. EOC-20 were purchased from ATCC. Cells were grown in EMEM with 10% FBS and 20% conditioned media from LADMAC at 37°C. Media was changed weekly and cells were split when confluence levels were approximately 80%.

Optimizing Transfection Procedure:

EOCs were harvested and counted prior to transfection. After counting the cells, the cells were centrifuged at 100g for 5 minutes. The pellet was resuspended in the transfection buffer and concentrations of 50 pmol, 100 pmol or 150 pmol of Flourescent Labeled Negative Control A Oligos (Exiqon) was added to 100,000 cells. Electroporation buffer was added to the NEON instrument. The EOCs were transfected using a NEON electroporator for 30 ms at 1900 V for one pulse. The optimal concentration of oligos was found to be 100 pmol. Next the conditions for transfection were optimized by varying the voltage and the number of pulses.

Transfections: (not optimal protocol)

EOCs were harvest and counted prior to transfection. After counting the cells, the cells were centrifuged at 100g for 5 minutes. The pellet was resuspended in the transfection buffer and

1ug of the miRNA 3'UTR target clone (Genecoppia) per 100,000 cells. Eletroporation buffer was added to the cell. The cells were then transfected using a NEON electroporator for 20ms at 1200V for two pulses. Cells were transferred from the electroporation tubes to fresh warmed media into a 6 well plate.

Luciferase Assay:

Binding affinity for miRNA to the 3-UTR of IGF was measured using the Dual-Glo Luciferase Assay System purchased from Promega. The protocol was run according to manufacturer's instructions.

CHAPTER III

RESULTS

Transfections of microglial cell line have resulted in a 20% transfection efficiency. The desired efficiency is at least 90% of positively transfected cells. This process must be optimized effectively before being able to proceed.

CHAPTER IV

DISCUSSION

Bioinformatic analysis was done using Targetscan. Due to the percentage of complementation it is likely that miR-1 and let-7f will directly bind to the IGF-1 3' UTR. In order to confirm this hypothesis, transfections of EOC-20 cells were attempted. A variety of parameters were manipulated in order to determine an optimal transfection protocol. These included the cell number, conditions of growth, concentration of oligos, voltage, number of pulses and path length. The efficiency of transfection has not reached the required 90% of positively transfected cells to proceed to the next step of the project.

CHAPTER V

CONCLUSION

Based on previous published data, we have concluded that there is an interaction between IGF-1 and several miRNA, including miR-1 and let-7f. To determine if the interaction demonstrated is a direct interaction, a series of transfections were set up. Unfortunately, the transfection efficiency has not reached the optimal 90% positively transfected cells in order to accurately confirm a direct interaction.

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