Vector Mediated RNAi Knockdown of SGK1 in Neuronal Cell Culture

INTRODUCTION

Serum-and gluco-corticoid-induced kinase 1, known as SGK1, is an aldosterone-stimulated protein, which plays a role in the regulation of epithelial sodium channels (ENaCs) in the distal nephron (Pearce et al., 2007). In addition, SGK1 has been implicated in memory consolidation of spatial learning (Tsai et al., 2002). Dr. Miles' lab reports that SGK is part of a gene network that responds to ethanol. In the paper "Ethanol-Responsive Brain Region Expression Networks: Implication for Behavioral Responses to Acute Ethanol in DBA/2J versus C57BL/6J Mice," Kerns et al. observe that acute ethanol caused a significant upregulation of *Sgk1* in the prefrontal cortex of DBA/2J (D2) mice. This upregulation implicates SGK1 in the differential responses of D2 and B6 mice to acute ethanol. The overall goal of the RNAi experiments with SGK1 is to study the role of SGK1 in acute ethanol in mice further through reduction of *Sgk1* protein levels.

The first trial of this experiment, completed during the summer of 2007, laid the groundwork for the proposed experiment. In the first trial, mouse and human neuroblastoma cells, N18TG2 and SH-SY5Y respectively, were transfected with siRNAs using a liposome transfection technique. The siRNAs included two targeted at SGK with a fluorescent FAM tag, a Gapdh positive control, and negative non-targeting sequences. Immunocytochemistry was used to stain for SGK and Gapdh levels, and the cells were imaged using a fluorescent microscope. After the primary antibody staining the FAM tag was no longer visible for unknown reasons. The inability to tell which cells had been successfully transfected with siRNAs, coupled with the wide range of SGK expression levels observed, did not allow for a qualitative assessment of SGK knockdown. In addition, it is possible that the 48-hour knockdown period resulted in cell death. The goal of the proposed project is to use a more stable transfection as well as a more quantitative evaluation method in order to determine if the RNAi technique can be used to knockdown levels of the SGK protein in mouse neuronal cells.

METHODS

In order to improve the results of the previously mentioned RNAi experiment it is necessary to use techniques that result in a stable transfection with a fluorescent reporter and allow for quantitative measure of knockdown. Mouse neuronal cells will be cultured, keeping with the desire to eventually move the experiment to live mouse brain. After culturing the cells to confluence, the cells will be transfected with a lentiviral vector. The vector will have a region that codes for shRNAs targeted against SGK as well as a GFP reporter. Liposomal transfection will be used for the initial experiment, though the viral vector will be used with the hope that in the future of the project a virus will be used to transduce the cells with the shRNA expression vector. After transfection, a fluorescent microscope will be used to determine if the plasmids are being transcribed along with the cell's DNA. If so, then after the appropriate amount of time, RNA extraction can be used to obtain the mRNA of the cell.

Quantitative PCR can be used to determine the number of SGK mRNA transcripts in the transfected cells versus the number of transcripts in the untransfected cells. If investigation with the fluorescent microscope reveals at least a 50% transfection rate, and quantitative PCR isn't feasible, then a western blot will be used to detect protein knockdown. If the results of the quantitative PCR reveal that transfected cells have lower levels of SGK mRNA, then it will be beneficial to future research to determine if SGK knockdown occurs over a time course. To test this possibility, RNA can be extracted at different time points after transfection with the plasmid, and quantitative PCR can be used to determine the course of SGK knockdown.

DISCUSSION

The overall goal of this experiment is to inject shRNA-expressing plasmids into mouse brain and study the effect that low levels of SGK have on the behavioral response to acute ethanol. In addition, transfected mouse brain tissue could be used to run microarrays that may help determine which genes are downstream of SGK. The microarray results would also be used to validate the genes thought to be involved in the ethanol response gene network in which SGK is involved. In order to come closer to the ultimate goal, the proposed experiment is working towards a viral transduction by using a lentiviral vector. A viral transduction would be preferable because of the reportedly high

transfection rate. Also, transfecting the cells with a plasmid that expresses shRNAs against SGK, rather than just the siRNAs, should yield a more stable transfection, and could, if necessary, allow us to sort the cells using flow cytometry. The continued use of mouse neuronal cells will keep the results as relevant to doing the experiment in live mouse brain as possible.

CONCLUSION

The use of shRNA producing plasmids and the more exact technique of quantitative PCR will hopefully lead to a significant knockdown in the levels of SGK, and allow us to continue toward our final mouse model goal.

SOURCES

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