Using RNAi to Knockdown *Sgk1* Levels in Mouse 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). SGK1 enhances sodium transport in the distal nephron by preventing internalization of ENaCs from the plasma membrane, and more recently suggested, by increasing the transcription of an ENaC subunit by preventing histone methylation. (Pearce et al., 2007). In addition, SGK1 has been implicated in memory consolidation of spatial learning (Tsai et al., 2002). 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 goal of the proposed experiment is to study the role of SGK1 in acute ethanol in mice further through reduction of *Sgk1* protein levels.

METHODS

Knocking down the expression of SGK1 with RNAi will allow for further study of SGK1's role in acute ethanol. The overarching goal is to use RNAi to knock down SGK1 expression *in vivo*, in this case, within a live mouse brain. With this long-term goal in mind, it follows that a technique for the RNAi against SGK1 should be developed within a cell culture of mouse neuronal cells. After preparing the cell culture and allowing it to reach confluence, a virus will be used to deliver vector mediated RNAi to the mouse cells. In vector mediated RNAi, a portion of the DNA sequence of the target gene is inserted into an expression vector with a promoter of a gene regularly expressed within the cell type being used. The target gene is

regularly transcribed along with the rest of the cell's DNA, "flooding" the cell with pre-miRNAs, or "pre-microRNAs." These pre-miRNAs are bound by the protein "dicer" and processed further to become miRNAs. The miRNAs bind with RISC (RNA-induced silencing complex), a protein complex, and then hybridize with the mRNA target sequence. This hybridization results in cleavage of the mRNA and its subsequent destruction.

In order to test whether the RNAi procedure was successful in knocking down the level of *Sgk1* in the cell, a Western Blot assay can be used. The Western Blot can be used to determine the amount of *Sgk1* expressed in the RNAi processed neuronal mouse cells and wild type neuronal mouse cells so an expression level comparison can be made. In a Western Blot analysis the cells in question are homogenized and the proteins within the cell are separated using gel electrophoresis. The protein samples are then transferred to a nitrocellulose membrane where they can be "probed" using an antibody against the protein in question. The antibody is tagged with something like a fluorescent marker so the sample containing the protein in question can be identified. Quantitative RT-PCR is an alternate technique that could be used to measure the decrease in *Sgk1* in the cells after RNAi. In Quantitative RT-PCR the PCR instrument can measure the amount of mRNA in the tube after amplification. The amount after amplification is proportional to the amount of mRNA added to the reaction so one would be able to compare the amount of mRNA for SGK1 in control and RNAi treated cells.

DISCUSSION

As mentioned before, the goal of a successful RNAi procedure in mouse cell culture is to be able to transfer the procedure to the brain of a living mouse. This would allow one to study the mouse's phenotype at low levels of *Sgk1*, as well as to study its phenotype at low levels of *Sgk1* and high levels of alcohol. These observations could allow one to more fully understand

the role of SGK1 in ethanol related behavior. In addition, and perhaps more importantly, DNA microarrays could be performed using brain tissue from the RNAi treated mouse. One could use the changes in gene expression of a mouse with lower *sgk1* levels to predict what proteins might be downstream of *sgk1*. These findings could allow for *sgk1*'s placement in some sort of signaling pathway.

In order to reach this long-term goal, it is necessary that the Western Blots or Quantitative RT-PCR show sufficiently lower levels of SGK1 expression in RNAi treated cells. If these techniques show no, or very little change, one might suspect that the cell is using alternate pathways to get around the RNAi disturbance, or that the virus is for some reason not successful in inoculating the cells with the RNAi expression vector.

CONCLUSION

The ability to successfully knock down a gene using RNAi technology in mouse could be a useful procedure in studying the function of proteins involved in acute ethanol. The use of microarray data has allowed researchers to identify many genes that are differently regulated by ethanol between two strains of mice with different drinking tendencies (Kerns et al., 2005). Further study of the differentially regulated genes using RNAi could lead to a greater understanding of the pathways behind increased alcohol consumption and addiction in some individuals.

SOURCES

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