Does the FKBP51 Promoter Cause Elevated Gene Expression in Squirrel Monkeys?

Caitlin Tidwell and Tina R. Hubler,
Department of Biology, University of North Alabama, Florence, AL 35632

Introduction

Glucocorticoid Receptor Function

1. Glucocorticoids are hormones released by the adrenal gland into the bloodstream.
2. Glucocorticoids diffuse into cells & combine with the glucocorticoid receptor to activate the receptor.
3. Activated receptors interact with DNA to cause gene expression.

FKBP51 Function

• FKBP51 is a glucocorticoid receptor (gr) protein that functions to modulate receptor response to cortisol.
• If FKBP51 levels are high, the gr receptors are less responsive.

FKBP51 levels are much lower in humans (h) than squirrel monkeys (sm)

Human Squirrel Monkey

Project Rationale and Objective

• According to the Central Dogma of Molecular Biology, protein levels are controlled by transcription and translation.
• Transcription is the most common level of control of gene expression.
• Transcriptional control is usually regulated by the gene promoter DNA sequence.

The focus of the research is:

To determine if the human FKBP51 gene promoter is more active than the squirrel monkey 51 gene promoter.

Promoter Sequence Luciferase Assay

• The sm or h 51 promoter sequences were inserted into a luciferase assay vector (pGL3 Basic)
• When transfected into cells, expression of the luciferase gene is controlled by the human or sm promoter

Reporter gene DNA transfected into cells

h or sm 51 Luciferase gene promoter

• Adherent, squirrel monkey kidney fibroblast cells were grown in tissue culture dishes
• DNA (luciferase assay vector containing either the squirrel monkey or human 51 promoter) and SuperFect Transfection Reagent were added to the cells
• Cells were incubated for 24 hours to allow for expression of the luciferase gene
• Media was removed from cells and a lysis buffer was placed in each dish
• Cells were scraped from the dishes and centrifuged for 2 minutes to produce a supernatant
• The supernatant (containing luciferase protein) was placed in a new tube and Luciferase Assay Reagent (containing the substrate for luciferase, luciferin) was added to each tube
• The tubes were placed in a luminometer and light output was measured as an indicator of luciferase activity
• Light output is proportional to h or sm 51 promoter activity.

Data

Comparison of human and sm promoter activity:
The activity of the human or sm promoter was divided by the activity of the control (pGL3 Basic vector without a promoter) to calculate fold change over pGL3.

Conclusions

According to the t-Test, there is no significant difference in the activities of the squirrel monkey and human 500 base pair promoter sequences.

\[ t_{\text{stat}} = 1.25 \quad < \quad t_{\text{critical}} = 2.10 \]

\[ \text{t-Test: Two-Sample Assuming Equal Variances} \]

\[ \begin{array}{cc}
\text{h51p} & \text{sm51p} \\
\text{Mean} & 42.3 & 26.5 \\
\text{Observations} & 10 & 10 \\
\text{t Stat} & 1.25 \\
\text{t Critical two-tail} & 2.10
\end{array} \]

Future Directions

1. Upon examining a larger promoter (>2kb) region, we have identified a difference in sizes of the human and squirrel monkey promoters, suggesting that 600 base pairs are deleted from the squirrel monkey 51 promoter.
2. Therefore, a promoter region (squirrel monkey and human) >2kb in length needs to be isolated and tested.

Acknowledgments

This work was supported by a College of Arts and Sciences Research Grant and the Department of Biology, University of North Alabama

Dr. Jonathan Scammell, University of South Alabama

References


Hubler TR et al. "The FK506-binding immunophilin FKBP51 is transcriptionally regulated by progestin and attenuates progestin responsiveness." Endocrinology 114.6 (2003): 2380-2387