CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

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Project Number

S0524

Project Title

shRNA-Mediation of UGGT1 to Modulate Excessive Procollagen Secretion in Cardiac Fibrosis

Abstract

Objectives

Pathological scarring of cardiac ECM through excessive collagen deposition is the primary cause of cardiac fibrosis, the predominant phenomenon characterizing the current heart failure epidemic. I aimed to target collagen post-translational modification as a novel strategy, specifically the N-linked glycosylation folding cycle in the ER of myofibroblasts. I hypothesized that in fibrosis, the enzymatic activities of UGGT1, the main regulator of collagen secretion, are increased, giving misfolded procollagen inappropriate folding time, and thus disrupting the ER folding cycle control machinery through the accumulation of procollagen. This project s main purpose is to answer the question: do excessive procollagen secretion levels decrease when UGGT1 is inhibited?

Methods

First, I calculated transfection efficiencies for myofibroblast cells obtained from fibrotic mice models and human donors using a beta-gal reporter vector to determine efficacy. I then identified three target sequences for the UGGT1 gene and designed a nonspecific control. To inhibit UGGT1, shRNA constructs were developed through ligation of an adenovirus plasmid vector with synthesized oligonucleotides specific to the selected target sequences. The constructs were cloned through transformation, screened with restriction digestion, and then used to transfect the myofibroblasts. UGGT1 expression levels, procollagen secretion, and intracellular retention or procollagen were analyzed by Western blot of cell lysates.

Results

Both mRNA and protein expression levels of UGGT1 were significantly inhibited in human and mouse fibrotic myofibroblast models, indicating the success of the constructed shRNA knockdown vectors. Procollagen secretion and intracellular retention levels of the control cells were significantly higher than healthy levels. Those levels decreased significantly in UGGT1 inhibited cells transfected with the most effective shRNA construct when compared to the control.

Conclusions

The results established UGGT1 and its myofibroblast ER folding cycle as a qualified therapeutic target to treat cardiac fibrosis. Additionally, my project defined increased UGGT1 activity as a major cause of excessive procollagen secretion, indicating that shRNA inhibition will be instrumental in the development of a clinical strategy. Further delineation of exact mechanisms will be the next step in this investigation.

Summary Statement

I constructed shRNA adenovirus vectors to inhibit UGGT1 in myofibroblasts, decreasing procollagen intracellular retention and secretion levels, and thus establishing the UGGT folding cycle as a novel therapeutic target for cardiac fibrosis.

Help Received

I utilized the lab equipment and materials of the Greenberg Lab at the University of California, San Diego and received limited mentorship from Dr. Randy Cowling.