shRNA-Mediation of UGGT1 to Modulate Excessive Procollagen Secretion: A Novel Approach to Treatment of Cardiac Fibrosis

Wang, Kaitlyn (School: Canyon Crest Academy)

Myocardial fibrosis is a significant global health problem associated with nearly all forms of heart disease. It is primarily caused by hypertrophic scarring of the heart's ECM through pathological collagen deposition by activated cardiac fibroblasts. As a novel approach to treating cardiac fibrosis, I inhibited UGGT1, the main enzymatic gatekeeper of the collagen glycosylation folding cycle in the endoplasmic reticulum (ER) of myofibroblasts, aiming to delineate the mechanisms behind UGGT1's post-translational control of excessive collagen secretion in fibrosis. To knockdown UGGT1 activity, shRNA constructs were developed through ligation of a recombinant adenovirus plasmid vector with synthesized oligonucleotides specific to three selected target sequences and a nonspecific control. The constructs were then optimized and used to transfect human and mouse cardiac myofibroblasts. UGGT1 expression and procollagen intracellular retention levels were analyzed by Western blot of cell lysates and differential procollagen and ECM glycoprotein secretion in conditioned media was examined through several protein assessment methods. mRNA and protein expression levels of UGGT1 were significantly inhibited in both fibrotic myofibroblast cell models. Pathologically high procollagen intracellular retention and secretion levels seen in control cells decreased significantly in UGGT1 inhibited cells transfected with the most effective shRNA construct. The results established UGGT1 and its fibroblast ER folding cycle as a qualified therapeutic target to treat cardiac fibrosis. Additionally, it was identified that increased UGGT1 activity is a major cause of excessive collagen deposition, indicating that shRNA inhibition will be instrumental in the development of a clinical strategy.

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