Nonalcoholic fatty liver disease (NAFLD) is a family of chronic liver disease characterized by inflammation and fat buildup in the organ. It currently affects over 30% of United States adults, especially those with type 2 diabetes and obesity. Liver fibrosis is a type of damage part of the NAFLD spectrum. Its onset comes after NAFLD has progressed to non-alcoholic steatohepatitis (NASH), and itself can increase in severity to cirrhosis. Fibrosis occurs when hepatocyte regeneration causes large amounts of scar tissue and collagen to build up in the organ. Unlike normal liver cells, scar tissue cells are unable to function or self-repair, thus reducing overall liver health.

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A recent study demonstrated a relationship between the onset of fibrosis in the body and the presence of glutaredoxin-1 (Glrx), a redox enzyme involved in systematic glutathione-disulfide catalyzation pathways. In idiopathic pulmonary fibrosis in the lung, overexpression of Glrx reversed fibrotic symptoms in mice. Glrx deficiency also accelerates symptoms of liver fibrosis through signaling pathways by inhibiting sirtuin-1, a protein involved in deacetylation, which leads to increased regulation of functional hepatic enzymes.

This experimental study tested the effects of wild-type (WT) and catalytically inactive C235/C265 mutant Glrx as adeno-associated virus (AAV) gene therapy in fibrotic cells.

**METHODS**

- **Vector Growth**
- **Miniprep**
- **Amplification & Digestion**
- **Gibson Assembly**
- **Cell Culture & Transfection**
- **Virus Production**

**RESULTS**

**Fig. 6** Gel results of WT construct, C235/C265 construct (both cut with EcoRI), and AAV capsid pAAV/DJ8 verification cut with PvuII, respectively. (A) and (B) have bands of 869, 1363, & 3203 bp reflecting 3 restriction sites of EcoRI. (C) has 3 bands reflecting 3 restriction sites of PvuII.

**Fig. 9** Sequencing results of WT and C235/C265 plasmid constructs. (A) and (B) are sequencing results from GeneWiz with Glrx noted from the arrows. Chromatograms in (C) show the difference in sequence between WT Glrx and C235/C265 Glrx.

**Fig. 10** Protein gel of virus titration. In an ideal gel, three bands within 50-100 kDa are visible with no smearing (contamination). This is shown in samples 2-4 under iodixanol.

**REFERENCES**

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