A Comparative Study of Fibrosis Treatments using Aptamer-Based Quantitative Proteomics in a Model of Nonalcoholic Steatohepatitis Cirrhosis

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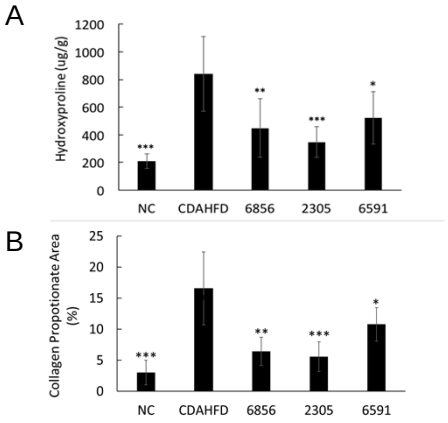
Background & Aims

Cirrhosis is the common endpoint of all chronic liver diseases, including nonalcoholic steatohepatitis (NASH). Several therapeutics are currently under clinical investigation for the treatment of NASH cirrhosis and they target key features of the disease including lipogenesis, inflammation, and fibrogenesis. Effective biomarkers to assess the efficacy of these therapeutics are urgently needed and could also provide insights into shared mechanisms of action. Here, we investigate three anti-fibrotic therapeutics in a pre-clinical model of NASH cirrhosis: two ASK1 inhibitors, EP-026856 and selonsertib, and one FXR agonist, EDP-305.

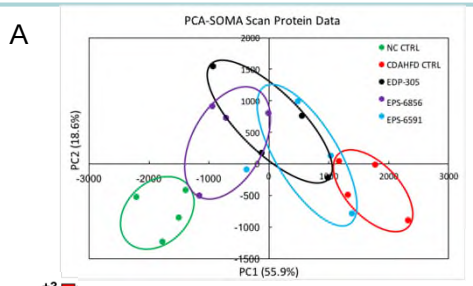
Methods

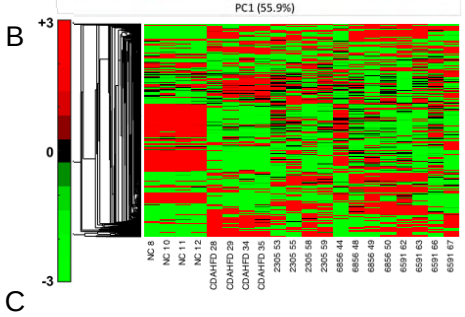
Male Wistar rats (175-200 g) were fed either normal chow (NC) or a choline-deficient, L-amino acid-defined, high fat diet with 60 kcal% fat and 0.1% methionine (CDAHFD) for 12 weeks. Animals were randomized to receive daily oral gavage of either vehicle (0.5% methylcellulose (MC)), 30 mg/kg EDP-305, 30 mg/kg EP-026856, or 30 mg/kg selonsertib at the first signs of fibrosis (5 weeks, n = 8 per group). At 12 weeks, animals were sacrificed and serum samples, collected via cardiac puncture, were sent for proteomics analysis using SOMAScan, which was then analyzed utilizing a multivariate approach

Results



Hydroxyproline content (A) and collagen proportionate area (CPA) (B) were measured in livers after NC (normal chow) alone, CDAHFD alone, 6856 (EP-026856), 2305 (EDP-305) and 6591 (selonsertib) revealing significant reduction in fibrosis across all treatment groups when compared to CDAHFD rats. Compared to CDAHFD * p<0.05; ** p< 0.005; *** p<0.001





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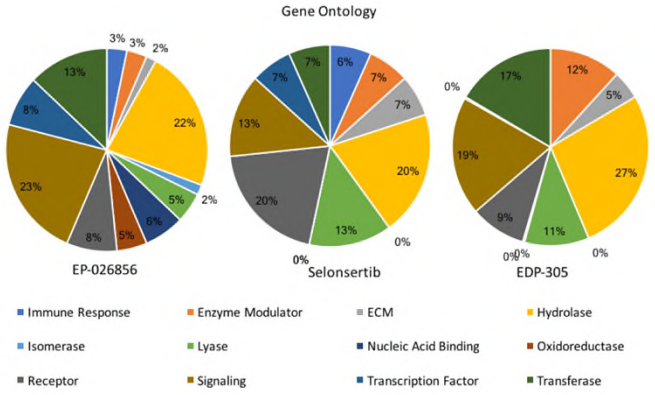
Protein	FC (CDAHFD:NC)	FC (EDP305:CDAHFD)	FC (EP-026856:CDAHFD)	FC (selonsertib:CDAHFD)
Serine protease HTRA2	12.91	0.32	0.92	1.24
60 kDa heat shock protein	9.80	0.26	0.55	0.71
Semaphorin-6A	9.79	0.73	0.61	0.63
Phosphoglycerate mutase 1	9.28	0.70	0.86	0.67
Formimidoyltransferase-cyclodeam.	8.82	0.36	1.65	1.97
IGF-binding protein 2	7.76	1.59	0.23	0.50
Bone morphogenetic protein 6	7.66	0.53	0.49	0.62
Cytochrome c	7.14	2.30	2.41	2.05
Aminoacylase-1	7.06	0.41	0.78	1.07
SPARC-like protein 1	6.04	0.59	0.63	0.77

Principle component analysis (PCA) (A) revealed highest similarity between normal chow rats and CDAHFD rats treated with EP-026856. These two groups separated across principle component 1 (PC1) from all other treatment groups. Hierarchical clustering (B) by protein (rows) revealed qualitative changes upon introduction of diet and subsequent treatments with EDP-305 (2305), EP-026856 (6856), or selonsertib (6591). Proteins were ranked (C) fold-changes (FC) and top differential proteins were selected for further analysis.



Differential proteins were classified by function based on gene ontology classifications, using PANTHER, and compared between EP-026856 and EDP-305. EP-026856 (ASK1 inhibitor) showed broader affects at the protein function level when compared against EDP-305 (FXR agonist)

Conclusions

By utilizing SOMAScan, we were able to identify non-invasive biomarkers of treatment response and potential pathways activated by different drugs currently in clinical trials. While all three drugs were effective in inhibiting fibrosis development in this rat model of NASH cirrhosis, PCA analysis identified EP-026856 has having the greatest effect on returning non-invasive serum markers back to baseline.

References

Krämer, A., Green, J., Pollard Jr, J. and Tugendreich, S., 2013. Causal analysis approaches in ingenuity pathway analysis. *Bioinformatics*, *30*(4), pp.523-530.

Paul D. Thomas, Michael J. Campbell, Anish Kejariwal, Huaiyu Mi, Brian Karlak, Robin Daverman, Karen Diemer, Anushya Muruganujan, Apurva Narechania. 2003. PANTHER: a library of protein families and subfamilies indexed by function. Res., 13: 2129-2141 .