THE IDENTIFICATION OF NOVEL SMALL MOLECULE COMPOUNDS WITH POTENT ANTI-FIBROTIC PROPERTIES BY PHENOTYPIC SCREENING OF PRIMARY HUMAN STELLATE CELLS

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INTRODUCTION
Fibrosis is a devastating outcome of many chronic liver diseases, where an excess deposition of extracellular matrix impairs liver function. There is presently approved therapeutic for liver fibrosis. Indeed, a number of small molecules or biologics are being investigated in clinical trials.

AIMS
To identify new drug candidates with potential anti-fibrotic properties by using a relevant and unbiased phenotypic screening approach in a library that contains both new chemical entities and FDA-approved drugs.

STUDY DESIGN

PHENOTYPIC HTS SET-UP AND VALIDATION

A phenotypic screening assay was implemented to identify small molecules with antifibrotic properties in hepatic stellate cells.

(A) General outline of the semi-robotized phenotypic screening assay. The HSC were pretreated for 5 min with the compound followed by TGF-β1 stimulation. The measure of α-SMA was performed by a frame-based sandwich ELISA on the HSC lysate.

(B) A time-course inhibitor (GFE2137) served as a positive control for inhibition of α-SMA production in TGF-β1 stimulated HSC. The factor of 0.5 was obtained by the time-scan rate, thus allowing to determine the inhibitory potential of a hit.

(C) The equation used to measure the inhibition percentage of a hit.

(D) Three-run variability was assessed on a subsample of 127 compounds. In order to limit the number of false positives, a stringent threshold of 49% was chosen. A related coefficient of 0.86% was obtained (positive hits: 5% > IC50 > 2% and IC50 > 12%). A calculated coefficient of variation of 0.7% was further investigated in phenotypically assays in both HSC and primary hepatocytes.

STATISTICAL ANALYSIS

Data was fitted a GMM

3p0.05, 3p0.01, 3p0.001 using GraphPad ANOVA and Benferroni post-hoc analysis

IDENTIFICATION OF HTS WITH ANTI-FIBROTIC PROPERTIES IN HSC

Various new chemical entities from GENFIT’s library and some FDA-approved drugs (diverse indications) showed strong anti-fibrotic properties in HSC, IC50 < 0.1µM and max inhibition > 60%.

(D) Representative diagram of hit compound efficacy on α-SMA inhibition in the screening campaign. HSCα from different series and compounds from the pharmacopeia are color coded.

(C) Dose-response analyses of best hits from the GENFIT’s library (in orange) and from the repositioning campaign. The hit compounds were further investigated in phenotypically assays in both HSC and primary hepatocytes.

IN VIVO PROOF OF CONCEPT STUDY WITH A PRIORITIZED CANDIDATE

Treatment with GFE2137 significantly attenuated fibrosis in the murine CDA Anch model. (A) C57BL/10Tg-f/f mice were fed a control diet (CSAA), or a choline-deficient diet (Choline-deficient diet supplemented with cholic acid) for 10 weeks. Hepatic total collagen content, and liver expression of the pro-fibrogenic markers COL1α1 and TGFβ1 were strongly reduced by treatment with GFE2137.

REFERENCES

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* p<0.05, ** p<0.01, *** p<0.001 using One-way ANOVA and Bonferroni post-hoc analysis.

FDA-approved drugs with unsuspected anti-fibrotic properties.

Several candidates that play to classical drug-therapeutic properties were identified among the hit compounds with strong anti-fibrotic activity in HSCs.

Anti-fibrotic properties of a prioritized hit compound (GFE2137) were confirmed in the CDA Anch model in vivo.

Other promising lead candidates will be further investigated in animal models of fibrosis. As well, complementary studies aiming to define the mechanism of action and molecular targets will be undertaken.

Drug repositioning is becoming a popular alternative to classical drug discovery programs. Our phenotypic screening approach has led to the discovery of known FDA-approved drugs with unsuspected anti-fibrotic properties.

"The identification of novel small molecule compounds with potent anti-fibrotic properties by phenotypic screening of primary human stellate cells."