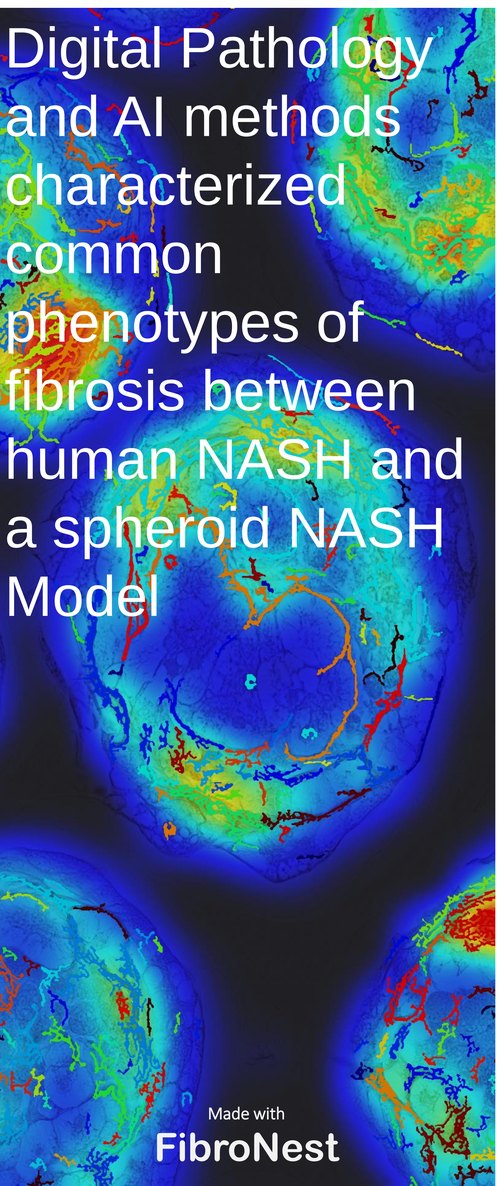

Description of Translational Fibrosis Phenotypes between the 3D NASH Spheroid Model and Human NASH

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1 Introduction

The adoption of spheroid models in the screening of anti-fibrotic compounds for Non-Alcoholic Steatohepatitis (NASH) has led to the need to understand the phenotypic relevance of the fibrosis histological phenotypes and their clinical translational relevance.

2 Aim

Here, we use a novel Digital Pathology quantitative image analysis and AI platform, FibroNest™, to generate and measure quantifiable Fibrosis Traits (qFTs) in Human in-vitro 3D NASH tissue models and natural cohort of patients diagnosed with NASH. Hundreds of histological parameters are measured in both models and a Venn diagram approach is used to identify those traits that describe histological fibrosis severity in both models.

3 Method

- Two 3D-NASH spheroid-colony groups **(Lean n=17, NASH n=14)**
  - 3D liver tissues were either treated for 10 days with free fatty acids and LPS or not to generate NASH and lean conditions respectively
- A retrospective clinical cohort (**n=104** patients) with NASH diagnosed by histologic assessment of liver biopsy according to NASH CRN criteria by pathologists (**F0(26), F1(24), F2(28), F3(20), F4(6)**).

Spheroid Information

- Human in vitro 3D InSight™ liver microtissues
- Contain primary hepatocytes, Kupffer cells, endothelial cells and hepatic stellate cells

Pathology, Digital Pathology and AI:

- 4µm thick Spheroid FFPE slices were stained with **Picro Sirus Red** and scanned at **40x**
- FFPE sections of liver human biopsies were stained with **Picro Sirus Red** and scanned at **20x**
- **Scanning** was done with bright-light whole slide imaging scanners.
- **Fibrosis severity continuous score** (**Ph-FCS**, 1 to 10). Quantitative image analysis extracts single-fiber quantitative traits (qFTs, N=315) from the fibrosis histological phenotype. Principal qFTs are automatically detected to best describe the progression of the fibrosis in both models and combined into a normalized Phenotypic Composite Fibrosis Score (Ph-FCS).
- **A Venn diagram** approach was used to identify those traits that describe histological fibrosis severity in both models. qFTs are normalized to their initial value and their folds describe relative levels of progression which can be benchmarked from a model to another
- Additional **sub-Phenotypic scores** (fine and assemble fiber sub-classes, morphometry, architecture, fibrosis scar) are used to further describe the fibrosis phenotypes and its remodeling as fibrosis progress or regresses

5 Conclusions

We identified 97 histological traits of fibrosis severity phenotype that can be translated from the human 3D NASH spheroid model to clinical F2 or F3 NASH CRN stages. These traits will be used to evaluate the anti-fibrotic compounds effect in 3D NASH model to predict their effect in human.

6 Contact information

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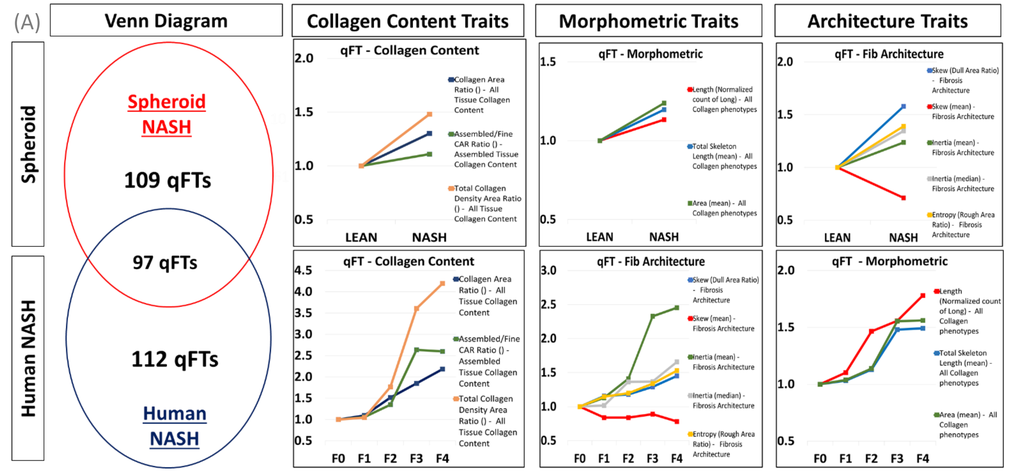
4 Results

- The Venn Diagram identifies 97 traits shared between the two fibrosis progression models (B).

(A)



- **10 traits described high-level collagen features** such as Collagen Area Ratio, Assembled/Fine Collagen fibers area ratio, and density (intensity weighted) area ratio (selected qFTs on figure A).
- **61 traits described common morphometric** features, such as the proportion of collagen fibers that are long, the mean fiber skeleton length, and the mean fiber area (selected qFTs on figure A).
- **26 traits described common architectural** features (selected qFTs on figure A).

- The spheroid NASH model cannot be directly associated to a specific human NASH CRN stage number. However, specific qFTs in the NASH spheroid model can be associated with either F2 or F3 stages based on their fold-change values.

(B)