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Collagen co-localized with macrovesicular steatosis better differentiates fibrosis progression in non-alcoholic fatty liver disease mouse models

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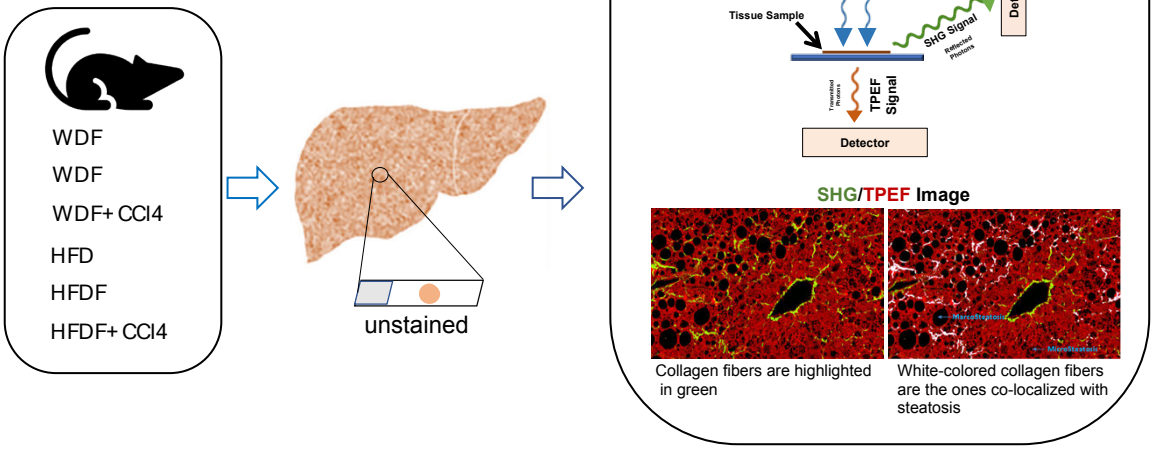
Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is a global commonly occurring liver disease. However, its exact pathogenesis is not fully understood. The purpose of this study was to quantitatively evaluate the progression of steatosis and fibrosis by examining their distribution, morphology, and co-localization in NAFLD animal models.

Method: Six mouse NAFLD groups were established: (1) western diet (WD) group; (2) WD with fructose in drinking water (WDF) group; (3) WDF + carbon tetrachloride (CCl4) group, WDF plus intraperitoneal injection of CCl4; (4) high-fat diet (HFD) group, (5) HFD with fructose (HFDF) group; and (6) HFDF + CCl4 group, HFDF plus intraperitoneal injection of CCl4. Liver tissue specimens from NAFLD model mice were collected at different time points. All the tissues were serially sectioned for histological staining and Second-harmonic generation (SHG)/ two-photon excitation fluorescence imaging (TPEF) imaging. The progression of steatosis and fibrosis was analyzed using SHG/TPEF quantitative parameters with respect to the NASH CRN scoring system.

Results: qSteatosis showed a good correlation with steatosis grade (R: 0.823–0.953, P<0.05) and demonstrated high performance (AUC: 0.617–1) in six mouse models. Based on their high correlation with histological scoring, qFibrosis containing four shared parameters (#LongStrPS, #ThinStrPS, #ThinStrPSAgg, and #LongStrPSDis) were selected to create a linear model that could accurately identify differences among fibrosis stages (AUC: 0.725–1). qFibrosis co-localized with macrosteatosis generally correlated better with histological scoring and had a higher AUC in six animal models (AUC: 0.846–1).

Conclusion: Quantitative assessment using SHG/TPEF technology can be used to monitor different types of steatoses and fibrosis progression in NAFLD models. The collagen co-localized with macrosteatosis could better differentiate fibrosis progression and might aid in developing a more reliable and translatable fibrosis evaluation tool for animal models of NAFLD.

Figure:



The collagen co-localized with macrosteatosis could better differentiate fibrosis progression and might aid in developing a more reliable and translatable fibrosis evaluation tool for animal models of NAFLD.

Collagen Co-Localized with Macrovesicular Steatosis Better Differentiates Fibrosis Progression in Non-Alcoholic Fatty Liver Disease Mouse Models

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a global commonly occurring liver disease. However, its exact pathogenesis is not fully understood. Precise, objective, and dynamic evaluation of liver histology in preclinical NAFLD animal models is valuable for developing more effective drugs.

Aim

The purpose of this study was to quantitatively evaluate the progression of steatosis and fibrosis by examining their distribution, morphology, and co-localization in NAFLD animal models.

Method

Six mouse NAFLD groups were established: 1) western diet (WD) group; 2) WD with fructose in drinking water (WDF) group; 3) WDF + carbon tetrachloride (CCl4) group, WDF plus intraperitoneal injection of CCl4; 4) high-fat diet (HFD) group, 5) HFD with fructose (HFDF) group; and 6) HFDF + CCl4 group, HFDF plus intraperitoneal injection of CCl4. Liver tissue specimens from NAFLD model mice were collected at different time points. All the tissues were serially sectioned for histological staining and second harmonic generation (SHG)/two-photon excitation fluorescence imaging (TPEF) imaging. The progression of steatosis and fibrosis was analyzed using SHG/TPEF quantitative parameters with respect to the non-alcoholic steatohepatitis Clinical Research Network scoring system.

Conclusions

Quantitative assessment using SHG/TPEF technology can be used to monitor different types of steatosis and fibrosis progression in NAFLD models. The collagen co-localized with macrosteatosis could better differentiate fibrosis progression and might aid in developing a more reliable and translatable fibrosis evaluation tool for animal models of NAFLD.

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References

1.Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: Trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol (2021) 18:223–38.
2.Liu F, Goh GB, Tiniakos D, et al. qFIBS: An automated technique for quantitative evaluation of fibrosis, inflammation, ballooning, and steatosis in patients with nonalcoholic steatohepatitis. Hepatology (2020) 71:1953–66.
3. Tandra S, Yeh MM, Brunt EM, et al. Presence and significance of microvesicular steatosis in nonalcoholic fatty liver disease. J Hepatol (2011) 55:654–9.

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Results

1.Good Correlations were Observed Between the Histopathology and SHG/TPEF Assessments of Steatosis in All Mouse Models

Liver steatosis progression was quantified by examining the fat vacuoles in the TPEF channel. Overall, 45 steatosis parameters were extracted from the whole-tissue images. Good correlations were observed for all steatosis parameters with steatosis scores in all six NAFLD mouse models (Figure 1). For representative parameters such as %Area (percentage of steatosis in the overall region), %MacroArea (percentage of macrosteatosis in the overall region), and %MicroArea (percentage of microsteatosis in the overall region), the Spearman correlations ranged from 0.823 to 0.953 (P<0.05) in all mouse models. In addition, steatosis area parameters demonstrated high AUC values for differentiating steatosis grades (0.810–1).

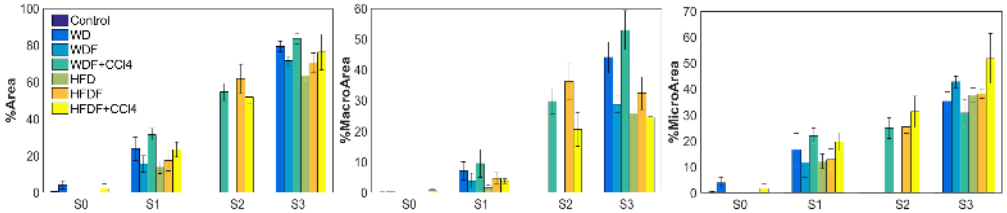

Figure 1. The trends of the representative steatosis parameters with respect to the steatosis grades from the six animal models.

2. The qFibrosis Index Combining Four Shared Morphological Parameters Faithfully Recapitulated the Fibrosis Staging

Based on their quantitative trends of fibrosis stages and systemic AUC analyses, four shared parameters of collagen string (#LongStrPS, #ThinStrPS, #ThinStrPSAgg and #LongStrPSDis) were selected to combine qFibrosis indices, which showed good correlation with fibrosis stages in all animal models (R: 0.501–0.911, P<0.05). These indices can also accurately identify differences among fibrosis stages (AUC: 0.725–1). Furthermore, the performances of qFibrosis indices versus CPA for scoring fibrosis were evaluated using receiver operating characteristic analysis; these AUC values were mostly higher than those using CPA in differentiating fibrosis stages (0.725–1 vs. 0.531–1).

3. qFibrosis Co-localized with Macrosteatosis Showed Superior Performance Compared to CPA, qFibrosis, and qFibrosis Co-localized with Microsteatosis in Evaluating Fibrosis Severity

The relationship between steatosis and fibrosis progression was further analyzed by examining the co-localization (Figure 2). The results revealed that qFibrosis co-localized with macrosteatosis was generally correlated better with histological scoring than qFibrosis (R: 0.598–0.914, P<0.05), and qFibrosis co-localized with microsteatosis (R: 0.516–0.946, P<0.05) in most animal models. Furthermore, using receiver operating characteristic analysis, the AUC values of qFibrosis co-localized with macrosteatosis for the detection of different stages were > 0.875 (AUC: 0.875–1), whereas the AUC values of qFibrosis were >0.725 (AUC: 0.725–1) and the AUC values of qFibrosis co-localized with microsteatosis were >0.615 (AUC: 0.615–1).

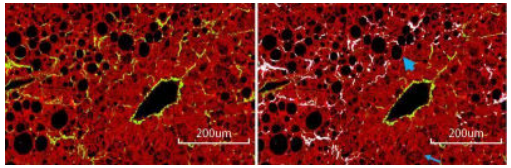

Figure 2. Collagen co-localized with steatosis. The subfigure on the left is a raw ROI image; the subfigure on the right is the corresponding annotated image, where white-colored collagen fibers are co-localized with steatosis. The bold and thin arrow points to an example of macro- and microsteatosis, respectively.