Dynamic changes in type III collagen formation and degradation are associated with fibrotic activity following hepatic injury in rats.

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BACKGROUND

Non-invasive biomarkers of collagen turnover enable assessment of drug effects on the extracellular matrix. Type III collagen formation (fibrogenesis) and degradation (fibrolysis) can be assessed with nordicPRO-C3™ and nordicCTX-III™, respectively. A shift toward fibrolysis has recently been associated with improvement of hepatic fibrosis in patients with metabolic dysfunction-associated steatohepatitis (MASH), making PRO-C3 and CTX-III relevant biomarkers for translational studies of hepatic fibrosis.

We developed rodent versions of CTX-III (rCTX-III) and PRO-C3 (rPRO-C3) and applied them in two preclinical models of hepatic fibrosis.

METHODS

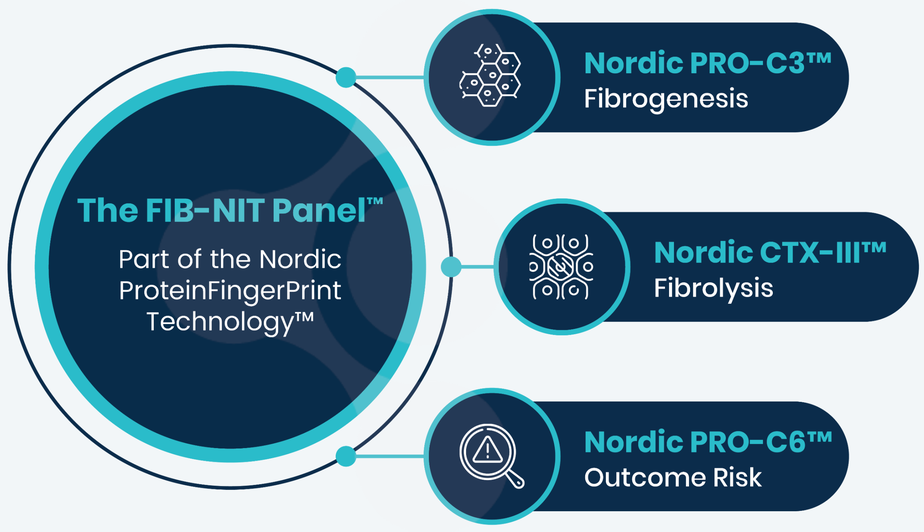
A chemiluminescent sandwich immunoassay for nordic rCTX-III and a competitive enzyme-linked immunosorbent assay for nordic rPRO-C3 were developed for rodent blood samples using monoclonal antibodies. **rCTX-III detects a protease-generated neo-epitope in a cross-linked dimeric analyte, while rPRO-C3 targets a pro-peptide neo-epitope.** rCTX-III and rPRO-C3 were measured in serum of two rat models: 83 rats with CCl4-induced fibrosis by inhalation (1 L/min) twice weekly and given water containing phenobarbital (0.3 g/L) for 8, 12, 16, or 20 weeks (wks) including regression groups with 15 or 20 wks of CCl4 followed by 6 wks of washout compared with healthy controls (n = 7 per group); and a bile-duct ligation model for 1 (n = 7), 2 (n = 7), or 3 (n = 8) wks of bile duct ligation, plus 3-week sham surgery (n = 8) and baseline (n = 13).

RESULTS

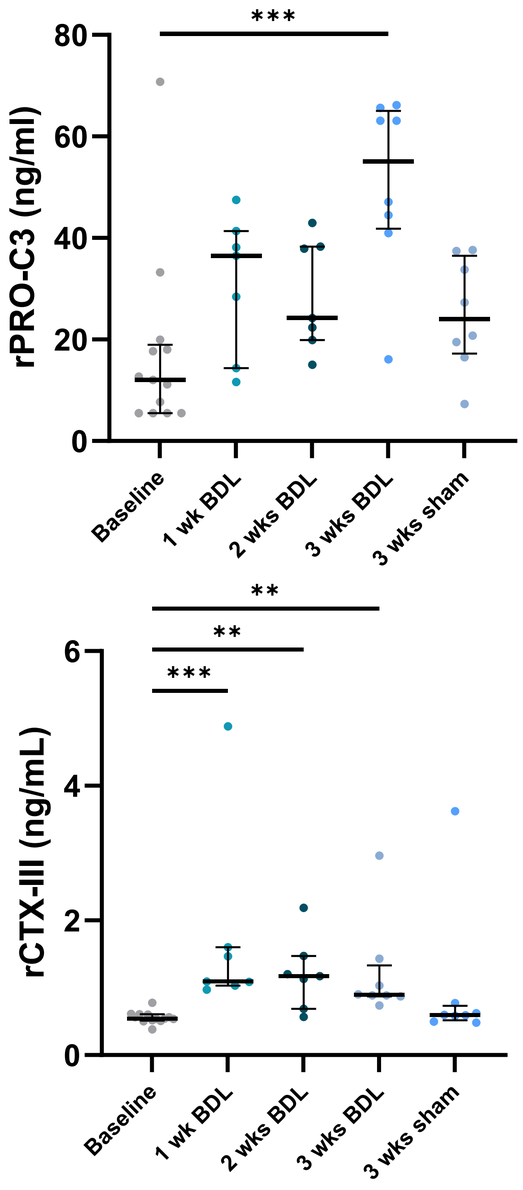
Assay performance

Technical performance	rPRO-C3	rCTX-III
Measurement range, LLOD-ULOD	5.52 - 552.71 ng/mL	0.30 – 5.56 ng/mL
Intra-assay variation, CV%	4%	10.7%
Inter-assay variation, CV%	17%	19.7%

LLOD: lower limit of detection; ULOD: upper limit of detection; CV: coefficient of variation; SD: standard deviation

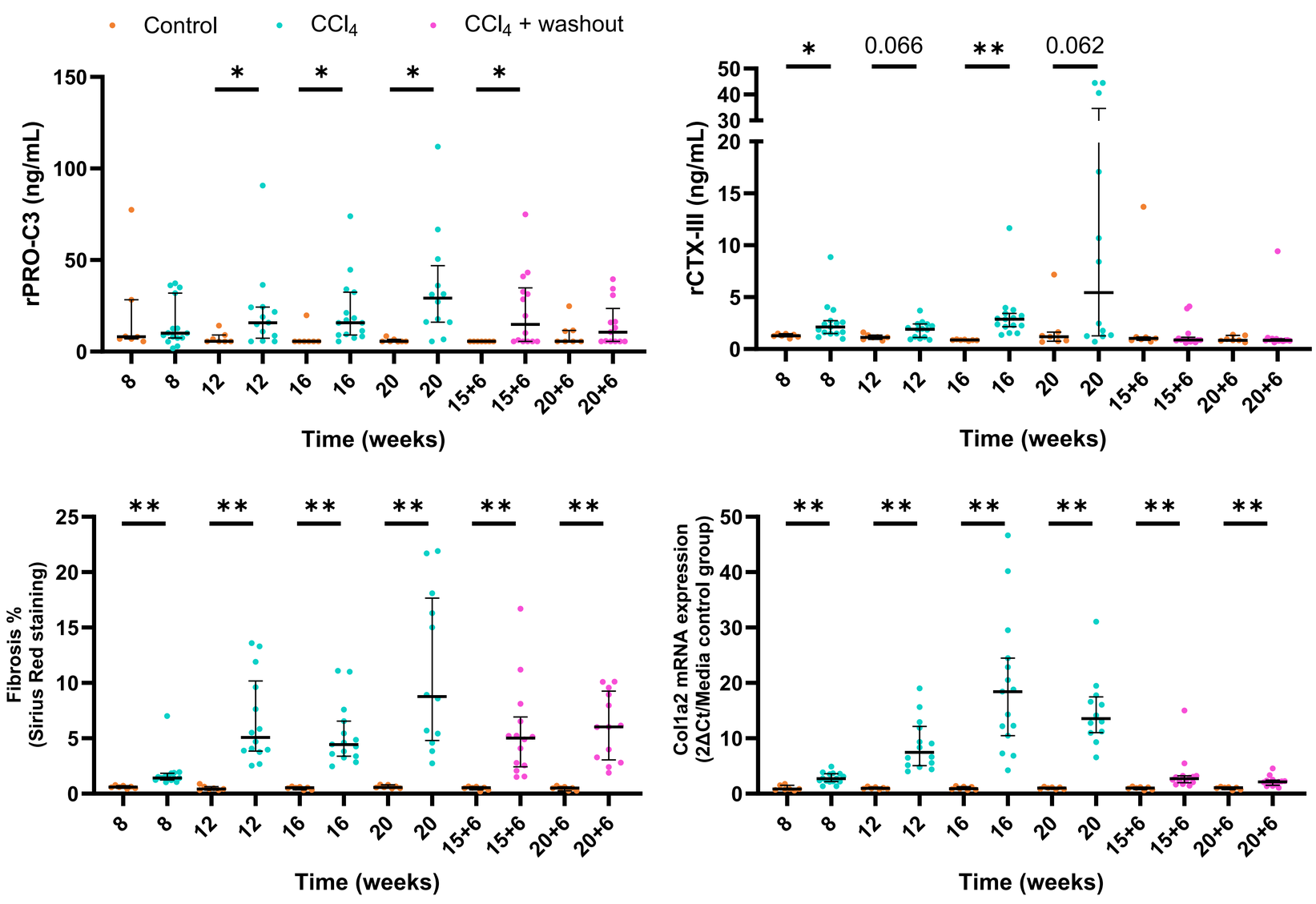


Bile duct ligation rat model



*Fibrogenesis and fibrolysis assessed by rPRO-C3 and rCTX-III respectively in rats following bile duct ligation surgery for 1, 2, and 3 weeks (wks), compared to baseline and 3 wks sham surgery. Between groups comparisons were performed with Kruskal-Wallis test with post-hoc Dunn's test for multiple comparisons. Number of rats per group: Baseline n=13, 1 wk BDL n=7, 2 wks BDL n=7, 3 wks BDL n=8, 3 wks sham n=8. Asterisks indicate: ** = p<0.01, *** = p<0.001.*

CCl_4-inhalation rat model



*Fibrogenesis assessed by rPRO-C3, fibrolysis assessed by rCTX-III, Col1a2 mRNA expression, and histologically assessed fibrosis percentage by Sirius Red staining of rat liver tissue, stratified by control and CCl_4-treatment, and CCl_4-treatment + six weeks of washout groups. Mann-Whitney test compared CCl4-treatment groups and corresponding control groups followed by Benjamini-Hochberg correction for false discovery. Number of rats per CCl_4-treatment group: 8 n=15, 12 n=14, 16 n=15, 20 n=12, 15+6 n=14, 20+6 n=13, and n=7 per control group. Asterisks indicate: * = p<0.05, ** = p<0.01.*

KEY MESSAGES

- **We developed robust assays for the rodent fibrolysis biomarker rCTX-III and fibrogenesis biomarker rPRO-C3**
- **The biomarkers were applied in two hepatic fibrosis rat models, showing increased type III collagen turnover after injury.**
- **rCTX-III and rPRO-C3 may serve as translational biomarkers in antifibrotic drug development.**

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Disclosures: TW, MKS, YMM, MK, and DJL are employed at Nordic Bioscience. MK and DJL hold stocks in Nordic Bioscience.