

PROCEEDINGS

A 3D Bio-Printed Liver Disease Model Revealed Notch1-Driven Copper–Mechanics Crosstalk in NASH-HCC

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ABSTRACT

Background: NASH-associated hepatocellular carcinoma (NASH-HCC) develops within a fibrotic, lipid-enriched liver niche. As extracellular matrix (ECM) stiffness increases, mechanosensitive signaling (including Piezo1) is engaged and can promote immunosuppressive macrophage polarization. Concurrently, disrupted copper handling contributes to oxidative stress, ECM remodeling, and diminished susceptibility to cuproptosis. Here, we aim to investigate how Notch1 connects copper dysregulation with mechanical signaling during the NASH-to-HCC transition, integrating a stiffness-controllable 3D bioprinted liver model.

Methods: We quantified Notch paralog expression using transcript-based analyses and immunohistochemistry in clinical cohorts, and evaluated survival associations with Cox regression. Fibrosis burden was measured by Sirius Red staining, and relationships among Notch1, ATP7B (copper export), Piezo1 (mechanosensing), and M2 macrophage markers (CD163/CD206) were assessed using both local specimens and public datasets. Cuproptosis-associated gene networks were characterized by WGCNA followed by GO/KEGG pathway enrichment. Mechanistic validation was performed by γ -secretase inhibitor (GSI) treatment in HepG2 cells. To model disease mechanics, we engineered AlgMA hydrogels spanning fibrosis-relevant stiffness and constructed a coaxial 3D-printed system representing soft NASH versus stiff NASH-HCC microenvironments.

Results: Notch1 was the most prominently upregulated Notch paralog in NASH-HCC and was associated with poorer overall and disease-free survival; importantly, Notch1 remained an independent prognostic factor alongside UICC stage (Figure 1A-C). Notch1 expression increased with fibrosis severity and correlated positively with ATP7B and Piezo1 (Figure 2A). Piezo1 was upregulated in NASH-HCC, associated with poorer survival, and spatially aligned with CD206⁺ macrophages in stiffer regions (Figure 2B-D). Cuproptosis-related genes were enriched for immune regulatory programs and collagen/ECM pathways (Figure 3A-B). Pharmacologic Notch blockade with GSI suppressed Notch1/HES1 signaling and produced dose-dependent decreases in ATP7B and Piezo1 (Figure 3C-D). The AlgMA and coaxial bioprinted constructs reproduced fibrosis-range stiffness and showed increased lipid staining within the stiff NASH-HCC compartment (Figure 4A-C).

Conclusion: Notch1 integrated copper-metabolic and mechanical signaling to promote ECM remodeling and induced M2-like macrophage mechano-reprogramming, ultimately accelerating NASH-HCC progression. In aggregate, our 3D bio-printed liver disease platform enabled mechanistic studies and therapeutic screening of this pathway.

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