Antifibrotic Drug Response in a Long-Term 3D Human Hepatic Stellate Cell Model of Progressive ECM Remodeling

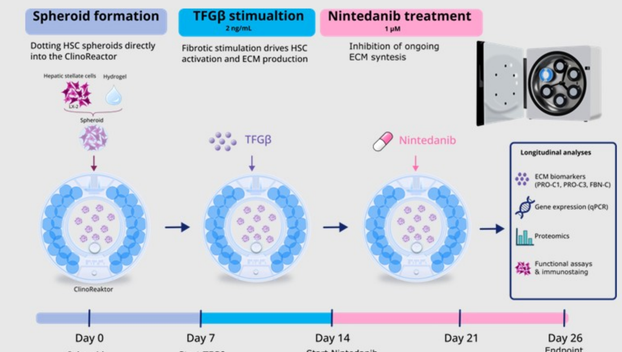
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Introduction

Liver fibrosis is driven by activation of hepatic stellate cells (HSCs) and progressive extracellular matrix (ECM) accumulation. The development of effective antifibrotic therapies is hindered by limited availability of human-relevant in vitro models that capture long-term fibrotic remodeling.

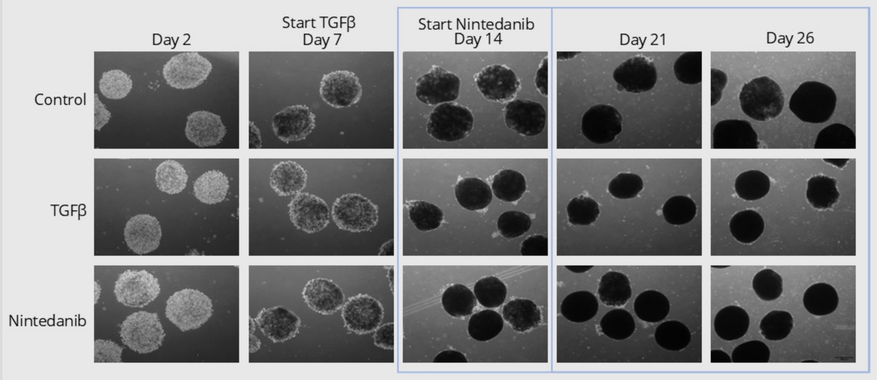
Workflow

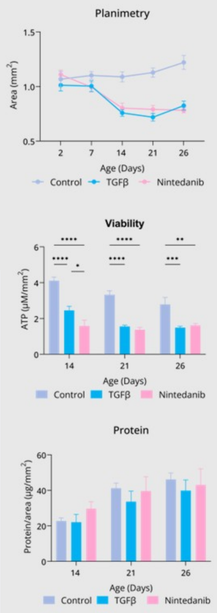
Experimental timeline illustrating fibrosis induction, therapeutic intervention, and longitudinal analysis in HSC spheroids.



Dynamic HSC spheroids remain stable during prolonged TGFβ and Nintedanib exposure

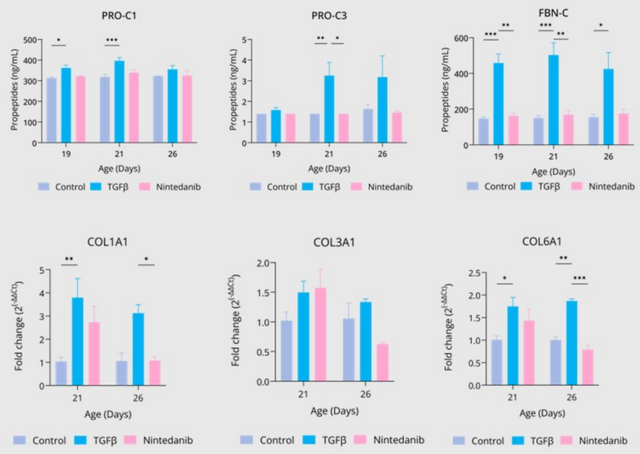
Dynamic culture maintained spheroid structure and viability throughout 26 days. The platform maintained spheroid integrity during prolonged TGFβ and Nintedanib exposure, enabling long-term fibrosis studies.



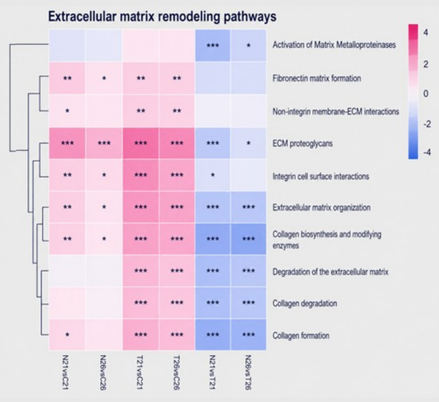


Nintedanib suppresses active fibrogenesis

Sustained TGFβ stimulation increased ECM biomarkers and collagen gene expression, consistent with active fibrogenesis. Nintedanib reduced both, indicating suppression of ongoing ECM production.



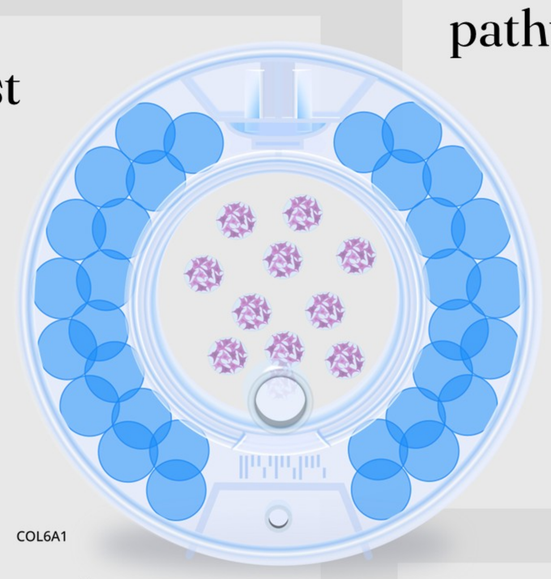
Nintedanib attenuates ECM remodeling pathways

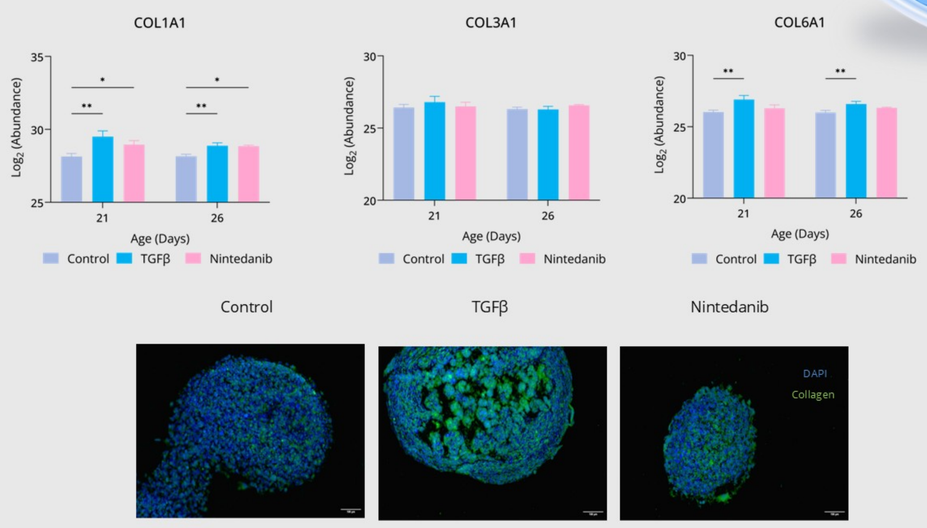


TGFβ promoted ECM remodeling pathways associated with collagen formation and matrix organization. Nintedanib reduced these responses, consistent with suppression of active fibrogenesis.

Structural collagen proteins persist following Nintedanib treatment

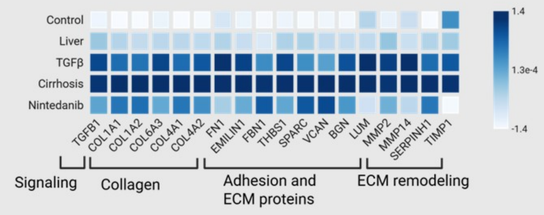
Despite reduced ECM production, structural collagens and collagen staining changed only modestly after Nintedanib treatment, suggesting it inhibits ongoing matrix production rather than reversing established ECM.





Proteomic comparison with human liver fibrosis

TGFβ-treated spheroids resembled cirrhotic liver, and despite Nintedanib reducing fibrosis-associated proteins, a fibrotic signature persisted.



Conclusions

This study demonstrates Nintedanib primarily suppressing ongoing matrix synthesis while preserving established collagen. This model distinguishes active fibrogenesis from pre-existing matrix and supports antifibrotic drug evaluation.

Acknowledgements

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