

Multi-omics investigation into the role of PTK7 as a driver of liver fibrosis

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ABSTRACT

Objective: Liver fibrosis is a critical stage in the progression of chronic liver disease to cirrhosis, and effective antifibrotic targets remain lacking. This study aims to investigate the association between protein tyrosine kinase 7 (PTK7) and liver fibrosis through multi-omics analyses, explore its potential role in hepatic stellate cell activation, and evaluate the value of circulating PTK7 as a candidate biomarker for assessing liver fibrosis severity.

Methods: Genome-wide association study (GWAS) summary statistics, cis-expression quantitative trait locus (cis-eQTL), and plasma protein quantitative trait locus (pQTL) datasets were integrated to identify candidate genes associated with liver fibrosis. Candidate genes were further validated using multiple machine-learning models in three independent Gene Expression Omnibus (GEO) cohorts (GSE84044, GSE25097, and GSE49541). Human and murine liver single-cell transcriptomic datasets were analyzed to characterize the expression profile of PTK7. In an exploratory clinical cohort, plasma PTK7 levels were measured by enzyme-linked immunosorbent assay (ELISA), and PTK7 expression was evaluated in a carbon tetrachloride (CCl₄)-induced mouse model of liver fibrosis. In LX-2 cells, PTK7 was silenced to assess changes in β -catenin and fibrosis-related proteins, followed by intervention with SKL2001. Molecular docking and molecular dynamics simulations were performed to evaluate the potential interaction between PTK7 and β -catenin.

Results: A total of 18 candidate genes were identified, among which PTK7 consistently showed upregulated expression in fibrotic liver tissues across multiple datasets. Single-cell transcriptomic analysis revealed enriched PTK7 expression in Kupffer cells and fibroblast-related populations. Plasma PTK7 levels were significantly elevated in patients with liver fibrosis and were positively correlated with liver stiffness measurements. In activated LX-2 cells, PTK7 knockdown reduced the expression levels of collagen type I alpha 1 chain (COL1A1), alpha-smooth muscle actin (α -SMA), and β -catenin. Furthermore, rescue

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experiments using SKL2001 supported the involvement of β -catenin-related signaling pathways in PTK7-mediated profibrotic effects. Computational analyses suggested a stable interaction pattern between PTK7 and β -catenin.

Conclusion: PTK7 is closely associated with liver fibrosis at both multi-omics and transcriptomic levels, may participate in hepatic stellate cell activation, and may be associated with β -catenin-related signaling pathways. Circulating PTK7 may serve as a candidate biomarker for reflecting the severity of liver fibrosis; however, further validation in larger clinical cohorts is warranted.

KEY WORDS liver fibrosis; PTK7; hepatic stellate cell; β -catenin; biomarker

多组学探讨PTK7在肝纤维化驱动中的作用

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[摘要] 目的: 肝纤维化是慢性肝病进展至肝硬化的关键环节, 目前缺乏有效的抗纤维化靶点。本研究旨在通过多组学探讨蛋白酪氨酸激酶7(protein tyrosine kinase 7, PTK7)与肝纤维化的关联, 探索其在肝星状细胞活化中的潜在作用, 并评估循环PTK7作为肝纤维化严重程度候选指标的价值。方法: 整合全基因组关联研究(genome-wide association study, GWAS)汇总统计数据、顺式表达数量性状位点(cis-expression quantitative trait locus, cis-eQTL)和血浆蛋白数量性状位点(protein quantitative trait locus, pQTL)数据, 筛选肝纤维化相关候选基因; 并在3个独立基因表达综合(Gene Expression Omnibus, GEO)队列(GSE84044、GSE25097和GSE49541)中结合多种机器学习模型进行验证。利用人和小鼠肝单细胞转录组数据分析PTK7的表达特征; 在临床探索性队列中采用酶联免疫吸附实验(enzyme-linked immunosorbent assay, ELISA)检测血浆PTK7水平, 并在四氯化碳(carbon tetrachloride, CCl₄)诱导的小鼠肝纤维化模型中评估PTK7表达。在LX-2细胞中, 通过敲低PTK7观察 β -catenin及纤维化相关蛋白质的变化, 并进一步采用SKL2001进行干预。采用分子对接和分子动力学模拟评估PTK7与 β -catenin的潜在相互作用。结果: 共筛选出18个候选基因, 其中PTK7在多个数据集中均表现为在纤维化肝组织中持续上调。单细胞转录组分析提示PTK7在Kupffer细胞和成纤维细胞相关群体中富集表达。肝纤维化患者血浆PTK7水平显著升高, 且与肝硬度值呈正相关。在活化的LX-2细胞中, 敲低PTK7可降低I型胶原蛋白 $\alpha 1$ 链(collagen type I alpha 1 chain, COL1A1)、 α -平滑肌肌动蛋白(alpha-smooth muscle actin, α -SMA)和 β -连环蛋白(β -catenin)的表达水平, 而SKL2001挽救实验支持 β -catenin相关信号通路参与PTK7介导的促纤维化过程。计算分析提示PTK7与 β -catenin之间存在稳定的相互作用模式。结论: PTK7在多组学和转录组数据层面与肝纤维化密切相关, 且可能参与肝星状细胞活化过程, 并可能与 β -catenin相关信号通路有关。循环PTK7有望作为反映肝纤维化严重程度的候选生物标志物, 但仍需在更大样本临床队列中进一步验证。

[关键词] 肝纤维化; PTK7; 肝星状细胞; β -连环蛋白; 生物标志物

Liver fibrosis is a major global health concern, characterized by excessive extracellular matrix (ECM) deposition that disrupts liver architecture and function. If left untreated, it can progress to cirrhosis and hepatocellular carcinoma (HCC), leading to significant

morbidity and mortality worldwide^[1-2]. Chronic liver injuries, such as those caused by viral infections (e.g., hepatitis B and C), alcohol abuse, and non-alcoholic fatty liver disease, are the primary drivers of fibrosis^[2-3]. With an estimated 844 million individuals affected by

chronic liver disease globally, liver fibrosis represents a pressing challenge for public health^[2]. However, despite significant research, the molecular mechanisms underlying liver fibrosis remain incompletely understood, and effective therapies are still lacking^[1,4].

A key event in liver fibrosis is the activation of hepatic stellate cell (HSC), which transform into myofibroblast-like cells that secrete large amounts of ECM proteins, including collagen and α -smooth muscle actin (α -SMA)^[1,5]. This transformation is driven by key signaling pathways, such as the Wnt/ β -catenin and phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) signaling pathway, which are crucial for HSCs survival, proliferation, and persistent fibrogenesis^[4,6-7]. Although ECM deposition is initially part of the wound-healing response to chronic liver injury, continued damage results in excessive ECM accumulation and fibrosis^[3]. Despite advances in understanding the downstream effects of these signaling pathways, the upstream regulators that drive fibrosis remain unclear, making targeted therapy development challenging.

Protein tyrosine kinase 7 (PTK7) is a transmembrane receptor involved in various biological processes, including cell polarity, migration, invasion, and survival^[8]. PTK7 plays a significant role in non-canonical Wnt signaling, which is essential for tissue organization and cell movement^[8]. In cancer biology, PTK7 has been implicated in tumorigenesis, with elevated expression observed in various tumors, including liver cancer, suggesting its involvement in disease progression and metastasis^[9]. However, its role in liver fibrosis has not been fully explored. Emerging evidence suggests that PTK7 may influence fibrogenesis in liver fibrosis by interacting with the Wnt/ β -catenin pathway, potentially affecting HSCs activation and ECM production^[8-10].

In this study, we aim to elucidate the role of PTK7 in liver fibrosis using a multi-omics approach, including genetic analysis [cis-expression quantitative trait locus (eQTL)/plasma protein quantitative trait locus (pQTL)], single-cell transcriptomics, and functional assays. Additionally, we employ molecular docking and dynamics simulations to investigate the interaction between PTK7 and β -catenin, providing insights into its potential mechanism in fibrogenesis. Our findings suggest that PTK7 may play an important role in liver

fibrogenesis and may represent a potential therapeutic target^[11].

1 Materials and methods

1.1 Ethics statements

All animal experiments were approved by the Institutional Animal Care and Use Committee of Xiangya Hospital of Central South University (Approval No. 2025081099) and were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and relevant Chinese regulations for experimental animal administration.

1.2 Animal model and treatment

Eight-week-old male wild-type C57BL/6 mice were obtained from the Department of Laboratory Animals, Central South University (Changsha, Hunan, China). Mice were housed under specific pathogen-free conditions [(22±2) °C, 50%±10% humidity, 12 hours light/dark cycle, with free access to food and water] and acclimatized for 1 week before random assignment to a liver fibrosis group ($n=5$) or a control group ($n=5$). Liver fibrosis was induced by twice-weekly intraperitoneal injections of carbon tetrachloride (CCl₄; Macklin, Shanghai, China) diluted 1:4 (v/v) in olive oil (Macklin, Shanghai, China) at 3 μ L/g body weight for 15 weeks. This regimen corresponds to approximately 0.6 μ L/g of pure CCl₄ and was selected based on a commonly used chronic CCl₄-induced liver fibrosis model in mice^[6,12]. Control mice received an equivalent volume of olive oil.

At the end of the 15-week treatment period, mice were anesthetized with isoflurane (4% for induction and 1.5% for maintenance in oxygen). Blood was collected from the retro-orbital sinus into ethylenediaminetetraacetic acid (EDTA)-containing tubes, and plasma was isolated by centrifugation and stored at -80 °C for PTK7 measurement. Mice were then euthanized by cervical dislocation under deep anesthesia, followed by bilateral thoracotomy to confirm death. Liver tissues were promptly harvested; some were fixed in 10% neutral-buffered formalin (Solarbio, Beijing, China) for histological and immunofluorescence analyses, and the remainder were snap-frozen in liquid nitrogen and stored at -80 °C for molecular and protein analyses.

1.3 Methods

1.3.1 eQTL and pQTL analysis for liver fibrosis

Liver fibrosis Genome Wide Association Study (GWAS) summary statistics (FinnGen: Finn-b-FIBROLIV and finn-b-K11_FIBROCHIRLIV) were integrated with cis-eQTL (eQTLGen) and plasma cis-pQTL (deCODE) datasets. For eQTL analysis, summary-based summary-data-based Mendelian randomization (SMR) was performed^[13]. Candidate genes were selected based on genome-wide significant cis-eQTL instruments ($P < 5 \times 10^{-8}$), false discovery rate (FDR) < 0.05 , heteroskedasticity identifies disconnected instruments (HEIDI) $P > 0.05$, and a positive causal effect ($\beta > 0$) with the 95% confidence interval (CI) not crossing zero. For pQTL analysis, two-sample Mendelian randomization using the inverse-variance weighted (IVW) method was performed with Benjamini-Hochberg FDR correction^[14]; proteins with FDR < 0.05 and nominal $P < 0.05$ were retained. Genes supported by both analyses were prioritized for downstream validation.

1.3.2 Validation using Gene Expression Omnibus datasets

Preliminary validation was performed using Gene Expression Omnibus (GEO) datasets: GSE84044 [hepatitis B virus (HBV) etiology, 43 controls, 81 cases]^[15], GSE25097 (unknown etiology, 6 controls, 40 cases)^[16], and GSE49541 [non-alcoholic fatty liver disease (NAFLD) etiology, 40 controls, 32 cases]^[17]. Datasets were preprocessed to remove batch effects using the sva package and standardized. Five machine learning algorithms, including least absolute shrinkage and selection operator (LASSO), support vector machine-recursive feature elimination (SVM-RFE), random forest (RF), generalized boosted regression modeling (GBM), and glmboost, were used for feature selection and model construction^[18-19]. To evaluate model robustness and predictive performance, we performed repeated 5-fold cross-validation with 10 repeats for hyperparameter tuning and internal validation. Model performance was quantified using the area under the curve (AUC) of receiver operating characteristic (ROC), accuracy, sensitivity, specificity, precision, and F1 score. Furthermore, ROC analysis, decision curve analysis (DCA), and calibration curves were applied to assess discrimination, clinical net benefit, and calibration

performance, respectively^[20].

1.3.3 Single-cell transcriptomic analysis

Single-cell RNA sequencing data were obtained from GEO (GSE136103), including scRNA-seq profiles from 5 healthy and 5 cirrhotic human livers, and mouse hepatic macrophage profiles from control and CCl₄-induced fibrotic mice^[21]. After quality control, 59 977 human liver cells and 5 901 mouse liver cells were retained. Human and mouse data were processed separately with species-matched annotations. Standard workflows were applied for normalization, dimensionality reduction, and clustering^[22]. Cell type annotation was performed using SingleR with the Human Primary Cell Atlas dataset (human samples) and Mouse RNA-seq Atlas dataset (mouse samples) from the celldex database^[23], followed by assessment of PTK7 expression across annotated cell populations. To further evaluate stromal localization, HSCs signature scoring based on classical HSCs marker genes was performed in the stromal subset, and the overlap between PTK7-positive cells and high HSCs-score cells was visualized. Co-expression analysis between PTK7 and classical HSCs markers was also performed within the stromal compartment.

1.3.4 Histology and immunofluorescence staining

Fixed liver tissues were paraffin-embedded, sectioned at 4 to 6 μm , and subjected to hematoxylin and eosin (HE), Masson's trichrome, and Sirius Red staining according to standard protocols^[6]. Fibrosis severity and collagen deposition were assessed qualitatively based on these staining results. For immunofluorescence staining, paraffin sections were deparaffinized in xylene and subjected to antigen retrieval in citrate buffer (pH 6.0) for 10 to 15 minutes. Sections were blocked with 5% BSA and incubated overnight with a primary antibody against PTK7 (1:400). After washing, Alexa Fluor-conjugated secondary antibody (1:500) was applied for 1 hour. Nuclei were counterstained with 4', 6-diamidino-2-phenylindole (DAPI). Fluorescence images were captured using a confocal microscope, and PTK7 expression was quantified using ImageJ.

1.3.5 Baseline patient characteristics for enzyme-linked immunosorbent assay analysis

A total of 114 participants were enrolled: 51 liver

fibrosis patients [Meta-analysis of histological data in viral hepatitis (METAVIR) F2 – F4 or Liver Stiffness Measurement (LSM) ≥ 7.3 kPa], 27 healthy controls, 23 non-fibrotic metabolic dysfunction-associated fatty liver disease (MAFLD) patients (LSM < 7.3 kPa), 13 HBV-infected patients without advanced fibrosis (LSM < 7.3 kPa). Age, sex and body mass index (BMI) were comparable across groups (all $P > 0.05$), while alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly elevated in the fibrosis group ($P < 0.001$). G*Power 3.1 analysis confirmed a statistical power of 0.96 ($\alpha = 0.05$, effect size = 0.65), meeting the requirements for biomarker exploratory research^[24]. Detailed baseline data are shown in Supplementary Table 1 (<https://doi.org/10.57760/sciencedb.38779>).

1.3.6 Plasma PTK7 quantification

Plasma PTK7 levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, Wuhan, China; Lot. Q12036892). Briefly, 100 μ L of plasma was added to pre-coated PTK7 capture antibody plates and incubated for 2 hours at 37 °C. After washing, biotin-conjugated PTK7 detection antibodies and horseradish peroxidase (HRP)-avidin were added sequentially. The reaction was visualized using tetramethylbenzidine (TMB) substrate, and absorbance was measured at 450 nm. Key quality control: Standard curve $R^2 \geq 0.99$; duplicate wells with coefficient of variation (CV) $< 20\%$; samples beyond the linear range were diluted and retested^[25]. PTK7 concentrations were quantified via linear regression of the standard curve.

1.3.7 LX-2 cell culture and PTK7 silencing

LX-2 human HSCs were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, Hubei, China). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Grand Island, New York State, USA) and 1% penicillin-streptomycin (Gibco) at 37 °C in a humidified incubator containing 5% CO₂. To induce fibrogenic activation, cells were serum-starved for 24 hours, and then treated with 5 ng/mL transforming growth factor-beta 1 (TGF- β 1) (R&D Systems, Minneapolis, Minnesota, USA) or 50 ng/mL platelet-derived growth factor (PDGF) (R&D Systems, Minneapolis, Minnesota, USA) for 48 hours. The concentrations of TGF- β 1 and PDGF were chosen based on previous studies that

effectively induce fibrotic activation in LX-2 cells^[5, 12]. For PTK7 silencing, cells were transfected with PTK7-specific small interfering RNA (siRNA) using riboFECTTM CP transfection reagent (Guangzhou RiboBio Co., Ltd., Guangzhou, Guangdong, China) according to the manufacturer's instructions. Transfections were performed 24 hours prior to TGF- β 1 treatment. Knockdown efficiency was confirmed by Western blotting.

1.3.8 Western blotting analysis

Protein extracts from LX-2 cells and liver tissues were prepared using Radioimmunoprecipitation Assay (RIPA) buffer with protease and phosphatase inhibitors (Beyotime, Shanghai, China). Total protein was quantified using the bicinchoninic acid (BCA) method (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Equal amounts (30 μ g) were separated by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Burlington, Massachusetts, USA), and blocked with 5% non-fat milk (Bio-Rad, Hercules, California, USA). Primary antibodies against PTK7, collagen type I alpha 1 chain (COL1A1), and α -SMA (all diluted 1 : 1 000; Abcam, Cambridge, UK) were incubated overnight at 4 °C. Membranes were incubated with HRP-conjugated secondary antibodies (1 : 5 000; Cell Signaling Technology, Danvers, Massachusetts, USA). Bands were visualized using ECL reagents (Thermo Fisher Scientific, Waltham, Massachusetts, USA), with Tubulin (Abcam, Cambridge, UK) used as a loading control.

1.3.9 SKL2001 treatment and rescue experiments

LX-2 cells were transfected with negative control siRNA (siNC) or PTK7-targeting siRNA (siPTK7), followed by stimulation with TGF- β 1 and treatment with DMSO or SKL2001. The cells were divided into 4 groups: Negative control (NC) + Dimethyl sulfoxide (DMSO), NC + SKL2001, siPTK7 + DMSO, and siPTK7 + SKL2001. SKL2001 (KKL Med, KM8835), a Wnt/ β -catenin pathway activator, was used at a final concentration of 50 μ g/mL^[7, 26]. Protein expression of β -catenin and COL1A1 was analyzed by Western blotting, with Tubulin as the loading control.

1.3.10 Molecular docking and dynamics simulations

Molecular docking was performed using AutoDock Vina to explore the potential interaction between PTK7

and β -catenin^[27]. The three-dimensional structure of PTK7 was obtained from the Protein Data Bank (PDB ID: 6VG3), and the structure of β -catenin was obtained from the Protein Data Bank (PDB ID: 7ZRB). The protein structures were prepared prior to docking by removing water molecules, adding hydrogen atoms, and assigning appropriate charges. Molecular dynamics simulations were subsequently performed using GROMACS for 100 ns to evaluate the stability of the complex^[28], with root mean square deviation (RMSD) and root mean square fluctuation (RMSF) used to assess conformational stability and residue flexibility.

1.4 Statistical analysis

All statistical analyses were performed using GraphPad Prism 8.0 and SPSS 26.0. All tests were two-sided, and $P < 0.05$ was considered statistically significant. Continuous variables were assessed for normality using the Shapiro-Wilk test. Because the ELISA data were not normally distributed, non-parametric methods were applied. Comparisons between two groups were performed using the Mann-Whitney U test, whereas comparisons among three or more groups were performed using the Kruskal-Wallis test followed by Dunn's multiple-comparison test with Bonferroni correction. The correlation between PTK7 and liver stiffness measurement (LSM) was evaluated using Spearman's rank correlation, and 95% CI were calculated where applicable. ELISA data are presented as median [interquartile range (IQR)], whereas baseline clinical variables are presented as mean $\pm$ standard deviation (SD). eQTL and pQTL analyses were conducted using the SMR and IVW methods, respectively, and machine-learning-based validation was performed in Python (v3.8).

2 Results

2.1 Identification of risk genes for liver fibrosis via eQTL and pQTL analysis

Through SMR analysis of eQTL data from the finn-b-FIBROLIV dataset, we identified 230 candidate genes associated with liver fibrosis (Figure 1A). Using pQTL instruments selected at genome-wide significance and IVW-based Mendelian randomization, we identified 113

plasma proteins showing nominal evidence of association with liver fibrosis (Figure 1B). Cross-validation between eQTL and pQTL analyses revealed 18 overlapping genes (Figure 1C), which were prioritized for downstream validation.

2.2 Machine learning-based validation of risk genes using GEO datasets

Overlap analysis of the five machine learning algorithms identified three shared candidate genes, PTK7, t-SNARE domain containing 1 (TSNARE1), and serine hydroxymethyltransferase 1 (SHMT1) (Figure 2A), and their chromosomal distribution is shown in Figure 2B. Using repeated 5-fold cross-validation with 10 repeats, the 5 models showed moderate to good discriminative ability, among which the RF model achieved the best performance ($AUC = 0.827$, 95% CI 0.810 to 0.843), followed by GBM (Figure 2C). Detailed model performance metrics are summarized in Supplementary Table 2 (<https://doi.org/10.57760/sciencedb.38779>). Decision curve analysis indicated a favorable net benefit of the three-gene signature (Figure 2D), while the calibration curve demonstrated good agreement between predicted and observed outcomes (Figure 2E). Variable importance analysis further identified PTK7 as a key informative feature (Figure 2F). In addition, PTK7 was significantly upregulated in hepatic fibrosis samples compared with controls (Figure 2G). Integrative genetic analyses further supported its potential role, as evidenced by the positive correlation between cis-eQTL and GWAS effect sizes (Figure 2H) and the overlapping regional association signals at the PTK7 locus (Figure 2I).

2.3 PTK7-centered molecular network in liver fibrosis

Protein-protein interaction (PPI) analysis identified PTK7 as a central node within the fibrosis-related candidate-gene network, with close connections to platelet-derived growth factor receptor beta (PDGFRB), lysosomal associated membrane protein 1 (LAMP1), fibroblast growth factor receptor 3 (FGFR3), and matrix metalloproteinase 14 (MMP14, Figure 3A). Correlation analysis further showed that PTK7 was positively correlated with several fibrosis-and ECM-related genes, including PDGFRB, collagen type I alpha 1 chain

(COL1A1), and MMP14 (Figure 3B). These findings suggest that PTK7 is embedded in a fibrosis-associated molecular network.

2.4 Functional enrichment of PTK7-related risk genes

Gene Ontology (GO) enrichment analysis showed that the prioritized risk genes were mainly enriched in ECM organization, extracellular structure organization,

collagen-containing ECM, ECM structural constituent, platelet-derived growth factor binding, heparin binding, and cytokine activity (Figure 4A). Pathway enrichment analysis linked these genes to fibrosis-related signaling pathways, including Wnt, Ras, PI3K-Akt, mitogen-activated protein kinase (MAPK), calcium signaling, focal adhesion, and adherens junction pathways (Figure 4B). These results indicate that PTK7-related genes may participate in ECM remodeling and profibrotic signaling.

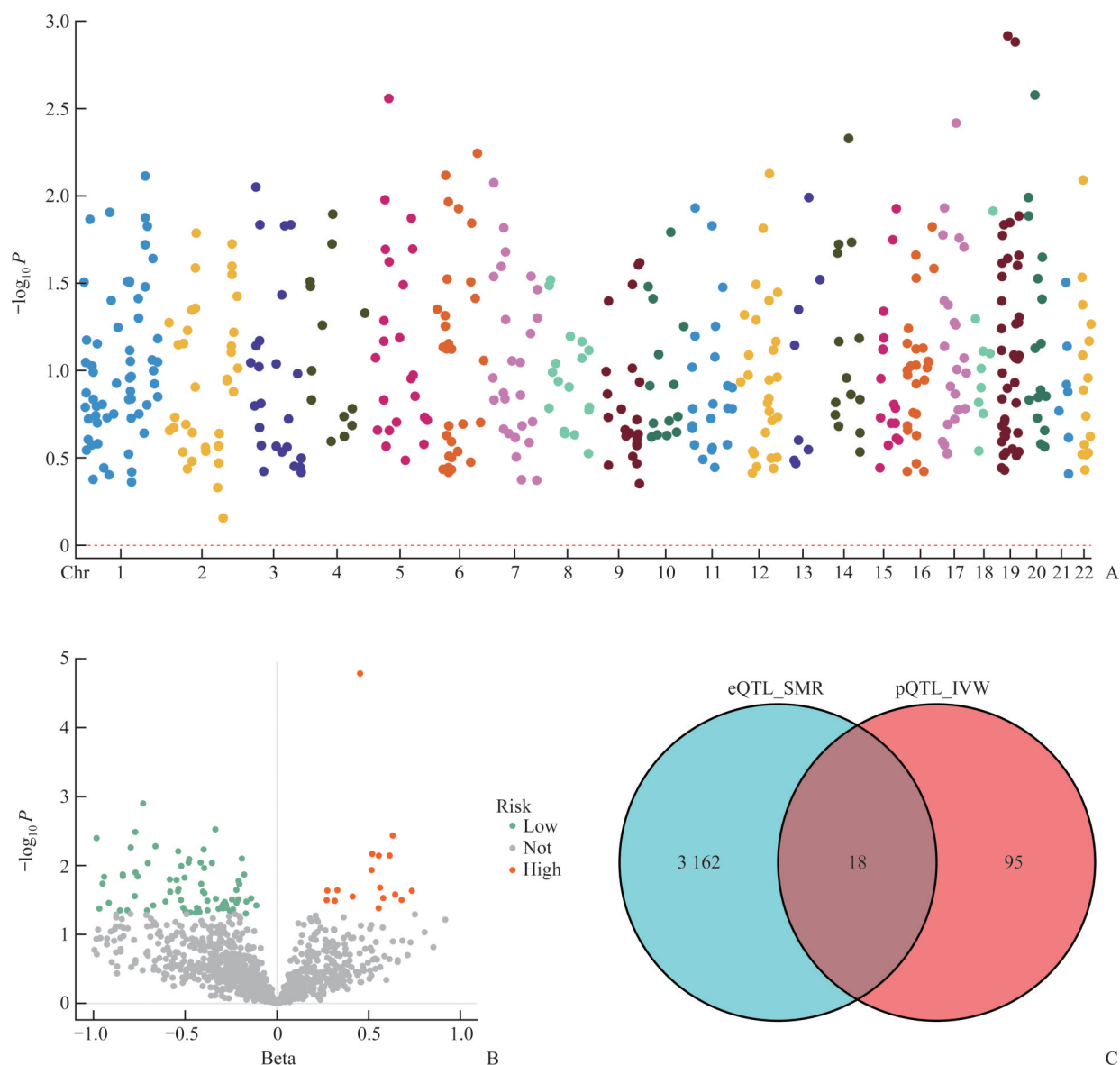


Figure 1 Integrated eQTL and pQTL analyses identify genetic associations in liver fibrosis

A: Manhattan plot from the SMR analysis using eQTL data and the FinnGen R10 FIBROLIV dataset, highlighting significant loci associated with liver fibrosis. B: pQTL analysis of plasma proteins related to liver fibrosis, based on the FinnGen B-K11 FIBROCHIRLIV dataset. C: Venn diagram showing the overlap of genes identified by both SMR and pQTL analyses, indicating shared genetic determinants involved in liver fibrosis. eQTL: Expression quantitative trait locus; pQTL: Protein quantitative trait locus; SMR: Summary-based mendelian randomization; IVW: Inverse-variance weighted.

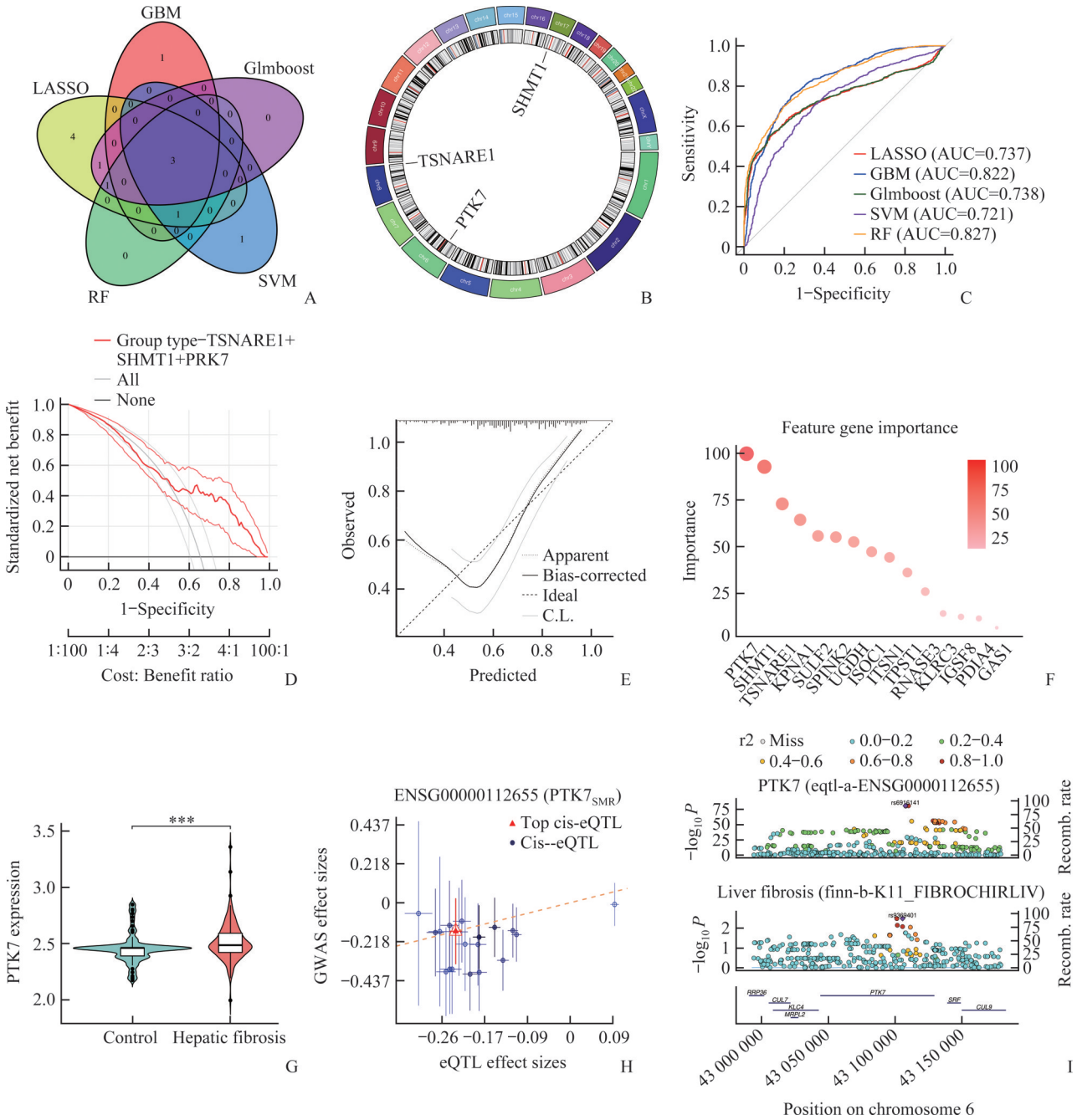


Figure 2 Machine-learning-based validation and prioritization of PTK7 in liver fibrosis

A: Overlap of candidate genes identified by 5 machine learning algorithms. B: Chromosomal distribution of the prioritized candidate genes. C: ROC curves of the 5 machine learning models. D: DCA showing the net benefit of the three-gene signature compared with the treat-all and treat-none strategies across a range of threshold probabilities. E: Calibration curve assessing the agreement between predicted and observed probabilities. F: Variable importance ranking of candidate genes in the machine learning model. G: PTK7 expression in control and hepatic fibrosis samples. Statistical significance was assessed using the Wilcoxon rank-sum test (** $P < 0.001$). H: Correlation between PTK7 cis-eQTL effect sizes and GWAS effect sizes. I: Regional association plot of the PTK7 locus showing cis-eQTL signals (top) and liver fibrosis GWAS signals (bottom), with rs6991441 highlighted. PTK7: Protein tyrosine kinase 7; ROC: Receiver operating characteristic; DCA: Decision curve analysis; cis-eQTL: Cis-expression quantitative trait locus; LASSO: Least absolute shrinkage and selection operator; GBM: Gradient boosting machine; SVM: Support vector machine; RF: Random forest; TSNARE1: t-SNARE domain containing 1; SHMT1: Serine hydroxymethyltransferase 1; KPNA1: Karyopherin alpha 1; SULF2: Sulfatase 2; SPINK2: Serine protease inhibitor Kazal-type 2; UGDH: Uridine diphosphate-glucose dehydrogenase; ISOC1: Isochorismatase domain containing 1; ITS1: Intersectin 1; TPST1: Tyrosyl protein sulfotransferase; RNASE3: Ribonuclease, RNase A family, 3; KLRC3: Killer cell lectin-like receptor subfamily C, member 3; IGSF8: Immunoglobulin superfamily, member 8; PDIA4: Protein disulfide isomerase family A, member 4; GAS1: Growth arrest-specific 1.

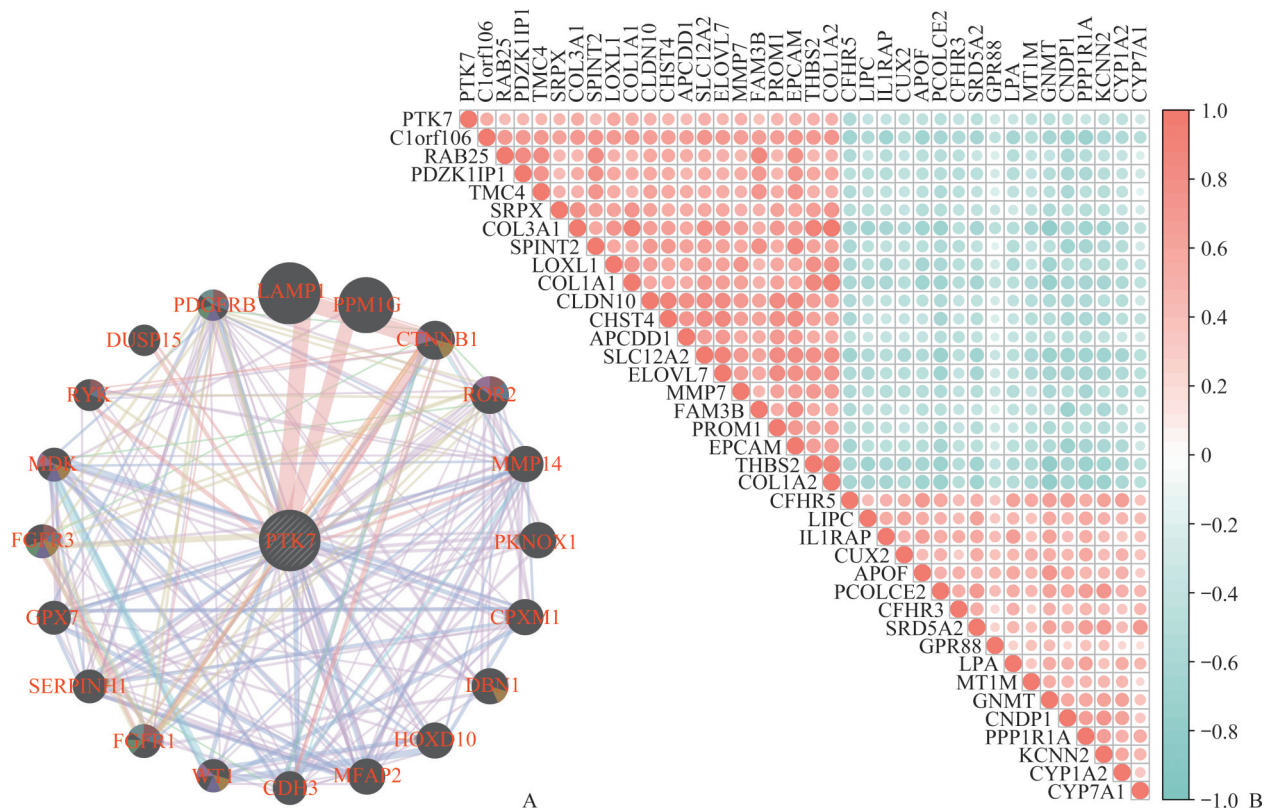


Figure 3 PTK7-centered molecular network in liver fibrosis

A: PPI network of PTK7 and fibrosis-related candidate proteins. Nodes represent proteins, and edge thickness indicates interaction strength. B: Correlation heatmap showing pairwise correlations between PTK7 and other candidate genes. Red indicates positive correlation, blue indicates negative correlation, and circle size reflects correlation magnitude. PPI: Protein-protein interaction; PDGFRB: Platelet-derived growth factor receptor beta; COL1A1: Collagen type I alpha 1 chain; MMP14: Matrix metalloproteinase 14; LAMP1: Lysosomal associated membrane protein 1; FGFR: Fibroblast growth factor receptor.

2.5 Single-cell transcriptomic analysis of human and mouse liver samples

Single-cell RNA-seq data from GSE136103 were analyzed to characterize the cellular distribution of PTK7 in human and mouse liver samples. In the human dataset, t-Distributed Stochastic Neighbor Embedding (t-SNE) clustering revealed multiple distinct cell populations, reflecting substantial cellular heterogeneity (Figure 5A). SingleR annotation identified major liver-resident and immune-associated cell types, including Kupffer cells, endothelial cells, stromal cells, neutrophils, fibroblasts, and hepatocytes (Figure 5B). PTK7 expression was mainly detected in Kupffer cells, with additional signals observed in endothelial cells and lower expression in other annotated cell types (Figure 5C). In the mouse dataset, t-SNE analysis similarly identified distinct cellular clusters (Figure 5D). SingleR annotation classified these clusters into several major

cell populations, including Kupffer cells, common myeloid progenitors (CMPs), neutrophils, epithelial cells, and natural killer (NK) cells (Figure 5E). PTK7 expression was detectable in Kupffer cells and epithelial cells, suggesting a cell-type-specific expression pattern in the mouse liver microenvironment (Figure 5F). CellChat analysis was then performed to evaluate potential intercellular communication. In both human and mouse datasets, Kupffer cells showed extensive interactions with stromal, epithelial, and immune-associated populations, indicating active cellular crosstalk within the liver microenvironment (Figure 5G and 5H). Finally, HSC signature scoring showed that PTK7-positive cells partially overlapped with cells exhibiting higher HSC-related scores (Figure 5I). These findings suggest a potential association between PTK7 expression and HSC-related stromal features, while PTK7 alone should not be considered a definitive marker of hepatic stellate cell identity.

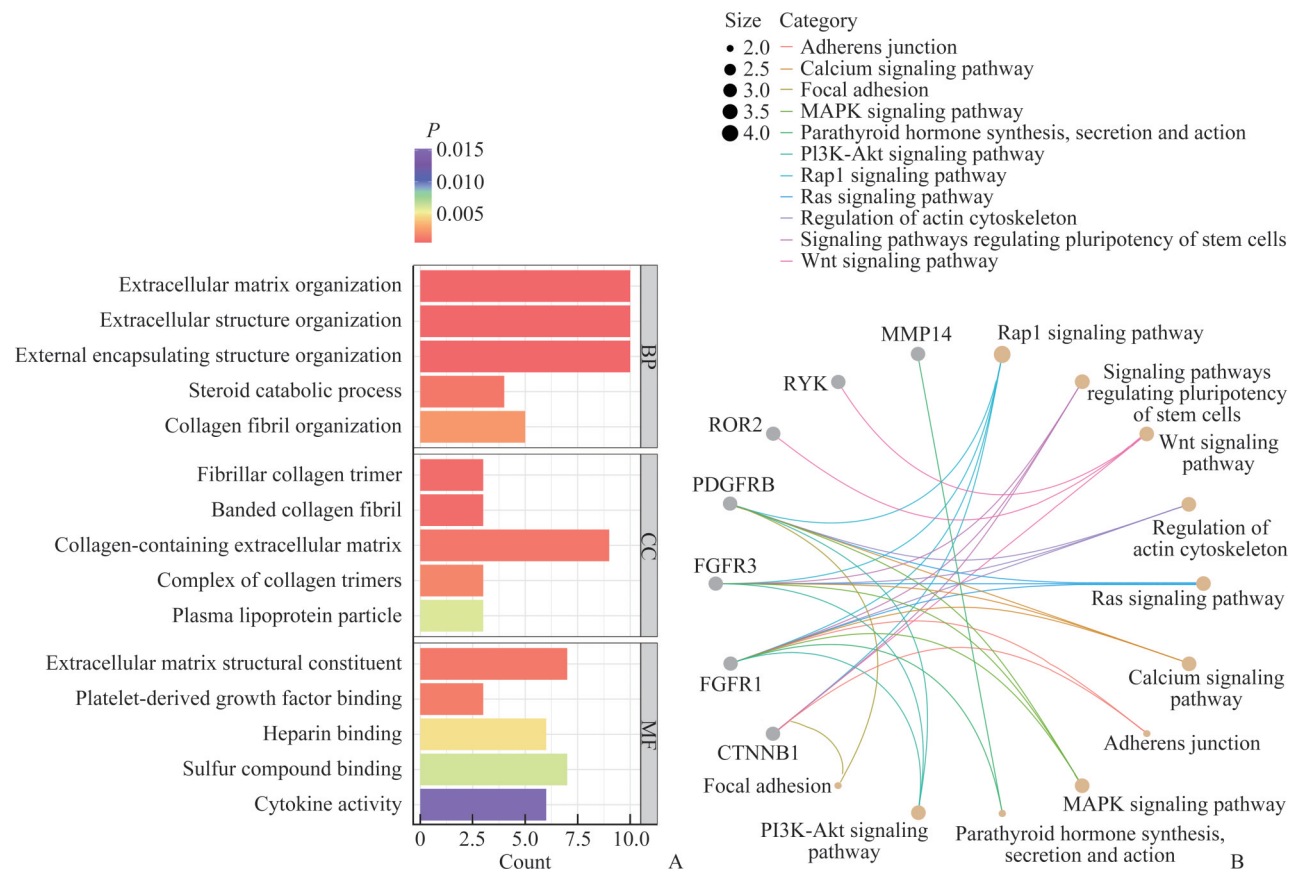


Figure 4 Functional enrichment and pathway analysis of PTK7-related risk genes

A: GO enrichment analysis of prioritized fibrosis-related risk genes. Enriched terms are classified into BP, CC, and MF. Bar length represents gene count, and color indicates the adjusted P value. B: Pathway enrichment network showing associations between candidate genes and enriched signaling pathways. Edges indicate gene-pathway associations, and edge thickness reflects the degree of pathway involvement. GO: Gene Ontology; BP: Biological process; CC: Cellular component; MF: Molecular function; PI3K: Phosphoinositide 3-kinase; Akt: Protein kinase B; MAPK: Mitogen-activated protein kinase; RYK: Receptor protein-tyrosine kinases; MMP14: Matrix metalloproteinase 14; CTNNB1: Catenin beta 1; FGFR1: Fibroblast growth factor receptor 1; PDGFRB: Platelet derived growth factor receptor beta; ROR2: Receptor tyrosine kinase-like orphan receptor 2.

2.6 Establishment of the liver fibrosis model and PTK7 expression analysis

Histological staining results confirmed the successful establishment of the liver fibrosis model in C57BL/6 mice following CCl_4 injection (Supplementary Figure 1 (<https://doi.org/10.57760/sciencedb.38779>)). Immunofluorescence showed significantly higher PTK7 expression in fibrotic mouse liver tissues (Mann-Whitney U test, $P < 0.05$, Figure 6A and 6B).

Plasma PTK7 levels differed significantly among four clinical groups (Kruskal-Wallis $H = 36.89$, $P < 0.001$, Figure 6C): the fibrosis group [median (IQR): 74.26 (53.55, 98.75)] had significantly higher PTK7 levels than healthy controls [44.27(32.14, 51.28), $P < 0.001$]

and MAFLD without fibrosis [47.72 (34.18, 50.97), $P < 0.01$], with no difference between the two control subgroups ($P = 0.172$). A strong positive correlation was observed between plasma PTK7 and LSM in fibrosis patients (Spearman $r = 0.77$, 95% CI 0.63 to 0.86, $P < 0.001$, Figure 6D).

2.7 Effect of PTK7 silencing on fibrosis markers in LX-2 cells

PTK7 silencing in TGF- β 1 or PDGF-activated LX-2 cells resulted in a significant reduction in COL1A1 and α -SMA expression by 45% and 52%, respectively, suggesting that PTK7 may contribute to the fibrogenic phenotype in activated LX-2 cells (Figure 6E).

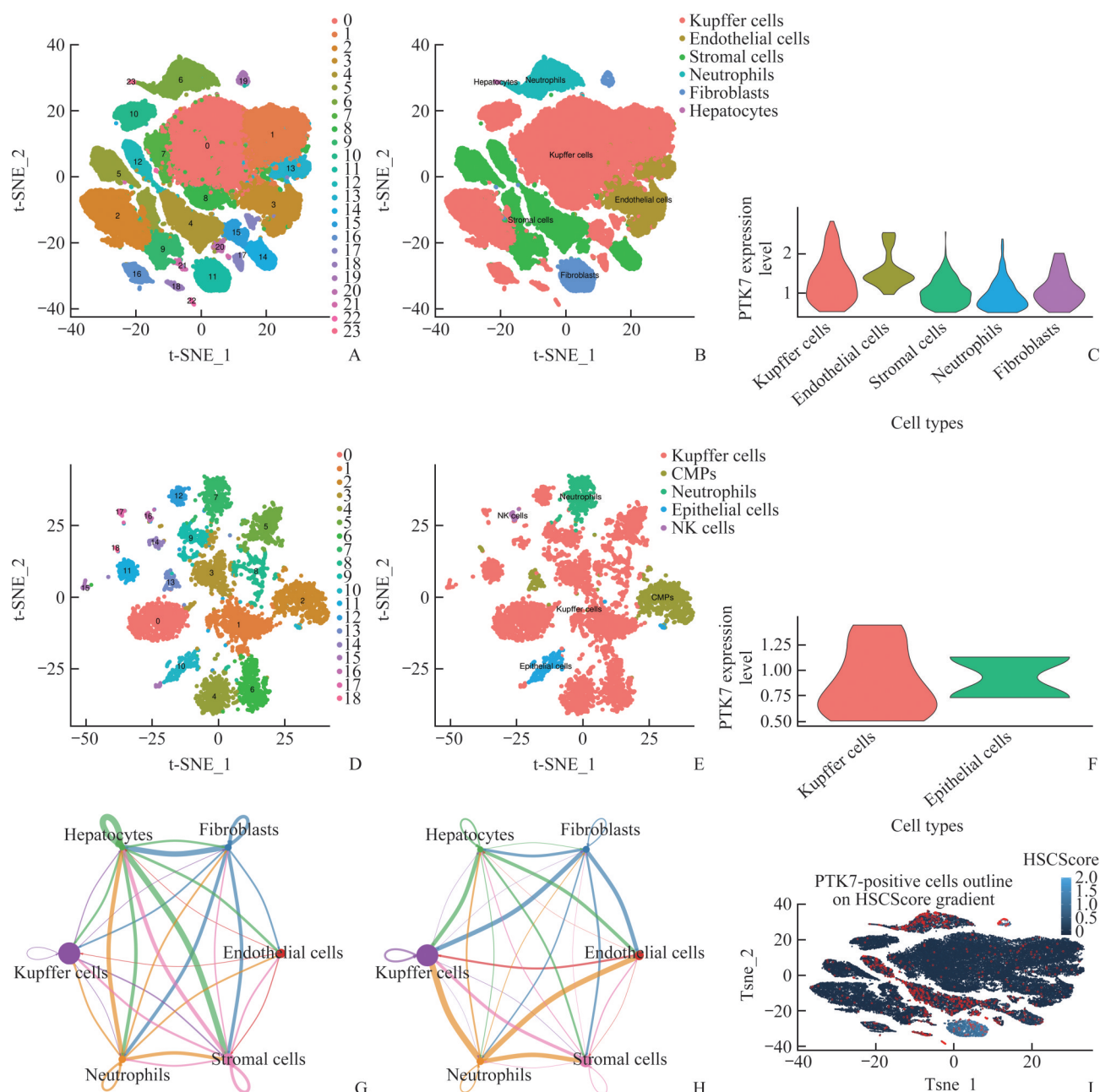


Figure 5 Single-cell transcriptomic and cell-cell communication analysis of human and mouse liver samples from GSE136103

A: t-SNE plot showing clustering of human liver cells based on transcriptomic data. Cells are grouped into distinct clusters representing different cell types. B: SingleR annotation of human liver cells, with each cluster labeled according to its most likely cell type based on reference data. C: Expression of PTK7 across different human liver cell types, visualized using a violin plot. The color scale represents normalized gene expression levels of PTK7 in various cell types. D: t-SNE plot showing clustering of mouse liver cells, highlighting distinct cellular subpopulations based on gene expression. E: SingleR annotation of mouse liver cells, with each cluster assigned to a specific cell type based on reference datasets. F: Expression of PTK7 across different mouse liver cell types, visualized using a violin plot. Expression levels of PTK7 are shown with a color scale representing normalized expression in each cell type. G: Cell-cell communication network in human liver cells based on ligand-receptor interactions. The plot shows the number of interactions between different cell types, with the size of the circles representing the number of interactions and the thickness of the edges representing the strength of the interactions. H: Cell-cell communication network in mouse liver cells based on ligand-receptor interactions. Stronger interactions are indicated by thicker lines, highlighting key signaling pathways between different cell types. I: Overlap analysis between PTK7-positive cells and HSCs signature scores within the stromal subset. Cells with higher HSCs-related scores showed partial overlap with PTK7-positive cells, supporting a potential association of PTK7 with HSCs-related stromal populations while not implying definitive lineage assignment. t-SNE: t-Distributed Stochastic Neighbor Embedding; HSCs: Hepatic stellate cells; CMPs: Common myeloid progenitors; NK: Natural killer.

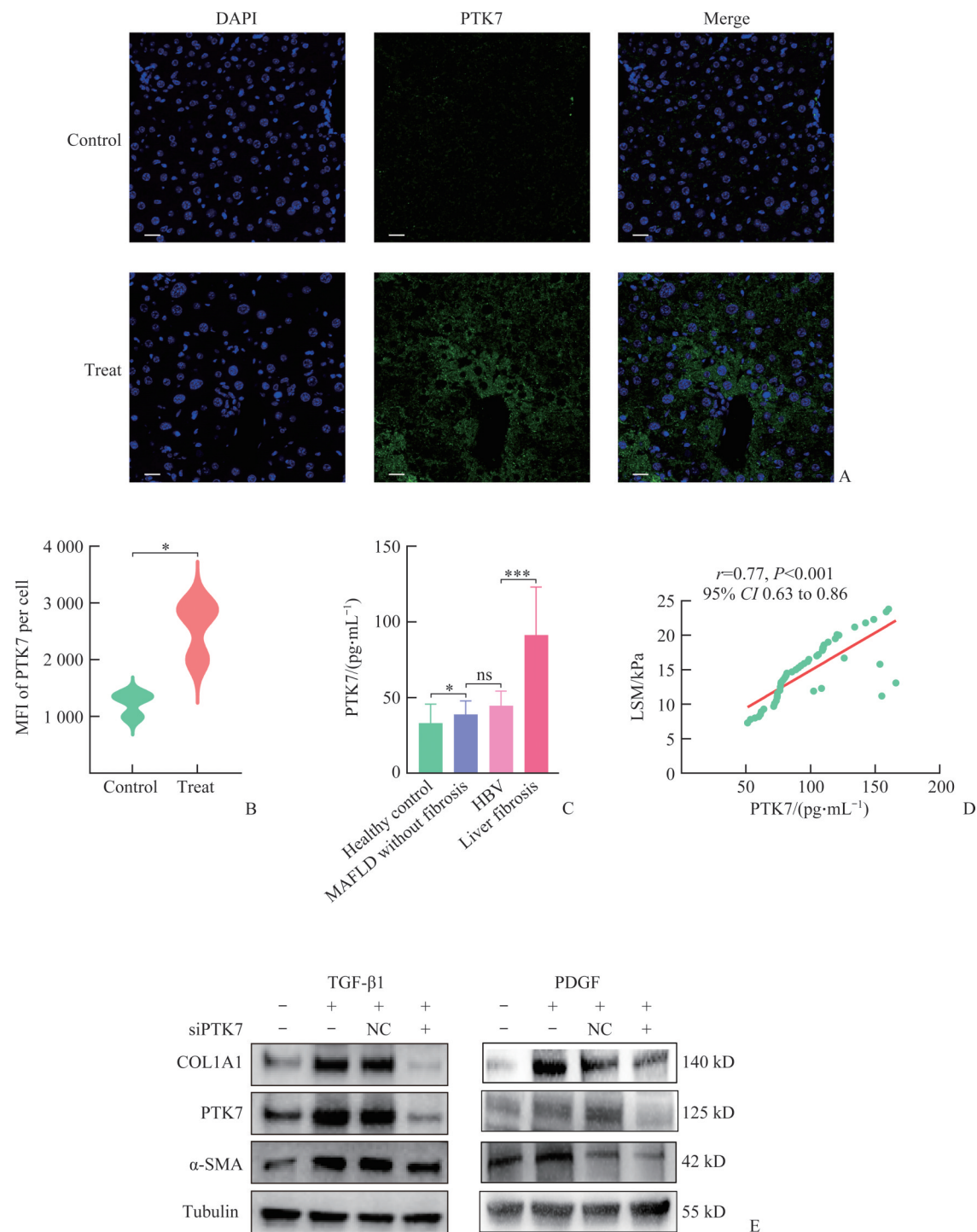


Figure 6 PTK7 expression and its potential as a fibrosis biomarker

A: Immunofluorescence staining of PTK7 in control and fibrotic mouse liver tissues. B: Quantification of PTK7 fluorescence intensity in control and fibrotic mouse liver tissues. Data are presented as mean±SD. Statistical analysis was performed using the Mann-Whitney *U* test. C: Plasma PTK7 levels in healthy controls, MAFLD without fibrosis, HBV infection without advanced fibrosis, and liver fibrosis. Data are presented as median (IQR). Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's multiple-comparison test. D: Correlation between plasma PTK7 and LSM in 51 patients with fibrosis, assessed using Spearman's rank correlation (Spearman $r=0.77$, 95% CI 0.63 to 0.86, $P<0.001$). E: Western blotting analysis of fibrosis markers after PTK7 silencing in LX-2 cells. Scale bars=20 μm. No statistical significance was defined as ns $P\geq0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$. DAPI: 4',6-Diamidino-2-phenylindole; MFI: Mean fluorescence intensity; DAB: 3,3'-Diaminobenzidine; HBV: Hepatitis B virus; MAFLD: Metabolic dysfunction-associated fatty liver disease; IQR: Interquartile range; LSM: Liver stiffness measurement; CI: Confidence interval; TGF-β1: Transforming growth factor-beta 1; PDGF: Platelet-derived growth factor; NC: Negative control.

2.8 Computational analyses and functional rescue support the involvement of PTK7 in β -catenin-related fibrogenic signaling

Molecular docking revealed a strong interaction between PTK7 and catenin beta 1 (CTNNB1), with a binding energy of -7.8 kcal/mol (1 kcal=4.186 kJ). Key residues, including ARG-925 and GLU-462, were identified as crucial for hydrogen bond formation and complex stabilization (Figure 7A). Molecular dynamics (MD) simulations further confirmed the stability of the PTK7- β -catenin complex. The RMSD stabilized between 4.2 and 5.0 Å after 20 ns, indicating a robust and optimized binding conformation (Figure 7B). RMSF

analysis showed minimal flexibility at the binding site residues, and secondary structure analysis demonstrated consistent structural integrity throughout the 100 ns simulation (Figure 7C and 7D). These results highlight the stable interaction between PTK7 and β -catenin, providing structural insights into PTK7's regulatory role in the Wnt/ β -catenin signaling pathway. Compared with the siNC+DMSO group, PTK7 silencing reduced the expression of β -catenin and COL1A1 (Figure 7E). SKL2001 treatment partially reversed these changes (Figure 7F). These findings suggest that PTK7 may promote fibrogenic activation, at least in part, through modulation of Wnt/ β -catenin signaling.

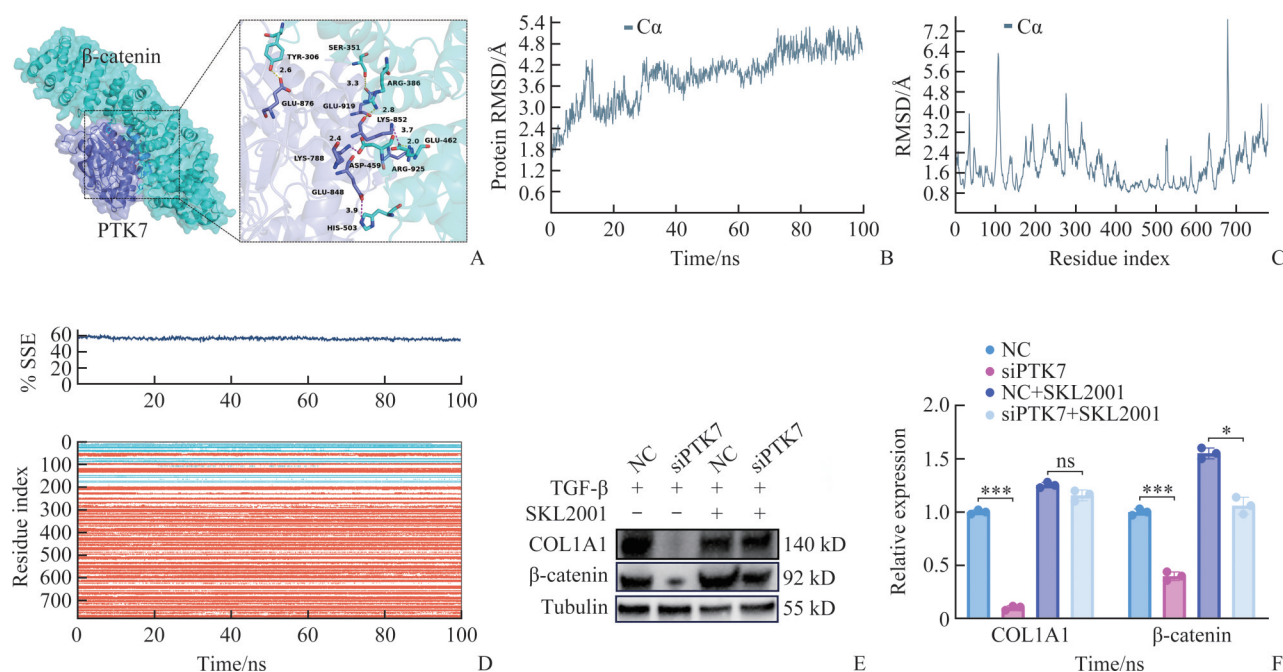


Figure 7 Computational analyses and functional rescue support the involvement of PTK7 in β -catenin-related fibrogenic signaling

A: Molecular docking results show the PTK7- β -catenin binding interface, with key residues (e.g., ARG-925 and GLU-462) highlighted. Hydrogen bonds are represented as dashed lines. B: RMSD analysis of the protein complex over a 100 ns simulation indicates stabilization between 4.2 and 5.0 Å after 20 ns. C: Residue-specific RMSF analysis demonstrates low fluctuations at the binding site residues. D: Secondary structure elements (SSE) analysis confirms the structural integrity of the PTK7- β -catenin complex throughout the simulation. E: Western blotting analysis of β -catenin and COL1A1 expression in TGF- β 1-activated LX-2 cells after PTK7 silencing and SKL2001 treatment. Cells were divided into four groups: NC+TGF- β 1+SKL2001(-), siPTK7+TGF- β 1+SKL2001(-), NC+TGF- β 1+SKL2001(+), and siPTK7+TGF- β 1+SKL2001(+). Tubulin was used as the loading control. F: Densitometric quantification of β -catenin and COL1A1 protein expression normalized to Tubulin. No statistical significance was defined as ns $P \geq 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. RMSD: Root mean square deviation; RMSF: Root mean square fluctuation.

3 Discussion

Liver fibrosis is a major pathological consequence of chronic liver injury and may progress to cirrhosis, liver failure, and HCC if left untreated^[1-2]. Although substantial progress has been made in understanding fibrogenesis, effective antifibrotic therapies remain limited^[1,3]. Therefore, identifying molecules that are not only associated with fibrosis severity but also functionally involved in fibrogenic activation remains of considerable clinical relevance^[11]. In the present study, by integrating genetic association analysis, transcriptomic and proteomic prioritization, machine-learning-based external validation, single-cell transcriptomics, clinical ELISA analysis, animal experiments, and *in vitro* functional assays, we identified PTK7 as a fibrosis-associated molecule with potential biomarker and therapeutic relevance.

A major strength of this study is the multi-omics prioritization framework. By integrating eQTL and pQTL evidence, we identified 18 overlapping candidate genes, among which PTK7 showed the most consistent association with liver fibrosis across datasets. This finding is in agreement with previous studies showing that disease-specific eQTL screening can identify regulatory genes involved in hepatic injury and fibrosis^[29]. In addition, validation across independent GEO cohorts and multiple machine-learning models further supported PTK7 as a robust fibrosis-related feature^[18-19]. Compared with single-dataset transcriptomic screening, this integrative strategy may improve the biological relevance and translational potential of candidate genes.

Our clinical data further suggest that circulating PTK7 may serve as a candidate indicator of fibrosis severity. Plasma PTK7 levels were significantly elevated in patients with liver fibrosis and positively correlated with liver stiffness measurement. In the current cohort, the fibrosis group showed higher PTK7 levels than healthy controls and MAFLD patients without fibrosis, while no difference was observed between the two control subgroups, supporting the view that PTK7 elevation may be more closely related to fibrotic remodeling than to chronic liver disease alone^[19, 30]. However, this clinical analysis should still be regarded as preliminary, and larger independent cohorts are

needed to determine the diagnostic performance, disease specificity, and incremental value of PTK7 over established non-invasive fibrosis markers.

From a mechanistic perspective, our findings support a role for PTK7 in hepatic stellate cell activation. Activation of HSCs is a central event in liver fibrogenesis and is characterized by increased expression of ECM proteins such as COL1A1 and α -SMA^[1,5]. In activated LX-2 cells, PTK7 knockdown reduced the expression of COL1A1 and α -SMA and was accompanied by decreased β -catenin abundance, suggesting that PTK7 contributes to maintenance of the profibrotic phenotype. Together with the rescue experiment using the Wnt/ β -catenin activator SKL2001, these findings support the involvement of Wnt/ β -catenin-related signaling in PTK7-mediated fibrogenic activation. Because dysregulation of the Wnt/ β -catenin pathway has been implicated in hepatic stellate cell activation and fibrosis progression^[7,31], our data support a model in which PTK7 may promote fibrogenic activation at least partly through modulation of β -catenin signaling. A plausible downstream consequence is altered β -catenin stabilization and subsequent regulation of profibrotic target gene expression, although this still requires direct validation.

The pathway enrichment and single-cell analyses provide additional biological context. PTK7-related genes were enriched in Wnt, Ras, PI3K-Akt, and calcium signaling pathways, all of which have been implicated in fibrosis progression^[4, 31-32]. In parallel, single-cell analysis suggested that PTK7 expression was preferentially enriched in Kupffer cell-annotated and fibroblast/stromal-related populations, rather than being uniformly distributed across liver cell types. Additional HSCs signature scoring in the stromal compartment showed partial overlap between PTK7-positive cells and HSCs-related transcriptional features, supporting a potential association with HSCs-like stromal cells. Nevertheless, because stromal and mesenchymal populations remain difficult to resolve precisely in single-cell datasets, these findings should be interpreted as providing cellular context rather than definitive lineage assignment^[20, 33].

Molecular docking and molecular dynamics simulations suggested a stable interaction pattern between PTK7 and β -catenin, providing preliminary computational

support for the observed reduction in β -catenin after PTK7 silencing. However, this finding should be interpreted cautiously. The PTK7- β -catenin relationship currently remains a mechanistic hypothesis supported by pathway analysis, knockdown data, rescue evidence, and computational modeling, rather than direct proof of a physical interaction. Importantly, whether PTK7 directly interacts with β -catenin remains to be experimentally validated, for example by co-immunoprecipitation. Additional studies, including β -catenin nuclear translocation assays and expanded downstream target-gene analyses, will also be required to further clarify this mechanism.

This study has several limitations. First, the single-cell data provide limited resolution for heterogeneous stromal and fibroblast-related populations, which may affect precise cellular localization of PTK7^[20, 33]. Second, the CCl₄-induced model does not fully recapitulate the etiological heterogeneity and chronic progression of human liver fibrosis^[6, 23]. Third, because PTK7 is not liver-specific and is expressed in multiple biological contexts, its specificity as a stand-alone biomarker requires further evaluation^[9, 34]. Finally, although the current results support the involvement of Wnt/ β -catenin-related signaling, whether PTK7 directly interacts with β -catenin still requires direct experimental confirmation, particularly by co-immunoprecipitation.

In conclusion, this study provides convergent evidence from multi-omics analyses, external transcriptomic validation, single-cell data, clinical samples, animal experiments, and in vitro functional assays that PTK7 is closely associated with liver fibrosis. PTK7 was elevated in fibrotic tissues and in plasma from patients with liver fibrosis, correlated with liver stiffness, and functionally contributed to the fibrotic phenotype in activated LX-2 cells. Taken together, these findings suggest that PTK7 may serve as a candidate biomarker of fibrosis severity and a potential therapeutic target, while warranting further validation in larger clinical cohorts and deeper mechanistic investigation^[35].

Contributions: YANG Yongwen Performed the research, collected, analyzed, and interpreted the data, conducted statistical analyses, and drafted the manuscript; CHEN Jun Collected, analyzed, and interpreted the data; HUANG Zebing Conceived and

designed the study, critically reviewed the manuscript, obtained research funding, and provided study supervision. All authors have read and approved the final version of the manuscript.

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