Journal of Clinical and Translational Hepatology **2026**
DOI: 10.14218/JCTH.2026.00482

Review Article

The CYB5R3–mARC1 Redox Axis in Metabolic Dysfunction-associated Steatotic Liver Disease: Mechanistic Basis and Therapeutic Prospects across the Steatosis–Cirrhosis–Hepatocellular Carcinoma Spectrum

Soon Woo Nam*

Hepatobiliary Unit, Division of Gastroenterology, Department of Internal Medicine, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Received: June 16, 2026 | **Revised:** July 16, 2026 | **Accepted:** August 03, 2026 | **Published online:** September 08, 2026

Abstract

Metabolic dysfunction-associated steatotic liver disease affects roughly 38% of adults, yet approved agents do not correct the upstream redox-metabolic perturbations—a depressed nicotinamide adenine dinucleotide (NAD^+/NADH) ratio, saturated lipid excess, and endoplasmic reticulum (ER) stress—that drive hepatocyte injury. Cytochrome b5 reductase 3 (CYB5R3) couples NADH oxidation to fatty acid desaturation, nuclear factor erythroid 2-related factor 2 (NRF2)-linked antioxidant and cholesterol-handling pathways, NAD^+/sirtuin signaling, and ER-phagy, and is the sole electron input to mitochondrial amidoxime-reducing component 1 (mARC1). This review grades every link in the axis across the steatosis–cirrhosis–hepatocellular carcinoma spectrum, reporting effect estimates with sample sizes and test statistics alongside a study-level appraisal of clinical relevance. The common MTARC1 p.A165T variant protects against all-cause cirrhosis (odds ratio, 0.91; 95% CI, 0.89–0.94; $P = 2.3 \times 10^{-11}$; 12,361 cases, 790,095 controls), with lower hepatic fat, liver enzyme levels, and low-density lipoprotein cholesterol levels. Germline mARC1 deletion reduces picrosirius red fibrosis area by 24–50% depending on diet, without altering histological disease activity, whereas partial protein reduction confers no protection. Critically, therapeutic hepatocyte-directed knockdown loses its anti-fibrotic effect when started at higher disease burden and in the choline-deficient model: efficacy depends on the depth, compartment, and timing of inhibition. Deep, hepatocyte-restricted mARC1 inhibition by GalNAc-conjugated oligonucleotides—which also avoids a male-predominant cardiac liability—is therefore the most credible near-term strategy, only in pre-cirrhotic F2–F3 disease. CYB5R3 activation, and its combination with mARC1 inhibition, remain unproven, and no clinical trial of this axis has been reported. Falsifiable *in vivo* and pharmacodynamic biomarker roadmaps are proposed, together with the efficacy, delivery, and safety uncertainties specific to advanced cirrhosis and a non-invasive pharmacodynamic framework.

Citation of this article: Nam SW. The CYB5R3–mARC1 Redox Axis in Metabolic Dysfunction-associated Steatotic Liver Disease: Mechanistic Basis and Therapeutic Prospects across the Steatosis–Cirrhosis–Hepatocellular Carcinoma Spectrum. J Clin Transl Hepatol 2026. doi: 10.14218/JCTH.2026.00482.

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects an estimated 38–39% of adults worldwide, and its progressive form, metabolic dysfunction-associated steatohepatitis (MASH), drives fibrosis, cirrhosis, and hepatocellular carcinoma (HCC); MASLD is now among the fastest-growing indications for liver transplantation and incident HCC.[1–7] A substantial minority of MASLD-related HCC arises before established cirrhosis, underscoring the oncogenic potential of the pre-cirrhotic milieu.[6,8]

The 2024 approval of resmetirom for non-cirrhotic MASH with F2–F3 fibrosis was the first disease-modifying milestone in the field,[9,10] but it acts on thyromimetic signaling rather than on the upstream redox-metabolic perturbations—nicotinamide adenine dinucleotide (NAD^+/NADH) imbalance, saturated fatty acid excess, and endoplasmic reticulum (ER) stress—or the heritable determinants of progression, such as PNPLA3 p.I148M, TM6SF2 p.E167K, and the protective MTARC1 p.A165T variant.[11–13] This motivates interest in targets positioned further upstream in hepatocyte injury.

This review advances a single central hypothesis: that the CYB5R3–mARC1 axis is an upstream redox-metabolic control node governing MASLD progression, whose most mature near-term therapeutic expression is deep, hepatocyte-restricted mARC1 inhibition, while CYB5R3 activation and the proposed combination of CYB5R3 activation with mARC1 inhibition remain biologically motivated but untested. CYB5R3 is an NADH-dependent diflavin oxidoreductase anchored to the ER and outer mitochondrial membrane (OMM); through

Keywords: Cytochrome b5 reductase; MTARC1; Metabolic dysfunction-associated steatotic liver disease; Liver cirrhosis; Hepatocellular carcinoma; Oxidation-reduction; NAD; Lipid metabolism; RNAi therapeutics.

***Correspondence to:** Soon Woo Nam, Hepatobiliary Unit, Division of Gastroenterology, Department of Internal Medicine, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul 21431, Korea. ORCID: https://orcid.org/0000-0001-5562-4775. Tel: +82-32-280-7493, E-mail: drswnam@catholic.ac.kr.

cytochrome b5, it supplies reducing equivalents to fatty acid desaturases, selected biosynthetic and drug-metabolizing cytochromes, and mARC1, and it participates in NAD^+ regeneration and antioxidant defense.[14–16] The strongest human evidence implicating the pathway comes from mARC1: large biobank analyses show that partial loss of mARC1 function (MTARC1 p.A165T) protects against liver disease across ancestries and etiologies.[11–13,17,18] Throughout, I distinguish three tiers of evidence—established in humans or *in vivo*; supported only by cell-based or preclinical data; and inferential—and, where possible, attach quantitative effect estimates. Table 1 grades every link in the axis by tier[11,14–17,19–30]; Table 2 gives the quantitative synthesis,[11,17,22–25,30–32] and Table 3 appraises each pivotal study for clinical relevance.[11,20,22,24,25,28,31,32] I also state at the outset what the evidence base does not contain: there are no clinical trials of this axis, and every interventional result cited here is preclinical.

Molecular architecture and regulation of CYB5R3

CYB5R3 is a 301-amino-acid flavoprotein with a flavin adenine dinucleotide-binding domain and an N-terminal hydrophobic anchor that tethers it to the ER and OMM.[14,16] It transfers electrons from NADH to cytochrome b5 isoforms (CYB5A, CYB5B), which reduce at least three acceptor systems: fatty-acid desaturases and elongases (notably stearoyl-CoA desaturase 1 (SCD1) and ELOVL5/6 [elongation of very long-chain fatty acids protein 5/6]) that govern membrane phospholipid composition; cholesterol-biosynthetic and drug-metabolizing cytochromes; and ubiquinone, contributing to antioxidant capacity.[14–16] A related but distinct NADPH-dependent reductase, cytochrome P450 oxidoreductase (POR/CPR), has separately been highlighted as an underappreciated regulator of hepatic lipid and redox metabolism in MASLD, illustrating the broader relevance of membrane reductases to this disease.[33]

Transcriptional control of CYB5R3 has been linked to nuclear factor erythroid 2-related factor 2 (NRF2), which binds antioxidant response elements in the promoter following electrophilic or apolipoprotein O (APOO)-associated stimuli, and to forkhead box (FOXO) factors that are inhibited by insulin/phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling and reactivated during fasting, coupling CYB5R3 expression to nutrient status.[14–16] A reciprocal relationship with NAD^+-dependent sirtuins has been proposed, whereby NAD^+ generated through CYB5R3 activity supports SIRT1 function.[14,30] A fourth regulatory layer is posttranslational: CYB5R3 is a substrate for UFMylation by the ER-membrane UFL1/UFBP1 ligase complex, which marks it as an ER-phagy receptor.[19] A downstream link to suppression of NLRP3 inflammasome activation and stellate-cell activation is biologically plausible but, to date, remains inferential rather than directly demonstrated in the liver.

Lipid remodeling, cholesterol handling, and ER proteostasis

Lipotoxicity in MASH depends less on absolute triglyceride content than on the balance between saturated fatty acids (SFAs) and long-chain polyunsaturated fatty acids (LC-PUFAs) in membrane and lipid-droplet phospholipids.[14,34] Excess SFAs rigidify membranes, perturb the unfolded protein response, and can activate proapoptotic stress kinases. CYB5R3 supplies the reducing equivalents for SCD1-mediated Δ9 desaturation and for ELOVL5/6 elongation, and in cell and rodent systems, its activity helps restore membrane fluidity and limit ER stress.[14–16]

A complementary cholesterol-handling route has been described in which APOO supports NRF2-dependent expression of CYB5R3 and biliary cholesterol elimination; hepatocyte APOO depletion reduced NRF2 signaling, lowered CYB5R3, and impaired cholesterol excretion through a low-density lipoprotein (LDL)-receptor-independent mechanism, with phenotypes worsened on Ldlr- and Apoe-deficient backgrounds, and CYB5R3 reconstitution rescued the phospholipid-unsaturation defect.[15] This pathway is attractive for settings such as statin intolerance, although it has been characterized principally in mouse models and cells. CYB5R3 additionally acts, under ER stress, as a selective ER-phagy receptor directing damaged ER to lysosomal degradation.[19] Whether this materially reduces stellate-cell-activating signals in the fibrotic liver is not yet established.

The CYB5R3–NAD^+–sirtuin axis

By oxidizing NADH at the ER and OMM, CYB5R3 can expand the NAD^+ pool available to NAD^+-dependent sirtuins, principally SIRT1 and SIRT3.[14,30,35] SIRT1-mediated deacetylation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and FOXO factors promotes mitochondrial biogenesis, fatty-acid oxidation, and antioxidant gene expression. Mice co-overexpressing CYB5R3 and NAD(P)H:quinone oxidoreductase 1 (NQO1) ("RedTg" mice) display features reminiscent of caloric restriction—improved insulin sensitivity, enhanced mitochondrial respiration, reduced inflammation, and modest lifespan extension.[30,36] These findings are relevant to MASH, in which the hepatic NAD^+/NADH ratio is typically depressed and NAD^+-repletion strategies have shown preclinical promise.[35] It must be emphasized that the RedTg phenotype reflects combined CYB5R3/NQO1 overexpression in a transgenic background and cannot be equated with the effect of a pharmacological CYB5R3 activator in established human disease.

Insulin resistance, CYB5R3, and fibrogenesis

Insulin resistance is among the strongest predictors of fibrosis progression in MASLD, and fibrosis stage—rather than steatohepatitis per se—is the histologic feature most tightly linked to liver-related mortality.[37–39] A self-reinforcing model has been proposed in which hyperinsulinemia suppresses FOXO1-driven CYB5R3 transcription, reducing desaturase activity and amplifying the SFA accumulation that provoked insulin resistance.[14,15] A consequent fall in NAD^+/NADH would impair respiratory-chain function, promote the release of danger-associated molecular patterns, and favor NLRP3 inflammasome and TGF-β-driven stellate-cell activation,[37,38] while CYB5R3-derived ubiquinol and NRF2-dependent antioxidant programs could counter the resulting reactive oxygen species.[14–16] This framework is internally coherent and supported by individual observations, but the complete sequence has not been demonstrated end-to-end in human liver and should be regarded as a working model.

CYB5R3, mARC1, and HCC

Liver-specific evidence

The most direct liver evidence linking this axis to hepatocarcinogenesis comes from mARC1. Transient knockdown of MTARC1 p.A165 lowered cell counts, averaged across three time points, by 34% in Hep3B2, 24% in Huh7, and 24% in HepG2 cells ($P \leq 0.01$), and by 16% in HepaRG cells ($P \leq 0.01$ at 48 h; $P < 0.001$ at 72 h). CRISPR-Cas9 knockout in

Table 1. Evidence on the CYB5R3–mARC1 axis across the MASLD–cirrhosis–HCC spectrum, graded by strength

Domain	Key finding	Model/source	Evidence tier	References
Human genetics (mARC1)	MTARC1 p.A165T (minor allele frequency 25% in the Emdin discovery cohorts; ~7% in East Asians): genome-wide-significant protection against all-cause cirrhosis across etiologies, with an allelic series extending to the protein-truncating p.R200Ter	UK Biobank, Partners Biobank, ARIC and two alcohol-cirrhosis case-control studies (discovery); BioVU, FinnGen, Million Veteran Program (replication); n > 800,000 total	Established (human)	Emdin *et al*. 2020[11]; Innes *et al*. 2020[17]; Ghouse *et al*. 2024[21]
Human hepatocyte biology	*MARC1* downregulation lowers neutral lipid by increasing beta-oxidation, upregulates ferroptosis-suppressor proteins and lowers ROS	Primary human hepatocytes genotyped for the risk and protective alleles; four hepatoma lines	Established (human cells)	Ciociola *et al*. 2025[22]
In vivo mARC1 loss	Complete mARC1 loss reduces picrosirius red fibrosis area by 24–50% depending on the dietary model, with lower *Col1a1* and *Timp1*; the effect is not seen with partial protein reduction, when therapeutic knockdown begins at higher disease burden, or in every diet	Merck; Broad/Bayer; academic (mouse)	Established (*in vivo*), with model- and timing-dependent limits	Coyne *et al*.2025[25]; Tie *et al*. 2026[23]
mARC1-phospholipid axis	mARC1 inactivation upregulates CEPT1/PEMT, increasing glycerophospholipids, reducing lipid-droplet size, enhancing lipolysis/lipophagy	Mouse KO, hepatocytes, lipidomics	Established (*in vivo*)	Tie *et al*. 2026[23]
Threshold effect	Murine A168T reduces mARC1 protein without affecting mRNA, yet does not reduce steatosis, inflammation or fibrosis in either sex; heterozygous Mtarc1 mice, whose protein is comparable to wild type, are likewise unprotected	Multiple mouse MASH models	Established (*in vivo*)	Pandovski *et al*. 2026[24]; Tie *et al*. 2026[23]
Cardiac safety	Cardiomyocyte-specific Cyb5r3 deletion causes male-predominant fatal cardiomyopathy; mandates hepatocyte-selective delivery	Inducible cardiac KO mice; human T117S	Established (*in vivo*/human)	Carew *et al*. 2022[28]
GalNAc delivery	GalNAc-siRNA achieves strong hepatic selectivity and durable knockdown; MTARC1-targeting siRNA programs are in preclinical development across several companies (patent filings), and no clinical trial has been reported	NHP; approved GalNAc agents	Established (platform)	Qin *et al*. 2025[29]
Caloric-restriction mimicry	CYB5R3+NQO1 overexpression improves insulin sensitivity and mitochondrial function; mean lifespan +4.2% (P = 0.04); in the diethylnitrosamine model, tumor number, volume and burden all reduced (P = 0.03, P = 0.01, P = 0.01)	RedTg transgenic mice	Preclinical	Diaz-Ruiz *et al*. 2018[30]
Lipid desaturation	CYB5R3 supplies electrons to SCD1; loss impairs SFA-to-LC-PUFA conversion and promotes ER stress	Hepatocyte/mouse models	Preclinical	Martin-Montalvo *et al*. 2016[14]; Siendones *et al*. 2014[16]
Cholesterol handling	APOO-NRF2-CYB5R3 supports LDLR-independent biliary cholesterol elimination	Mouse APOO-null, hepatocytes	Preclinical	Chen *et al*. 2024[15]
ER-phagy/fibrosis link	UFMylated CYB5R3 acts as ER-phagy receptor; proposed suppression of stellate-cell activation	Cell lines; inference to liver	Preclinical to Hypothesis	Ishimura *et al*. 2022[19]
CYB5R3 activator (THII)	THII improves glucose handling and beta-cell function (Cyb5r3-dependent); no anti-fibrotic *in vivo* data	Rodent pharmacology	Preclinical	Martin-Montalvo *et al*. 2016[14]; Watanabe *et al*. 2023[26]; Watanabe *et al*. 2024[27]
HCC apoptosis mechanism	CYB5R3-PARP16-PERK/IRE1α ER-stress apoptosis, cancer-cell-selective	NSCLC cells/xenografts (not liver)	Hypothesis for HCC	Im *et al*. 2024[20]
CYB5R3 activation + mARC1 inhibition	Proposed complementarity of CYB5R3 activation + mARC1 inhibition	None (rational only)	Hypothesis (untested)	None yet

Evidence tiers: Established = human genetics/cells or *in vivo* knockout data; Preclinical = cell- or single-model mechanistic data; Hypothesis = extrapolated or untested. ALT, alanine aminotransferase; APOO, apolipoprotein O; ARIC, Atherosclerosis Risk in Communities study; CEPT1, choline/ethanolamine phosphotransferase 1; Col1a1, collagen type I alpha 1 chain; CYB5R3, cytochrome b5 reductase 3; DEN, diethylnitrosamine; ER, endoplasmic reticulum; GalNAc, N-acetylgalactosamine; HCC, hepatocellular carcinoma; IRE1α, inositol-requiring enzyme 1 alpha; KO, knockout; LC-PUFA, long-chain polyunsaturated fatty acid; LDLR, low-density lipoprotein receptor; mARC1/MTARC1, mitochondrial amidoxime-reducing component 1; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; NHP, nonhuman primate; NQO1, NAD(P)H:quinone oxidoreductase 1; NRF2, nuclear factor erythroid 2-related factor 2; NSCLC, non-small-cell lung cancer; PARP16, poly(ADP-ribose) polymerase family member 16; PEMT, phosphatidylethanolamine N-methyltransferase; PERK, protein kinase R-like endoplasmic reticulum kinase; RedTg, CYB5R3/NQO1 double-transgenic mouse; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase 1; SFA, saturated fatty acid; siRNA, small interfering RNA; THII, tetrahydroindenoindole; Timp1, tissue inhibitor of metalloproteinase 1; UFMylation, ubiquitin-fold modifier 1 conjugation.

Table 2. Quantitative synthesis of pivotal evidence on the CYB5R3–mARC1 axis. Rebuilt in this revision from the primary sources

Study/ evidence	Design and sample size	Effect estimate (95% CI where derivable)	*P*-value or test statistic	Evidence tier
Emdin *et al*. 2020[11]/Human genetics	Multi-cohort GWAS with replication. Combined 12,361 all-cause cirrhosis cases/790,095 controls, 8 cohorts. Discovery 3,754/444,791, 5 cohorts; replication BioVU, FinnGen, Million Veteran Program	All-cause cirrhosis per p.A165T allele: OR 0.91 (95% CI 0.89–0.94)[†]; Discovery OR 0.87; BioVU 0.92; FinnGen 0.89; MVP 0.92; ALT −0.025 SD; ALP −0.025 SD; total cholesterol −0.030 SD; LDL-C −0.027 SD; Hepatic fat on CT (FHS+MESA) −0.08 SD; Physician-diagnosed fatty liver OR 0.83	Cirrhosis $P = 2.3 \times 10^{-11}$; Discovery $P = 8.7 \times 10^{-7}$; BioVU $P = 0.045$; FinnGen $P = 0.044$; MVP $P = 7.4 \times 10^{-5}$; ALT $P = 3.7 \times 10^{-43}$; ALP $P = 1.2 \times 10^{-37}$; TC $P = 1.9 \times 10^{-36}$; LDL-C $P = 5.1 \times 10^{-30}$; Hepatic fat $P = 8.2 \times 10^{-6}$; fatty liver $P = 1.90 \times 10^{-8}$; Heterogeneity $P = 0.64$	Established (human genetics)
Emdin *et al*. 2020[11]/allelic series	Protein-truncating p.R200Ter carriers versus non-carriers	0 cirrhosis cases among 238 R200Ter carriers versus 17,046 among 759,027 non-carriers	$P = 0.04$	Established (human genetics)
Innes *et al*. 2020[17]	GWAS for alcohol-related cirrhosis, discovery plus phase-2 validation across 5 European cohorts; adjusted for age, sex, BMI, type 2 diabetes	rs2642438 minor A allele, adjusted OR 0.76 (95% CI 0.64–0.91)[†]	$P = 0.0027$	Established (human genetics)
Ciociola *et al*. 2025[22]	siRNA knockdown in primary human hepatocytes genotyped for the risk and protective alleles; four HCC cell lines; UK Biobank n = 239,075	70% knockdown lowered neutral lipid in risk-allele hepatocytes only; β-oxidation nearly doubled under 300 μM oleate–palmitate; protective-allele hepatocytes carry ~50% less mARC1 protein; no change in ApoB100 secretion, media triglyceride or de novo lipogenesis; minor allele associated with higher plasma 3-hydroxybutyrate	Reported as significant in the primary source; UK Biobank association by REGENIE adjusted for age, sex, BMI and 10 principal components	Established (human cells and population)
Kovooru *et al*. 2025[31]	Transient knockdown in 4 HCC lines; CRISPR-Cas9 knockout in Hep3B2 (>90% protein reduction); subcutaneous xenograft in BALB/c nude mice, 48 days	Cell count −34% (Hep3B2), −24% (Huh7), −24% (HepG2), −16% (HepaRG); Knockout: proliferation −35%/−41%/−43% at 24/48/72 h; wound closure inhibited 73%/55%/60%; Xenograft tumor volume −45%; tumor weight −38%; Ki-67 −42%; Proteomics: 375 up/228 down of 8,381 proteins	Cell count $P \leq 0.01$; Proliferation $P < 0.001$, $P \leq 0.01$, $P < 0.001$; Migration $P < 0.001$, $P \leq 0.01$, $P \leq 0.01$; Volume $P < 0.001$; weight $P < 0.05$; Ki-67 $P < 0.05$; Proteomics FDR-adjusted, $\lvert \log_2 FC \rvert \geq 0.58$	Preclinical (liver-specific)
Coyne *et al*. 2025[25]/germline knockout	Male Mtarc1 knockout versus wild type. GAN diet 32 wk, n = 13–14. High-fat diet with 30% fructose water 20 wk, n = 10–13. CDAHFD and CCl_4 additionally tested	GAN: picrosirius red area ≈−50%; plasma ALT ↓; *Col1a1* and *Timp1* ↓; HFD-HFr: picrosirius red area −24%; ALT ↓; liver triglycerides ↓; Steatosis, inflammation, ballooning and MASLD activity score unchanged in both models	$^*P < 0.05$, $^{***}P < 0.001$ as reported (mean ± SEM)	Established (*in vivo*)
Coyne *et al*. 2025[25]/therapeutic knockdown	Hepatocyte-directed GalNAc-siRNA, Q2W × 8 wk, n = 10–13, started at different disease burdens	GAN 16 wk: 75% mRNA knockdown; collagen area ↓; fibrosis and inflammatory gene expression ↓; plasma ALT and AST unchanged; HFD-HFr 12–13 wk: >85% knockdown; steatosis and ballooning ↓; fibrosis trend only; GAN 24 wk (higher burden): no effect on any liver endpoint; CDAHFD: no effect on fibrosis	GAN 16 wk significant; HFD-HFr fibrosis not significant; GAN 24 wk and CDAHFD not significant; Authors' conclusion: disease burden at the time of intervention is critical	Established (*in vivo*), including negative results

(continued)

Table 2. *(continued)*

Study/ evidence	Design and sample size	Effect estimate (95% CI where derivable)	*P*-value or test statistic	Evidence tier
Pandovski *et al*. 2026[24]	First knock-in of the murine orthologous substitution mARC1 A168T; male and female mice; multiple MASH and fibrosis models	mARC1 protein significantly reduced with unchanged mRNA; neither sex showed significantly reduced steatosis, inflammation or fibrosis in any model	Not significant for all liver endpoints	Established (*in vivo*, null result)
Yin *et al*. 2026[32]	Whole-body Mtarc1 knockout (CRISPR/Cas9, C57BL/6J). High-fat versus low-fat diet obesity model, male, 16 wk, n = 9–11 per group. CDAHFD, female wild-type/heterozygous/knockout, 2 wk, n = 8–15 per group (also 4 and 11 wk). Untargeted metabolomics n = 9–21 per group (581 known metabolites). Primary hepatocytes, n = 3–4 independent experiments	CDAHFD 2 wk: liver-to-body-weight ratio 5.3% versus 6.6%; liver weight 1.156 g versus 1.398 g; plasma ALT 179.5 versus 268.1 mg/dL; plasma triglycerides 47.4 versus 60.2 mg/dL; Lipid-droplet area 14.9% versus 24.6% (a 39% relative reduction); Profibrotic genes: *Tgfb1* 2.4 versus 3.1 fold, αSMA 1.4 versus 2.1 fold, *Col1a1* 9.4 versus 18.6 fold; Inflammatory genes: Tnf-α 10.9 versus 14.2 fold; Mcp1 11.4 versus 16.1 fold; Histological fibrosis: reduction was a trend only; Obesity model: liver mass and cholesterol lower; no effect on glucose tolerance, fasting glucose or insulin; Hepatocytes: fatty-acid uptake reduced 32%; ApoB secretion increased; Hepatic phosphatidylcholine and phosphatidylethanolamine enriched in both sexes at 2, 4 and 11 wk	Liver-to-body-weight ratio $P < 0.0001$; liver weight $P = 0.0051$; ALT $P = 0.0028$; triglycerides $P = 0.0393$; Lipid-droplet area $P = 0.1587$ (not significant); *Tgfb1* $P = 0.0008$; αSMA $P = 0.0001$; *Col1a1* $P = 0.0056$; Tnf-α $P = 0.07$ and Mcp1 $P = 0.07$ (not significant); Histological fibrosis $P > 0.05$ (not significant); GraphPad Prism 10; unpaired two-tailed t test, one-way or two-way ANOVA with post hoc correction	Established (*in vivo*) for steatosis and profibrotic gene expression; fibrosis endpoint not met
Tie *et al*. 2026[23]	Global and liver-specific Mtarc1 knockout plus AAV-mediated knockout; male and female mice. CDAHFD 7–8 wk (global n = 7–9/group; liver-specific n = 8–11/group; AAV n = 6–8/group). High-fructose/high-fat diet (HFHFD) tested separately. RNA-seq n = 8/group; TMT proteomics n = 4/group; lipidomics n = 9/group	CDAHFD: liver-to-body-weight ratio ↓, hepatic triglycerides ↓ (cholesterol unchanged), ALT, AST and total bilirubin ↓, *Tgfb1* and *Il1b* ↓, *Col1a1*, *Spp1* and *Timp1* ↓, COL1A1 protein ↓ and Sirius red staining ↓; comparable protection in females; Heterozygous mice showed no phenotypic difference from wild type, matching their unchanged mARC1 protein; No significant phenotype on HFHFD; Protection abolished in Pnpla2/Lipa double-knockout livers and reversed by CEPT1/PEMT knockdown	Student's t-test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; HFHFD: not significant; Heterozygotes: not significant	Established (*in vivo*), including negative results
Diaz-Ruiz *et al*. 2018[30]	RedTg mice overexpressing CYB5R3 and NQO1. Longevity cohort n = 64 wild type/n = 75 RedTg. Diethylnitrosamine at 16 days with high-fat diet from weaning; MRI at 9 months (wild type n = 12, RedTg n = 17)	Liver tumor number 25.8 ± 4.8 versus 42.7 ± 5.7; Tumor volume 449.8 ± 105.4 versus 942.8 ± 177.5 mm^3; Tumor burden 21.9 ± 4.1% versus 40.6 ± 5.6% of liver volume; Liver volume 1,848.2 ± 129.1 versus 2,115.6 ± 220.2 mm^3; Mean lifespan +4.2%; 20% survival +4.8%	Tumor number $P = 0.03$; tumor volume $P = 0.01$; tumor burden $P = 0.01$; Liver volume $P = 0.27$ (not significant); Lifespan $P = 0.04$ (log-rank, $\chi^2 = 3.9$); 20% survival $P = 0.01$; Two-tailed Student's t-test	Preclinical (*in vivo*)

[†]The 95% confidence intervals marked with a dagger are derived from the reported point estimate and p value on the log-odds scale using a normal approximation because the primary reports present their confidence intervals graphically rather than in text. All other statistics are reproduced exactly as reported in the primary source. Where a primary study did not report a given statistic or where it could not be obtained from the accessible record, the cell states this rather than substituting an inferred value. Evidence tiers: Established = human genetics, human cells, or *in vivo* knockout data; Preclinical = cell- or single-model mechanistic data; Hypothesis = extrapolated or untested. ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; CCl_4, carbon tetrachloride; CDAHFD, choline-deficient amino acid-defined high-fat diet; CI, confidence interval; CT, computed tomography; FDR, false discovery rate; FHS, Framingham Heart Study; GAN, Gubra Amylin NASH diet; HFD-HFr, high-fat diet with fructose water; LDL-C, low-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis; MVP, Million Veteran Program; OR, odds ratio; SD, standard deviation; SEM, standard error of the mean; TC, total cholesterol; ↑, increased; ↓, decreased.

Table 3. Study-level appraisal: strengths, limitations, and what each study does and does not support in clinical practice

Study	Strengths	Limitations	What it does and does not tell the clinician
Emdin *et al*. 2020[11]/ Human genetics	Very large and adequately powered (12,361 cases, 790,095 controls, 8 cohorts). Genome-wide significant with independent replication in three separate biobanks and no heterogeneity (P = 0.64). An allelic series — p.A165T, p.M187K and truncating p.R200Ter — supplies an internal dose–response argument. No apparent excess of cardiovascular events	A common variant models lifelong partial inhibition beginning before disease onset, which is not the experiment of giving a drug to a patient who already has fibrosis. The per-allele effect is small (OR 0.91), and the effect of complete inhibition is estimated only indirectly from the truncating allele. Cirrhosis was largely ICD-code ascertained; cohorts were predominantly of European ancestry and the variant is far less common in East Asian populations (~7%)	Supports mARC1 as a credible target for preventing progression in at-risk populations and supports genotype as a stratification variable. It does not predict the benefit obtainable by starting an inhibitor in established disease, and its per-allele effect should not be quoted as an expected treatment effect
Coyne *et al*. 2025[25]/*In vivo* efficacy	Four independent disease models (GAN, HFD-fructose, CDAHFD, CCl_4) and two orthogonal interventions — germline knockout and therapeutic hepatocyte-directed GalNAc-siRNA. Group sizes of 10–14 with pathologist-scored histology. AI-assisted digital pathology adds zonal fibrosis quantitation, collagen fibrillar properties and fibrosis–steatosis co-localization. Negative results are reported alongside positive ones	The anti-fibrotic effect is model-dependent (≈50% GAN versus 24% HFD-fructose) and did not extend to the MASLD activity score in either model. Hepatocyte-directed knockdown produced no significant effect on any liver endpoint when started at higher disease burden (GAN 24 wk) and no effect on fibrosis in CDAHFD. Pivotal arms used male mice only. No model of cirrhosis and no test of regression of established fibrosis	The most clinically decisive dataset available: efficacy is contingent on intervening before advanced disease, not merely on achieving deep knockdown. Supports a pre-cirrhotic F2–F3 target population, argues against expecting benefit in decompensated cirrhosis, and implies that any trial must stratify by baseline fibrosis stage and pre-specify the treatment window
Pandovski *et al*. 2026[24]/*In vivo*, null	The first knock-in of the murine ortholog of the human protective substitution, tested in both sexes across multiple models — the correct experiment for asking whether the variant itself is causal *in vivo*	A null result cannot exclude a small effect, and the degree of protein reduction achieved may not correspond exactly to that produced by the human allele. Mouse and human mARC1 biology diverge[18], which limits inference in both directions	Partial protein reduction is insufficient. A therapeutic must achieve deep knockdown, raising the bar simultaneously for potency, dosing interval and hepatocyte-selective delivery — which makes the cirrhotic delivery problem more, not less, important
Ciociola *et al*. 2025[22]/ Human hepatocyte biology	Genotype-matched primary human hepatocytes, with the effect present only in risk-allele carriers, replicated in four cell lines and in a second donor, and anchored to a population-scale biomarker association (3-hydroxybutyrate, n = 239,075)	Short-term siRNA in culture does not model chronic pharmacological inhibition; the population association uses a circulating proxy for β-oxidation rather than a direct measurement; no fibrosis endpoint	Identifies β-oxidation as the mechanism of lipid lowering and identifies 3-hydroxybutyrate as a candidate pharmacodynamic marker of target engagement that could be measured in an early-phase trial
Kovooru *et al*. 2026[31]/ HCC, liver-specific	The only liver-specific interventional evidence linking the axis to hepatocarcinogenesis, combining knockdown and CRISPR knockout in human HCC lines with xenograft confirmation, two independent clones, proteomic read-out and a CPT1A rescue experiment that ties the phenotype to β-oxidation	Cell lines and subcutaneous xenografts do not reproduce the fibrotic, immunologically intact microenvironment in which human HCC arises; no orthotopic or genetically engineered model; no chemoprevention endpoint	Enough to justify mARC1 as an HCC-relevant target for further study; not enough to support any claim about treating established HCC in patients
Im *et al*. 2024[20]/ Apoptosis mechanism	A detailed and internally consistent mechanism (CYB5R3 → NAD^+ → PARP16 → PERK/ATF4 and IRE1α/JNK), with silencing experiments demonstrating pathway redundancy and apparent cancer-cell selectivity	Demonstrated entirely in non-small cell lung cancer. No hepatocyte or HCC replication, and the proposed selective window rests on the untested premise that normal hepatocytes have low basal ER stress	Carries no weight for clinical decision-making in liver disease at present. It is included as a hypothesis to be tested, and this review does not rely on it

(continued)

Table 3. *(continued)*

Study	Strengths	Limitations	What it does and does not tell the clinician
Carew *et al.* 2022[28]/ Safety	Inducible, cardiomyocyte-specific deletion in adult mice with a corroborating human partial loss-of-function variant — a clean demonstration of an on-target, tissue-specific liability	The phenotype is male-predominant, and the mechanism of female protection is unresolved; the relevance of partial pharmacological reduction, as opposed to deletion, is not established	Makes hepatocyte-restricted delivery a safety requirement rather than a convenience, and argues for cardiac monitoring and sex-stratified analysis in any first-in-human program
Yin *et al.* 2026[32]/*In vivo*, steatosis	Whole-body knockout with orthogonal readouts — histology, plasma biochemistry, bulk RNA-seq, untargeted metabolomics (581 metabolites) and primary hepatocyte assays — in both sexes and at three diet durations. The authors report their non-significant results explicitly and discuss where their findings conflict with others'	The 2-week CDAHFD model does not induce meaningful fibrosis, so the fibrosis endpoint could not be met; histological steatosis (P = 0.1587) and inflammatory gene expression (P = 0.07) also fell short of significance, leaving liver mass, ALT, triglycerides and profibrotic transcripts as the significant findings. On RNA-seq, Mtarc1 was the only differentially expressed gene, so the mechanism is inferred at pathway level. The authors' increased ApoB secretion contradicts human hepatocyte data, and their absence of change in fatty-acid-oxidation genes conflicts with Ciociola *et al.*	Supports mARC1 loss as protective against steatosis and against the profibrotic transcriptional program and provides reassurance on glucose homeostasis — a specific clinical concern raised by the mild excess type 2 diabetes risk seen in variant carriers. It does not demonstrate an anti-fibrotic effect, and should not be cited as if it did

Each pivotal study is additionally appraised at the point where it is first discussed in the main text; this table consolidates those appraisals for comparison at a glance. AI, artificial intelligence; ALT, alanine aminotransferase; ATF4, activating transcription factor 4; CCl4 (subscript 4), carbon tetrachloride; CDAHFD, choline-deficient amino acid-defined high-fat diet; CPT1A, carnitine palmitoyltransferase 1A; CRISPR, clustered regularly interspaced short palindromic repeats; CYB5R3, cytochrome b5 reductase 3; ER, endoplasmic reticulum; F2–F3, fibrosis stages 2–3; GalNAc, N-acetylgalactosamine; GAN, Gubra Amylin NASH diet; HCC, hepatocellular carcinoma; HFD, high-fat diet; HFD-HFr, high-fat/high-fructose diet; HPA, Human Protein Atlas; ICD, International Classification of Diseases; IRE1α, inositol-requiring enzyme 1 alpha; JNK, c-Jun N-terminal kinase; Ki-67, marker of proliferation Ki-67; mARC1/MTARC1, mitochondrial amidoxime-reducing component 1; MASLD, metabolic dysfunction-associated steatotic liver disease; NAD+, nicotinamide adenine dinucleotide, oxidized form; NOX4, NADPH oxidase 4; OR, odds ratio; PARP16, poly(ADP-ribose) polymerase family member 16; PERK, protein kinase R-like endoplasmic reticulum kinase; RNA-seq, RNA sequencing; siRNA, small interfering RNA; TCGA-LIHC, The Cancer Genome Atlas liver hepatocellular carcinoma cohort; wk, week.

Hep3B2 cells achieved more than 90% reduction in mARC1 protein and reduced intracellular neutral lipid (P < 0.001) while raising [^{3}H]-palmitate oxidation (P ≤ 0.01); proliferation fell by 35%, 41%, and 43% at 24, 48, and 72 h, and wound closure was inhibited by 73%, 55%, and 60% at the same time points. In BALB/c nude mice, subcutaneous xenografts of knockout cells were 45% smaller in volume by day 48 (P < 0.001) and 38% lower in weight (P < 0.05), with Ki-67 expression reduced by 42% (P < 0.05). Whole-cell proteomics of xenograft tumors detected 8,381 proteins, of which 375 were upregulated and 228 downregulated (FDR-adjusted, $|\log_2 FC| \geq 0.58$), with suppression of oncogenic pathways and activation of antiproliferative proteins; knockdown of CPT1A partially reversed the benefit, tying the phenotype to β-oxidation.[31] Complementary chemopreventive data come from RedTg mice subjected to diethylnitrosamine. On magnetic resonance imaging nine months after carcinogen exposure (wild type n = 12, RedTg n = 17), the number of liver tumors was 25.8 ± 4.8 versus 42.7 ± 5.7 (P = 0.03), tumor volume was 449.8 ± 105.4 versus 942.8 ± 177.5 mm^3 (P = 0.01), and tumor burden was 21.9 ± 4.1% versus 40.6 ± 5.6% of liver volume (P = 0.01), while total liver volume did not differ (P = 0.27); inflammatory and pro-proliferative transcriptomic signatures were also reduced.[30] Appraised for clinical relevance, the Kovooru dataset is the only liver-specific interventional evidence tying this axis to hepatocarcinogenesis, and it is internally consistent across four cell lines, two clones, and an *in vivo* model; however, cell lines and subcutaneous xenografts do not reproduce the fibrotic, immunologically intact microenvironment in which human HCC arises, there is no orthotopic or genetically engineered model, and no chemoprevention endpoint was tested. It is sufficient to justify mARC1 as an HCC-relevant target for further study and insufficient to support any claim about treating established HCC in patients (Table 3). Human descriptive context (CYB5R3 and MTARC1 expression and prognostic associations in public HCC datasets such as TCGA-LIHC and the Human Protein Atlas) is consistent with a tumor-restraining role but is correlative and hypothesis-generating only.

A lung-derived apoptotic mechanism, presented as a hypothesis

By contrast, the frequently cited ER-stress apoptotic mechanism—in which CYB5R3 overexpression raises NAD$^+$, activates the ER mono-ADP-ribosyltransferase PARP16, and engages PERK–ATF4 and IRE1α–JNK signaling together with NOX4-derived oxidative stress to trigger cancer-cell-selective apoptosis—was established in non-small-cell lung cancer cells and xenografts, not in hepatocytes.[20] I therefore present this pathway (Fig. 1) explicitly as a mechanistic hypothesis for HCC that requires validation in hepatocyte and liver-tumor systems before any therapeutic inference is drawn.[20,30,31]

Dual, context-dependent, and temporally specific effects

The axis is unlikely to act unidirectionally, and this nuance is central to its therapeutic interpretation. On the tumor-restraining side, CYB5R3-driven ER-stress apoptosis (in the lung model) and mARC1-loss-driven restriction of neutral lipid[22,31] and of reactive oxygen species and ferroptotic susceptibility[22] would oppose tumor initiation. On the opposing side, the same NAD$^+$-generating and lipid-desaturating functions of CYB5R3 could, in principle, support the anabolic and redox demands of an established, metabolically reprogrammed tumor—so the desirable direction of intervention may differ between chemoprevention and treatment of an

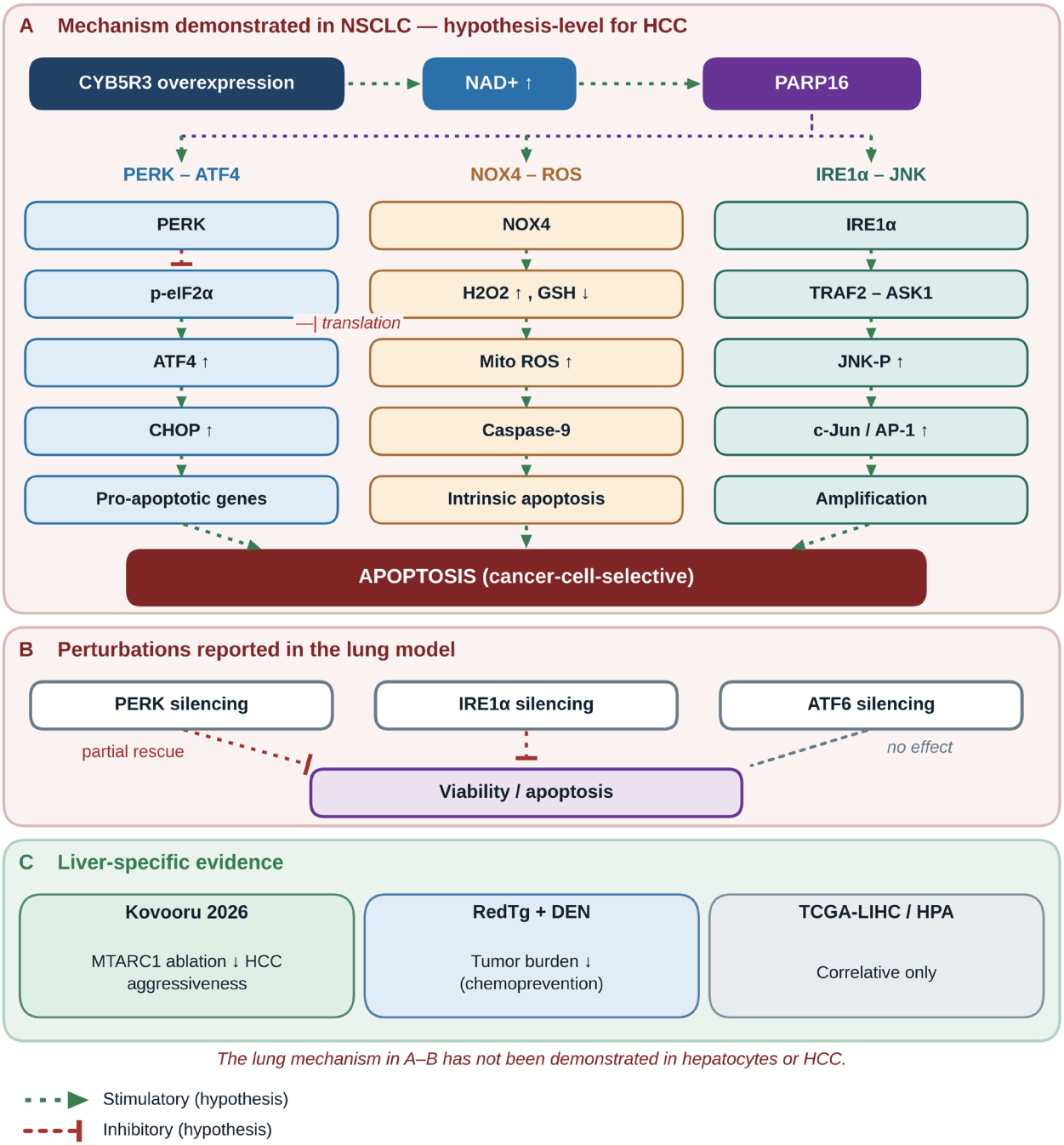

Fig. 1. The CYB5R3–PARP16 ER stress–apoptotic program: a lung-derived mechanism and the liver evidence that must replace it. Arrowheads denote stimulatory or activating interactions, and bar-headed lines denote inhibitory interactions; dashed and dotted lines denote preclinical and hypothesis-level evidence, respectively. (A) The apoptotic program characterized in non-small-cell lung cancer[20]: CYB5R3 overexpression raises NAD^+ and activates PARP16, which engages three limbs—PERK–eIF2α–ATF4–CHOP, including the inhibitory action of phosphorylated eIF2α on global translation; NOX4-dependent H_2O_2 with glutathione depletion and caspase-9 intrinsic apoptosis; and IRE1α–TRAF2–ASK1–JNK—converging on apoptosis reported to be cancer-cell selective. (B) Signed perturbation experiments: silencing PERK or IRE1α partially rescues viability, whereas silencing ATF6 does not, indicating pathway redundancy. (C) The liver-specific evidence that actually supports the axis in HCC—MTARC1 ablation in HCC lines and xenografts,[31] diethylnitrosamine chemoprevention in RedTg mice,[30] and correlative human expression data. Panels A and B are hypothesis-level for HCC: this mechanism has not been demonstrated in hepatocytes or HCC, and the review does not rely on it. NSCLC, non-small-cell lung cancer; HCC, hepatocellular carcinoma; CYB5R3, cytochrome b5 reductase 3; NAD^+, nicotinamide adenine dinucleotide, oxidized form; PARP16, poly(ADP-ribose) polymerase family member 16; PERK, protein kinase R-like endoplasmic reticulum kinase; ATF4, activating transcription factor 4; p-eIF2α, phosphorylated eukaryotic translation initiation factor 2 alpha; CHOP, C/EBP homologous protein; NOX4, NADPH oxidase 4; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; GSH, reduced glutathione; Mito ROS, mitochondrial reactive oxygen species; IRE1α, inositol-requiring enzyme 1 alpha; TRAF2, tumor necrosis factor receptor-associated factor 2; ASK1, apoptosis signal-regulating kinase 1; JNK, c-Jun N-terminal kinase; JNK-P, phosphorylated c-Jun N-terminal kinase; c-Jun, c-Jun transcription factor; AP-1, activator protein 1; ATF6, activating transcription factor 6; MTARC1, mitochondrial amidoxime reducing component 1; DEN, diethylnitrosamine; TCGA-LIHC, The Cancer Genome Atlas liver hepatocellular carcinoma cohort; HPA, Human Protein Atlas;↑, increased; ↓, decreased.

established tumor. Temporally and spatially, restoring CYB5R3/NAD^+ and lowering mARC1 activity is most plausibly beneficial in the preneoplastic, redox-stressed parenchyma (chemoprevention), whereas effects in an established tumor may be cell-type-specific across hepatocytes, activated stellate cells, and tumor-associated macrophages. Proposed effects on macrophage polarization remain speculative and are not relied upon. Defining this temporal-spatial and dual-regulatory behavior is a priority for future hepatocyte- and tumor-specific studies.

The mARC1–CYB5R3 axis and the MTARC1 p.A165T variant

The N-reductive electron-transfer system

mARC1 (gene MTARC1) is a molybdo-flavoenzyme that, together with CYB5B and CYB5R3, forms a three-component N-reductive system on the OMM. CYB5R3 donates NADH-derived electrons through cytochrome b5 to the molybdenum cofactor of mARC1, which reduces N-hydroxylated substrates (Fig. 2B). Because mARC1 is wholly dependent on the CYB5R3–cytochrome b5 relay, CYB5R3 is the rate-limiting electron input; conversely, mARC1 draws on the same NADH-derived reductive flux used for desaturation and antioxidant regeneration, providing a biochemical rationale for crosstalk.

Human genetics and mechanisms of protection (quantitative)

The missense variant rs2642438 (c.493G>A; p.A165T) in MTARC1 has a minor allele frequency of approximately 25% in the predominantly Northern European discovery cohorts of Emdin and colleagues and approximately 7% in East Asian populations, with lower frequencies in African and admixed American groups.[11,13] In the pivotal multi-cohort analysis by Emdin and colleagues (12,361 all-cause cirrhosis cases; 790,095 controls across eight cohorts), each p.A165T allele was associated with lower odds of all-cause cirrhosis (odds ratio 0.91; 95% CI, 0.89–0.94; derived as described in the footnote to Table 2; $P = 2.3 \times 10^{-11}$). The estimate was consistent across the discovery analysis (3,754 cases/444,791 controls; OR 0.87, $P = 8.7 \times 10^{-7}$) and three independent replication cohorts (BioVU OR 0.92, $P = 0.045$; FinnGen OR 0.89, $P = 0.044$; Million Veteran Program OR 0.92, $P = 7.4 \times 10^{-5}$), with no evidence of heterogeneity ($P = 0.64$). The same allele was associated with lower alanine aminotransferase (ALT) (−0.025 SD; $P = 3.7 \times 10^{-43}$), lower alkaline phosphatase (−0.025 SD; $P = 1.2 \times 10^{-37}$), lower total cholesterol (−0.030 SD; $P = 1.9 \times 10^{-36}$) and LDL cholesterol (−0.027 SD; $P = 5.1 \times 10^{-30}$), lower hepatic fat on computed tomography (−0.08 SD; $P = 8.2 \times 10^{-6}$), and lower odds of physician-diagnosed fatty liver (OR 0.83; $P = 1.90 \times 10^{-8}$), without an apparent increase in cardiovascular events.[11] An allelic series strengthens the causal argument: among 238 carriers of the protein-truncating p.R200Ter variant, there were no cases of cirrhosis, compared with 17,046 cases among 759,027 non-carriers ($P = 0.04$).[11] An independent genome-wide association study confirmed MTARC1 as a protective locus in alcohol-related cirrhosis (rs2642438 minor A allele, adjusted OR 0.76, 95% CI 0.64–0.91 [derived], adjusted for age, sex, body mass index, and type 2 diabetes across five European cohorts; $P = 0.0027$), consistent with the broader cirrhosis-risk architecture defined by PNPLA3, MBOAT7, and MTARC1.[17,21] These effect estimates, together with the preclinical findings below, are compiled in Table 2. Mechanistically, p.A165T destabilizes the protein and produces partial loss of function—primary human hepatocytes homozygous for the protective allele carry approximately 50% less mARC1 protein than risk-allele homozygotes. Silencing MTARC1 by 70% in risk-allele primary human hepatocytes lowered intracellular neutral lipid levels and nearly doubled β-oxidation under a 300 μM oleate–palmitate load, without changes in ApoB100 secretion, medium triglycerides, or de novo lipogenesis. The same manipulation had no effect on protective-allele hepatocytes, and the finding was reproduced in HepG2, Huh7, Hep3B2, and HepaRG cells. In 239,075 UK Biobank participants, the minor allele was associated with higher plasma 3-hydroxybutyrate, a circulating proxy for β-oxidation.[22] In the same primary hepatocytes, MTARC1 downregulation selectively upregulated ferroptosis-suppressor proteins, with no change in ferroptosis drivers, and lowered cellular reactive oxygen species.[22] Remodeling of lipid droplets and activation of lipophagy are reported separately in mouse models.[23]

A quantitative threshold: variant versus complete loss

An important nuance has emerged from *in vivo* work. The murine orthologous substitution A168T significantly lowers steady-state mARC1 protein levels without affecting mRNA; however, neither male nor female A168T mice showed significantly reduced steatosis, inflammation, or fibrosis in any of several models of MASH and liver fibrosis, indicating that partial protein reduction alone is insufficient—consistent with the reported divergence of MTARC1 biology between humans and mice.[18,24] Two independent lines of evidence now converge on this threshold. Tie and colleagues found that Mtarc1 heterozygous mice, whose mARC1 protein levels are comparable to those of wild-type mice, showed no phenotypic difference from wild-type mice on a choline-deficient amino acid-defined high-fat diet across every endpoint measured (n = 7–9 per group), whereas homozygous knockouts were robustly protected.[23] Partial reduction of mARC1, whether produced by a variant or by haploinsufficiency, is therefore insufficient in mice.

The magnitude of the anti-fibrotic effect obtained with complete loss is model-dependent and requires careful interpretation. In Coyne and colleagues, germline Mtarc1 knockout reduced picrosirius red-stained area by approximately 50% on the Gubra Amylin NASH (GAN) diet at 32 weeks (n = 13–14 per genotype; $P < 0.05$), with lower *Col1a1*, *Timp1*, and plasma ALT levels, but by 24% on a high-fat diet supplemented with fructose water at 20 weeks (n = 10–13). In neither model did steatosis, inflammation, ballooning, or the composite MASLD activity score change significantly.[25] The effect of mARC1 loss in these systems is therefore specific to fibrosis rather than a general improvement in steatohepatitis, and its magnitude is model-dependent. A third program reinforces this model-dependence from the opposite direction. Tie and colleagues showed robust protection on the choline-deficient amino acid-defined high-fat diet—lower liver-to-body-weight ratio, hepatic triglycerides, ALT, aspartate aminotransferase (AST), and bilirubin levels; lower *Tgfb1*, *Il1b*, *Col1a1*, *Spp1*, and *Timp1* expression; and reduced COL1A1 protein and Sirius red staining in both sexes—yet no significant phenotype at all on a high-fructose/high-fat diet, which they attributed to the choline dependence of the glycerophospholipid biosynthetic route through which the protection is mediated.[23] Their protection was also abolished in Pnpla2/Lipa double-knockout livers and reversed by CEPT1 or PEMT knockdown, establishing that it requires intact lipolysis and lipophagy. Taken together with Coyne, the pattern is that mARC1 loss is anti-fibrotic in some dietary contexts but not others, and that the responsible mechanism is specific

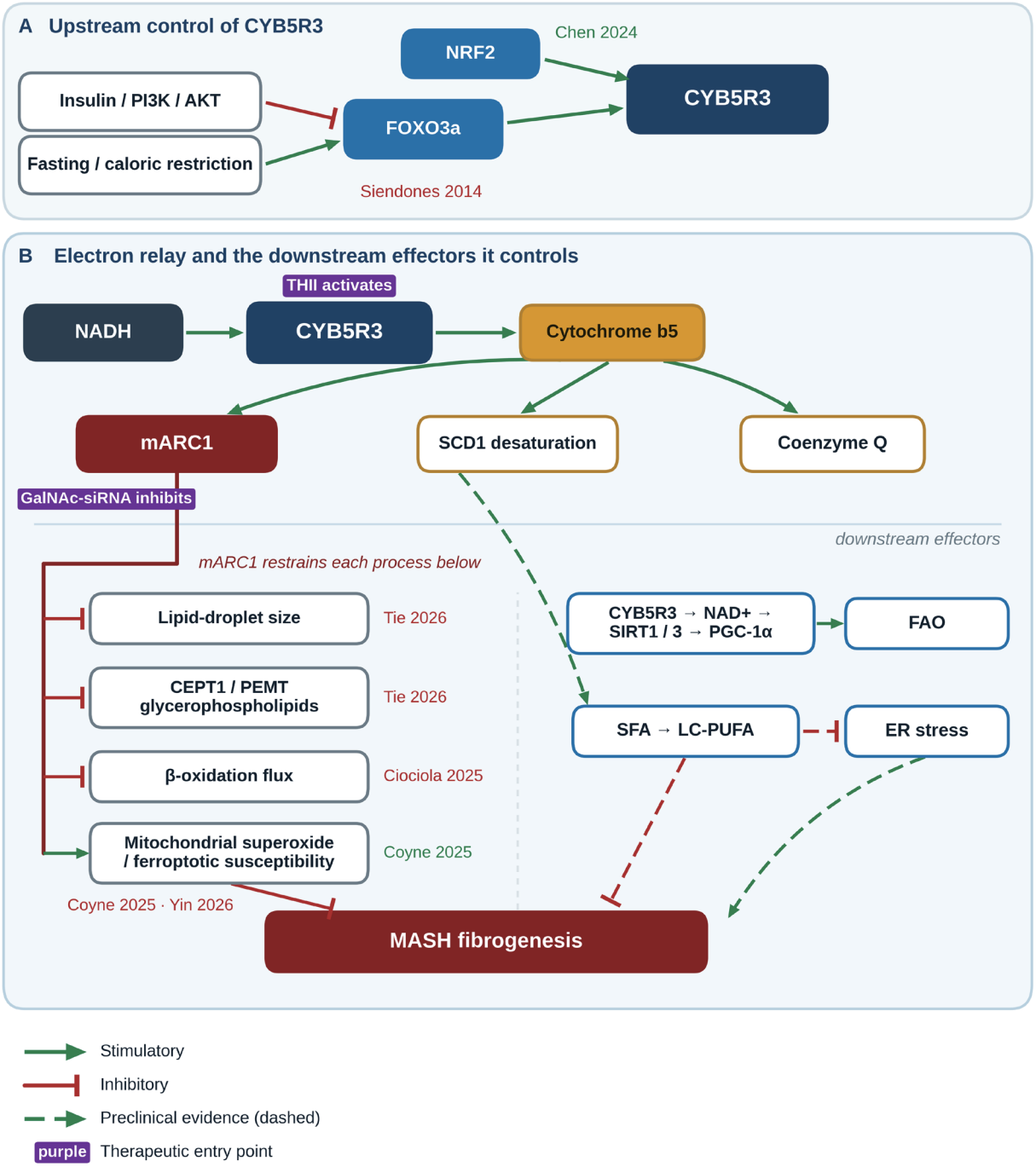

Fig. 2. The CYB5R3–mARC1 electron-transfer axis: signed mechanistic interactions and therapeutic entry points. Arrowheads denote stimulatory or activating interactions, and bar-headed lines denote inhibitory interactions; dashed lines denote preclinical evidence; every edge is annotated with the study that established it. (A) Upstream control of CYB5R3: insulin/PI3K/AKT signaling inhibits FOXO3a, whereas fasting and NRF2 provide stimulatory input. (B) The NADH→CYB5R3→cytochrome b5 relay and its three acceptor arms—mARC1, SCD1-dependent desaturation, and coenzyme Q—showing that CYB5R3 is the sole electron input on which mARC1 activity wholly depends, together with the downstream effectors each arm controls: mARC1 restrains lipid droplet size and CEPT1/PEMT-dependent phospholipid remodeling and restrains β-oxidation while promoting mitochondrial superoxide, and the CYB5R3 arm acts through NAD^+-dependent sirtuin signaling and, via SCD1, through the SFA→LC-PUFA balance that restrains ER stress; both converge on fibrogenesis. The two therapeutic entry points are annotated on the network itself: tetrahydroindenoindole (THII) activating CYB5R3 and GalNAc-conjugated siRNA inhibiting mARC1. CYB5R3, cytochrome b5 reductase 3; NRF2, nuclear factor erythroid 2-related factor 2; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; FOXO3a, forkhead box O3a; NADH, nicotinamide adenine dinucleotide, reduced form; mARC1/MTARC1, mitochondrial amidoxime reducing component 1; GalNAc-siRNA, N-acetylgalactosamine-conjugated small interfering RNA; SCD1, stearoyl-CoA desaturase 1; NAD^+, nicotinamide adenine dinucleotide, oxidized form; SIRT1/3, sirtuin 1 and sirtuin 3; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; FAO, fatty acid oxidation; SFA, saturated fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; ER stress, endoplasmic reticulum stress; MASH, metabolic dysfunction-associated steatohepatitis; CEPT1, choline/ethanolamine phosphotransferase 1; PEMT, phosphatidylethanolamine N-methyltransferase.

rather than general.

A further distinction, which I regard as the most clinically consequential observation currently available for this target, separates germline deletion from therapeutic knockdown. Hepatocyte-directed N-acetylgalactosamine (GalNAc)-conjugated small interfering RNA (siRNA) administered after 16 weeks of GAN diet achieved 75% knockdown of Mtarc1 mRNA and significantly reduced collagen area and fibrosis-associated gene expression, although plasma ALT and AST levels did not change. The identical intervention started at 24 weeks, when disease burden was higher, produced no significant effect on any liver endpoint; and in the choline-deficient amino acid-defined high-fat diet model, hepatocyte-specific knockdown had no effect on fibrosis at all, even though germline deletion in the same model did. The authors' own conclusion is that disease burden at the time of intervention is critical for fibrosis prevention through loss of mARC1.[25] Efficacy in this system is thus contingent not only on the depth of knockdown but also on when and in which compartment that knockdown is achieved. It is also worth stating what the a fourth does not show. Yin and colleagues reported significant reductions in liver mass, plasma ALT, triglycerides, and profibrotic transcripts (*Tgfb1*, $P = 0.0008$; αSMA, $P = 0.0001$; *Col1a1*, $P = 0.0056$) on a 2-week choline-deficient amino acid-defined high-fat diet; however, histological steatosis ($P = 0.1587$), inflammatory gene expression (Tnf-α and Mcp1, both $P = 0.07$), and histological fibrosis ($P > 0.05$) did not reach significance, and they explicitly stated that this model does not induce meaningful fibrosis at 2 weeks.[32] Their study should therefore be cited for protection against steatosis and the profibrotic transcriptional program, not as a demonstration of anti-fibrotic efficacy.

Appraised critically, this body of work has real strengths for clinical inference: four independent disease models, two orthogonal interventions (germline deletion and therapeutic hepatocyte-directed knockdown), group sizes of 10–14 with pathologist-scored histology, and artificial intelligence–assisted digital pathology that resolves zonal fibrosis, collagen fibrillar properties, and fibrosis–steatosis co-localization beyond the reach of conventional scoring. Its limitations bear just as directly on practice: the pivotal arms used male mice only, no model reached cirrhosis, regression of established fibrosis was never tested, and the negative results above define the outer boundary of the claim. What this evidence supports is prevention of progression in pre-cirrhotic disease; what it does not support is an expectation of benefit once advanced fibrosis is established. A study-level appraisal of this and the other pivotal studies is given in Table 3.

A testable roadmap for the combination hypothesis

It is biologically reasonable that increasing CYB5R3 activity and reducing mARC1 activity could be complementary: mARC1 loss diverts reductive flux toward desaturation while remodeling droplet phospholipids, and CYB5R3 activity could reinforce SFA→LC-PUFA conversion, cholesterol handling, and NAD^+-dependent signaling. However, no published study has tested combined CYB5R3 activation plus mARC1 inhibition, and there is no direct evidence of synergy. Rather than leaving the proposal purely theoretical, I specify a falsifiable program.

In vitro (2 × 2 factorial)

Human hepatocyte systems—primary human hepatocytes (including PXB), HepG2/Huh7, and iPSC (induced pluripotent stem cell)-derived hepatocyte spheroids/organoids—are exposed to a lipotoxic load and randomized across four arms: vehicle, CYB5R3 activation (tetrahydroindenoindole [THII] or genetic overexpression), MTARC1 knockdown (siRNA/ASO [antisense oligonucleotide]), and the combination. Readouts span neutral lipid (BODIPY), the SCD1 desaturation index, NAD^+/NADH, mitochondrial respiration and superoxide (Seahorse/MitoSOX), β-oxidation flux, lipid-droplet morphology, and lipidomic phosphatidylcholine species reflecting the CEPT1/PEMT axis. Synergy is assessed formally by Bliss independence or Loewe additivity.

In vivo (factorial)

Dietary MASH models (GAN, CDAHFD, HFD-HFr) receive hepatocyte-directed GalNAc-siRNA against MTARC1 with or without a hepatocyte-restricted CYB5R3 activator (or hepatotropic AAV-CYB5R3), in factorial arms powered for fibrosis endpoints. The primary endpoint is the fibrosis area (picrosirius red/collagen) with *Col1a1/Timp1*. Secondary endpoints include ALT, steatosis, and mitochondrial bioenergetics. A sex-balanced design is essential given the sex effects on both cardiac risk and progression.

Pharmacodynamic (PD) biomarker panel

Target engagement and pathway responses are tracked with a defined panel: for mARC1 engagement, accumulation of specified N-hydroxylated substrate/prodrug markers; for CYB5R3/desaturation, serum SCD1 desaturation indices (16:1n-7/16:0; 18:1n-9/18:0) and circulating sterol/cholesterol-biosynthetic intermediates; for redox/energetics, the NAD^+ metabolome and 3-hydroxybutyrate (β-oxidation); for phospholipid remodeling, phosphatidylcholine/phosphatidylethanolamine species (CEPT1/PEMT); and systemically, FGF21 and imaging PD (MRI-PDFF [magnetic resonance imaging proton density fat fraction], cT1 [iron-corrected T1]). I continue to label the combination as an untested hypothesis, but this roadmap renders it falsifiable.

Pharmacological activation of CYB5R3

THII is the best-characterized small-molecule activator of CYB5R3. It binds near the flavin adenine dinucleotide domain and increases catalytic turnover without transcriptional upregulation, and in rodent studies, it improved glucose handling and β-cell function through a Cyb5r3-dependent mechanism.[14,26,27] THII is also an antioxidant, and its CYB5R3-dependent and -independent actions have not been fully disentangled. Critically, no controlled study has demonstrated that THII (or any CYB5R3 activator) reduces hepatic fibrosis *in vivo*, and its pharmacology in established MASH is unknown; it should be regarded as a tool compound and a medicinal chemistry starting point rather than a clinical candidate. Adjunctive routes to raising CYB5R3 activity include NRF2 activators and dietary isothiocyanates that induce CYB5R3 transcription,[15,34] and insulin sensitizers that relieve FOXO1 suppression. A 2025 meta-analysis of 13 phase 2–3 trials (n = 1,811) found that GLP-1 receptor agonists, particularly semaglutide, improved MASH resolution and fibrosis relative to placebo;[40] any contribution of FOXO1-driven CYB5R3 upregulation to these benefits is speculative.

Hepatocyte-selective delivery and cardiac safety

Systemic modulation of CYB5R3 carries a defined cardiac liability. Inducible cardiomyocyte-specific deletion of Cyb5r3 in adult male mice causes cardiac hypertrophy, bradycardia, coenzyme Q depletion, and sudden death, whereas females are largely protected; a human partial loss-of-function vari-

ant (T117S) is associated with worse outcomes in heart failure.[28] Consequently, any oligonucleotide program targeting this axis—and any future systemic CYB5R3 activator—must achieve hepatocyte-restricted exposure (Fig. 2B). The GalNAc–conjugate platform exploits the asialoglycoprotein receptor (ASGPR), expressed at very high copy numbers on hepatocytes and essentially absent on cardiomyocytes, to achieve strong hepatic selectivity with durable knockdown after subcutaneous dosing, as established by approved agents such as inclisiran.[29] Several companies have filed patents on siRNA duplexes targeting MTARC1, but these programs remain preclinical, and no clinical trial of mARC1 inhibition has been reported. For small-molecule CYB5R3 activators, hepatocyte-selective prodrug or nanoparticle strategies would be needed to avoid the male-predominant cardiac risk of global perturbation. A second selectivity requirement applies within the mARC family itself: mice lacking mARC2, the paralog that carries most N-reductive activity in rodents,[18] develop rapidly progressing hindlimb paralysis at 7–10 weeks of age — although other groups have not reported a neurological phenotype — whereas mARC1-null mice are viable and fertile with no developmental abnormality.[32] Any oligonucleotide or small molecule directed at this axis must therefore be selective for mARC1 over mARC2.

The advanced-cirrhosis gap: delivery, efficacy, and safety

A candid limitation of the current evidence is that all pivotal *in vivo* efficacy derives from pre-cirrhotic, mild-to-moderate MASH. The decompensated cirrhotic population encountered in clinical practice raises distinct concerns that the field has not yet addressed and that any development program must confront.

Delivery in the cirrhotic liver

GalNAc conjugates depend on hepatocyte ASGPR expression and adequate sinusoidal perfusion. In cirrhosis, hepatocyte ASGPR density falls, sinusoidal capillarization and pericellular fibrosis impede parenchymal access, and portosystemic shunting from portal hypertension diverts drug away from functional hepatocytes—together predicting reduced and more variable knockdown than in pre-cirrhotic livers. These considerations bear on dose, dosing interval, and the potential need for delivery systems less dependent on ASGPR density in advanced disease.

Efficacy in established fibrosis

Regression of established bridging fibrosis or cirrhosis has not been demonstrated for this target; the available signals concern prevention of progression, not reversal of an established scaffold. I therefore position deep mARC1 inhibition as most credible for pre-cirrhotic F2–F3 disease, with cirrhosis-stage efficacy an explicit open question requiring dedicated models (e.g., established-fibrosis reversal designs) and, ultimately, cirrhotic-cohort trials.

Safety in impaired hepatic function

Sustained mARC1 inhibition in the failing liver raises specific concerns: accumulation of N-hydroxylated substrates and of prodrugs that require mARC1 for activation; potential effects on nitrite/nitric oxide reduction relevant to portal and systemic hemodynamics; and altered handling in patients with sarcopenia, coagulopathy, or polypharmacy. Prudent early development would stratify dosing by hepatic function, incorporate cardiometabolic and hemodynamic monitoring, and either exclude decompensated patients or study them under dedicated safety protocols.

An integrated non-invasive PD framework

Clinical translation requires validated non-invasive readouts spanning several domains, distinguishing markers of target engagement from markers of disease modification. (i) Imaging: MRI-PDFF and MR elastography, multiparametric MRI/cT1, and vibration-controlled transient elastography with controlled attenuation parameter. (ii) Serum fibrogenesis: enhanced liver fibrosis score, PRO-C3 (N-terminal type III collagen propeptide), FIB-4 (fibrosis-4 index), and APRI (aspartate aminotransferase-to-platelet ratio index). (iii) Pathway-engagement PD markers: the SCD1 desaturation index, circulating sterol intermediates, the NAD^+ metabolome, 3-hydroxybutyrate, and target-specific N-hydroxylated substrate accumulation. (iv) Lipidomic signatures of CEPT1/PEMT-driven phospholipid remodeling. (v) Genotype stratification (MTARC1, PNPLA3, TM6SF2). Assembled prospectively, these constitute an integrated PD evaluation system in which desaturation and substrate markers confirm engagement, while imaging and fibrogenesis markers track modification.

Sex, ancestry, and clinical-trial design

Sex modifies this biology in ways that bear on trial design. The cardiac phenotype of Cyb5r3 deletion is male-predominant,[28] and the relative protection of premenopausal women from MASH progression may involve sex-specific regulation of hepatic redox pathways;[37,38] sex-stratified randomization and capture of menopausal status are warranted. Population genetics adds a second axis: the ~three-fold difference in p.A165T frequency between Northern Europeans and East Asians, and its lower frequency in African and Indigenous American populations, predicts variation in baseline mARC1 activity and in the expected benefit from mARC1 inhibition.[11,13] Adequate enrollment of under-represented populations is essential for generalizable conclusions.

Strengths, limitations, and clinical implications

The evidence base has clear strengths and equally clear boundaries, which I set out explicitly because they determine clinical relevance; Figure 3 maps the effect estimate and evidence tier available at each disease stage, Table 3 gives the study-level appraisal, and the paragraphs below summarize what follows from both. The mARC1 human genetic evidence is unusually strong—large, multi-ancestry, genome-wide-significant, and consistent across biobanks and etiologies, with effect estimates compiled in Table 2 and reinforced by an allelic series in which a protein-truncating variant produced no cirrhosis among its carriers. Its principal limitation is that a common variant models lifelong, partial inhibition beginning before disease, which is not equivalent to acute pharmacological therapy of established disease; the per-allele effect is also small (OR 0.91) and should not be quoted as an expected treatment effect, and the variant is far less frequent in East Asian than in Northern European populations, which bears on where such a therapy would be tested. The depth-of-knockdown threshold defined by the A168T mouse both clarifies the mechanism and raises the therapeutic bar. The *in vivo* efficacy evidence is more nuanced: the anti-fibrotic effect ranged from 24% to 50%, depending on the model, did not extend to histological disease activity, and—critically for practice—was absent when hepatocyte-directed knock-

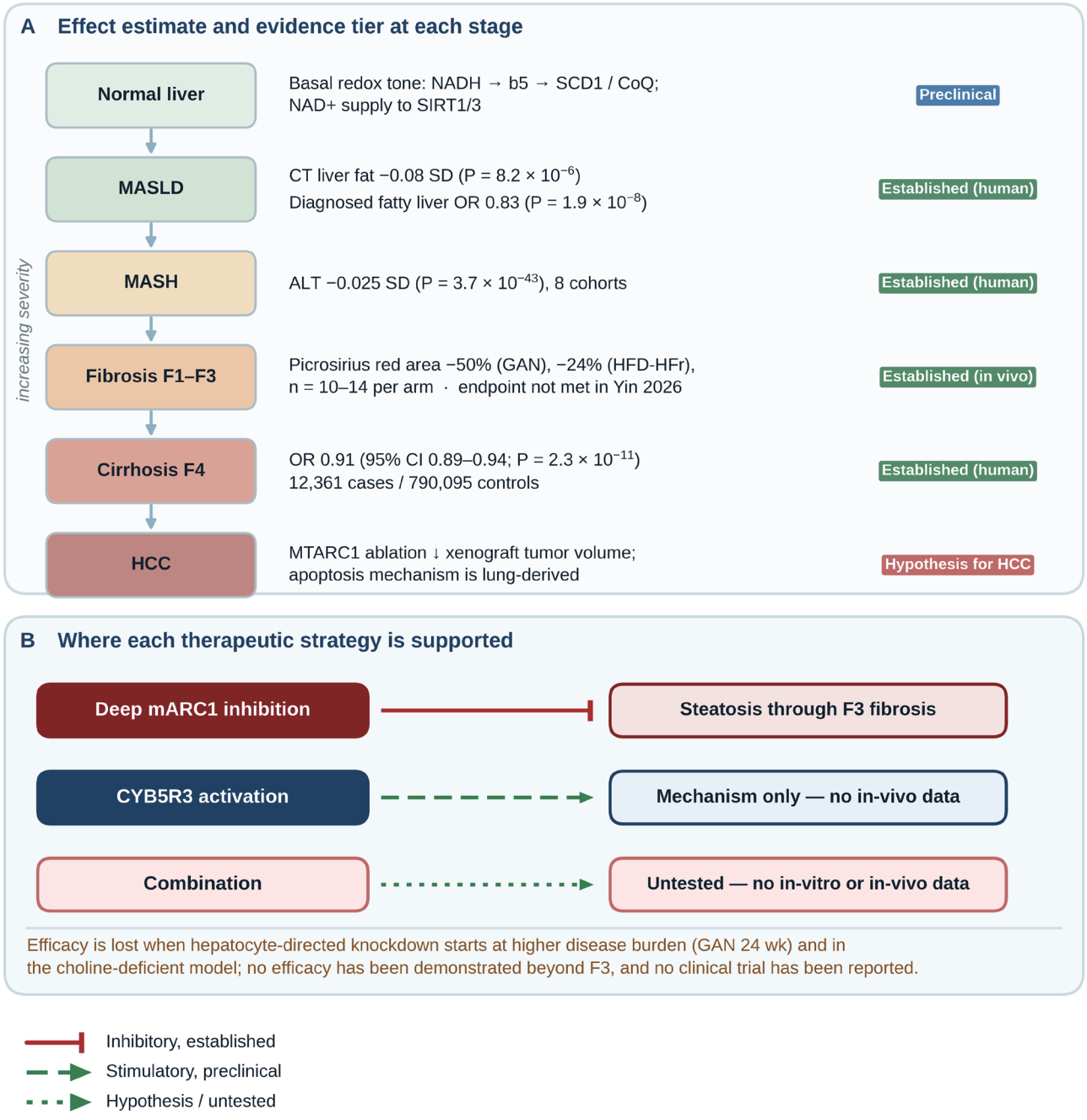

Fig. 3. The CYB5R3–mARC1 redox node across the MASLD–cirrhosis–HCC continuum. (A) Disease stages are arranged from normal liver to HCC, each carrying the quantitative effect estimate available at that stage and an evidence-tier chip: hepatic fat −0.08 SD ($P = 8.2 \times 10^{-6}$) and physician-diagnosed fatty liver OR 0.83 ($P = 1.9 \times 10^{-8}$) at the steatosis stage; ALT −0.025 SD ($P = 3.7 \times 10^{-43}$) at the steatohepatitis stage; picrosirius red area −50% (GAN) and −24% (HFD-HFr), n = 10–14 per arm, at the fibrosis stage, annotated to show that this endpoint was not met in Yin 2026; and all-cause cirrhosis OR 0.91 (95% CI 0.89–0.94; $P = 2.3 \times 10^{-11}$) at the cirrhosis stage. (B) Signed edges show where each of the three therapeutic strategies is supported—deep mARC1 inhibition from steatosis through F3 fibrosis, CYB5R3 activation on mechanism alone, and the combination untested—together with a statement of where the evidence stops. CYB5R3, cytochrome b5 reductase 3; NADH, nicotinamide adenine dinucleotide, reduced form; b5, cytochrome b5; SCD1, stearoyl-CoA desaturase 1; CoQ, coenzyme Q; NAD^+, nicotinamide adenine dinucleotide, oxidized form; SIRT1/3, sirtuin 1 and sirtuin 3; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; ALT, alanine aminotransferase; SD, standard deviation; OR, odds ratio; CI, confidence interval; HCC, hepatocellular carcinoma; mARC1/MTARC1, mitochondrial amidoxime reducing component 1; CT, computed tomography; F1–F3, fibrosis stages 1–3; F4, fibrosis stage 4; wk, week.

down was started at higher disease burden or applied in the CDAHFD model. Deep mARC1 inhibition is therefore best understood as a strategy for preventing progression in pre-cirrhotic disease, which argues for an F2–F3 target population, for stratification by baseline fibrosis stage, and for a prespecified treatment window in any trial. The CYB5R3 arm is weaker: much of it rests on transgenic overexpression (RedTg), single-model or non-hepatic data, and, for the HCC

apoptosis mechanism, on lung-cancer experiments. It should also be stated plainly that there are no clinical trials of this axis: human evidence is genetic-epidemiological, and all interventional evidence is preclinical. Key gaps for clinical practice include the absence of any *in vivo* demonstration that a CYB5R3 activator reduces fibrosis or prevents HCC; the lack of direct evidence for synergy between CYB5R3 activation and mARC1 inhibition; the untested efficacy, delivery, and safety of deep mARC1 knockdown in cirrhotic as opposed to pre-cirrhotic livers; and the need for validated PD biomarkers of pathway engagement. The accumulation of N-hydroxylated substrates during sustained mARC1 inhibition and the long-term consequences of chronic hepatic redox modulation also require systematic safety evaluation.

Future directions: an urgent need for liver-specific evidence and collaborative research

A recurring theme of this review is that the mechanistic case for CYB5R3 in hepatocarcinogenesis remains disproportionately thin outside a single tissue context. The most detailed apoptotic program attributed to CYB5R3—the PARP16–PERK/IRE1α ER-stress cascade—has been demonstrated only in non-small-cell lung cancer,[20] and the liver-specific evidence, though encouraging, rests on a small number of studies: MTARC1 knockout in HCC cell lines and xenografts[31] and diethylnitrosamine chemoprevention in RedTg mice.[30] This is an insufficient foundation on which to build clinical development, and I therefore argue explicitly that the field cannot advance on the strength of extrapolation from other tumor types. Direct, liver-specific validation is not merely desirable but urgently required.

Meeting this need will demand sustained effort from many investigators working in parallel. Priorities include: (i) replication of the CYB5R3–PARP16–PERK/IRE1α mechanism in hepatocyte and HCC systems, including patient-derived organoids and orthotopic and genetically engineered mouse models with hepatocyte-conditional gain- and loss-of-function; (ii) resolution of the dual, context- and cell-type-dependent behavior of the axis—distinguishing chemoprevention from treatment of an established, metabolically reprogrammed tumor, and hepatocyte from stellate-cell and tumor-associated macrophage contributions; (iii) human tissue studies that move beyond correlative expression (TCGA-LIHC, Human Protein Atlas) to functional and prognostic validation in well-annotated MASLD-HCC cohorts; (iv) factorial *in vivo* testing of the CYB5R3-activation plus mARC1-inhibition combination with the PD biomarker panel proposed above; and (v) dedicated evaluation of efficacy, delivery, and safety in advanced, cirrhotic livers rather than pre-cirrhotic models alone.

No single laboratory can span molecular mechanism, human genetics, delivery technology, and clinical translation simultaneously. Progress will therefore depend on collaboration—shared MASLD-HCC biobanks and multi-omics resources, standardized redox and PD assays, common reporting of quantitative effect sizes, and sex- and ancestry-stratified study designs—across academic groups, population biobanks, and industry programs. I offer this review not as a settled account but as an invitation: the CYB5R3–mARC1 axis is a biologically compelling yet still under-tested target and realizing (or refuting) its therapeutic promise for MASLD, cirrhosis, and HCC will require a coordinated, community-wide research effort that is, at present, only beginning.

Conclusions

The CYB5R3–mARC1 axis integrates NADH-derived reducing equivalents with lipid remodeling, cholesterol handling, NAD^+-dependent signaling, and ER quality control, and it is supported by unusually strong, quantifiable human genetic evidence on the mARC1 side. Convergent 2025–2026 *in vivo* data establish that near-complete mARC1 loss is anti-fibrotic in dietary MASH models—by 24–50% of picrosirius red area in the one program that quantified it, without a corresponding change in histological disease activity—while also showing that the effect is diet-dependent, that it was not reached at all in a third program, that partial protein reduction is insufficient, and that therapeutic hepatocyte-directed knockdown loses its effect when started at higher disease burden. This points to deep, hepatocyte-restricted mARC1 inhibition as the most credible near-term clinical strategy in pre-cirrhotic F2–F3 disease specifically, for which GalNAc-conjugated oligonucleotides offer a delivery solution that also mitigates the male-predominant cardiac liability of systemic perturbation. CYB5R3 activation and the proposed combination of CYB5R3 activation with mARC1 inhibition are biologically motivated but presently unproven and should be pursued through the *in vivo* efficacy, synergy, and PD-biomarker studies outlined here. No clinical trial of this axis has yet been reported, and this should be borne in mind when weighing the strength of the genetic signal. Efficacy, delivery, and safety in advanced cirrhosis remain a defined gap, and sex- and ancestry-stratified design will be essential at every stage. Positioned with appropriate caution, the axis is a promising addition to the emerging precision-hepatology toolkit rather than a substitute for existing therapy.

Acknowledgments

During preparation, the author used a generative AI assistant to help organize the literature and draft schematic figures. The author verified all references against primary sources and reviewed and edited all output, taking full responsibility for the content.

Funding

None to declare.

Conflict of interest

The author has no conflict of interest related to this publication.

Author contributions

SWN is the sole author, responsible for conception, literature analysis, drafting, and critical revision.

References

[1] Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology 2023;77(4):1335–1347. doi:10.1097/HEP.0000000000000004, PMID:36626630.

[2] Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, *et al*. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology 2023;78(6):1966–1986. doi:10.1097/HEP.0000000000000520, PMID:37363821.

[3] Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, *et al*. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol 2019;71(4):793–801. doi:10.1016/j.jhep.2019.06.021, PMID:31279902.

[4] Lazarus JV, Mark HE, Anstee QM, Arab JP, Batterham RL, Castera L, *et al*. Advancing the global public health agenda for NAFLD: a consensus statement. Nat Rev Gastroenterol Hepatol 2022;19(1):60–78. doi:10.1038/s41575-021-00523-4, PMID:34707258.

[5] Teng ML, Ng CH, Huang DQ, Chan KE, Tan DJ, Lim WH, *et al*. Global inci-

dence and prevalence of nonalcoholic fatty liver disease. Clin Mol Hepatol 2023;29(Suppl):S32–S42. doi:10.3350/cmh.2022.0365, PMID:36517002.
[6] Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 2019;16(7):411–428. doi:10.1038/s41575-019-0145-7, PMID:31028350.
[7] Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. N Engl J Med 2017;377(21):2063–2072. doi:10.1056/NEJMra1503519, PMID:29166236.
[8] Xiao S, Liu Y, Fu X, Chen T, Xie W. Modifiable Risk Factors for Hepatocellular Carcinoma in Patients with Metabolic Dysfunction-Associated Steatotic Liver Disease: A Meta-Analysis. Am J Med 2024;137(11):1072–1081.e32. doi:10.1016/j.amjmed.2024.06.031, PMID:39047929.
[9] Harrison SA, Bedossa P, Guy CD, Schattenberg JM, Loomba R, Taub R, *et al*. A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis. N Engl J Med 2024;390(6):497–509. doi:10.1056/NEJMoa2309000, PMID:38324483.
[10] European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). J Hepatol 2024;81(3):492–542. doi:10.1016/j.jhep.2024.04.031, PMID:38851997.
[11] Emdin CA, Haas ME, Khera AV, Aragam K, Chaffin M, Klarin D, *et al*. A missense variant in Mitochondrial Amidoxime Reducing Component 1 gene and protection against liver disease. PLoS Genet 2020;16(4):e1008629. doi:10.1371/journal.pgen.1008629, PMID:32282858.
[12] Luukkonen PK, Juuti A, Sammalkorpi H, Penttilä AK, Orešič M, Hyötyläinen T, *et al*. MARC1 variant rs2642438 increases hepatic phosphatidylcholines and decreases severity of non-alcoholic fatty liver disease in humans. J Hepatol 2020;73(3):725–726. doi:10.1016/j.jhep.2020.04.021, PMID:32471727.
[13] Mancina RM, Valenti L, Romeo S. Human genetics of steatotic liver disease: insights into insulin resistance and lipid metabolism. Nat Metab 2025;7(11):2199–2211. doi:10.1038/s42255-025-01394-8, PMID:41107482.
[14] Martin-Montalvo A, Sun Y, Diaz-Ruiz A, Ali A, Gutierrez V, Palacios HH, *et al*. Cytochrome b(5) reductase and the control of lipid metabolism and healthspan. NPJ Aging Mech Dis 2016;2:16006. doi:10.1038/npjamd.2016.6, PMID:28721264.
[15] Chen J, Hu J, Guo X, Yang Y, Qin D, Tang X, *et al*. Apolipoprotein O modulates cholesterol metabolism via NRF2/CYB5R3 independent of LDL receptor. Cell Death Dis 2024;15(6):389. doi:10.1038/s41419-024-06778-4, PMID:38830896.
[16] Siendones E, SantaCruz-Calvo S, Martín-Montalvo A, Cascajo MV, Ariza J, López-Lluch G, *et al*. Membrane-bound CYB5R3 is a common effector of nutritional and oxidative stress response through FOXO3a and Nrf2. Antioxid Redox Signal 2014;21(12):1708–1725. doi:10.1089/ars.2013.5479, PMID:24450884.
[17] Innes H, Buch S, Hutchinson S, Guha IN, Morling JR, Barnes E, *et al*. Genome-Wide Association Study for Alcohol-Related Cirrhosis Identifies Risk Loci in MARC1 and HNRNPUL1. Gastroenterology 2020;159(4):1276–1289.e7. doi:10.1053/j.gastro.2020.06.014, PMID:32561361.
[18] Smagris E, Shihanian LM, Mintah IJ, Bigdelou P, Livson Y, Brown H, *et al*. Divergent role of Mitochondrial Amidoxime Reducing Component 1 (MARC1) in human and mouse. PLoS Genet 2024;20(3):e1011179. doi:10.1371/journal.pgen.1011179, PMID:38437227.
[19] Ishimura R, El-Gowily AH, Noshiro D, Komatsu-Hirota S, Ono Y, Shindo M, *et al*. The UFM1 system regulates ER-phagy through the ufmylation of CYB5R3. Nat Commun 2022;13(1):7857. doi:10.1038/s41467-022-35501-0, PMID:36543799.
[20] Im JY, Kim SJ, Park JL, Han TH, Kim WI, Kim I, *et al*. CYB5R3 functions as a tumor suppressor by inducing ER stress-mediated apoptosis in lung cancer cells via the PERK-ATF4 and IRE1α-JNK pathways. Exp Mol Med 2024;56(1):235–249. doi:10.1038/s12276-024-01155-9, PMID:38253797.
[21] Ghouse J, Sveinbjörnsson G, Vujkovic M, Seidelin AS, Gellert-Kristensen H, Ahlberg G, *et al*. Integrative common and rare variant analyses provide insights into the genetic architecture of liver cirrhosis. Nat Genet 2024;56(5):827–837. doi:10.1038/s41588-024-01720-y, PMID:38632349.
[22] Ciociola E, Dutta T, Sasidharan K, Kovooru L, Noto FR, Pennisi G, *et al*. Downregulation of the MARC1 p.A165 risk allele reduces hepatocyte lipid content by increasing beta-oxidation. Clin Mol Hepatol 2025;31(2):445–459. doi:10.3350/cmh.2024.0642, PMID:39716370.
[23] Tie M, Hu L, Yang Y, Song S, Zhu Q, Li J, *et al*. MTARC1 Inactivation Remodels Lipid Droplets to Protect Against Metabolic Fatty Liver Disease. Liver Int 2026;46(3):e70539. doi:10.1111/liv.70539, PMID:41641916.
[24] Pandovski S, Yang T, Zhou H, Rosahl TW, Carballo-Jane E, Talukdar S, *et al*. A mitochondrial amidoxime-reducing component 1 (mARC1) A168T amino acid substitution does not confer protection from MASH and fibrosis in multiple mouse models of chronic liver disease. Biochem J 2026;483(2):89–105. doi:10.1042/BCJ20253411, PMID:41428769.
[25] Coyne ES, Nie Y, Lee D, Pandovski S, Yang T, Zhou H, *et al*. Loss of mitochondrial amidoxime-reducing component 1 (mARC1) prevents disease progression by reducing fibrosis in multiple mouse models of chronic liver disease. Hepatol Commun 2025;9(2):e0637. doi:10.1097/HC9.0000000000000637, PMID:39927988.
[26] Watanabe H, Du W, Son J, Sui L, Asahara SI, Kurland IJ, *et al*. Cyb5r3-based mechanism and reversal of secondary failure to sulfonylurea in diabetes. Sci Transl Med 2023;15(681):eabq4126. doi:10.1126/scitranslmed.abq4126, PMID:36724243.
[27] Watanabe H, Asahara SI, Son J, McKimpson WM, de Cabo R, Accili D. Cyb5r3 activation rescues secondary failure to sulfonylurea but not β-cell dedifferentiation. PLoS One 2024;19(2):e0297555. doi:10.1371/journal.pone.0297555, PMID:38335173.
[28] Carew NT, Schmidt HM, Yuan S, Galley JC, Hall R, Altmann HM, *et al*. Loss of cardiomyocyte CYB5R3 impairs redox equilibrium and causes sudden cardiac death. J Clin Invest 2022;132(18):e147120. doi:10.1172/JCI147120, PMID:36106636.
[29] Qin ZX, Zuo L, Zeng Z, Ma R, Xie W, Zhu X, *et al*. GalNac-siRNA conjugate delivery technology promotes the treatment of typical chronic liver diseases. Expert Opin Drug Deliv 2025;22(4):455–469. doi:10.1080/17425247.2025.2466767, PMID:39939158.
[30] Diaz-Ruiz A, Lanasa M, Garcia J, Mora H, Fan F, Martin-Montalvo A, *et al*. Overexpression of CYB5R3 and NQO1, two NAD(+) -producing enzymes, mimics aspects of caloric restriction. Aging Cell 2018;17(4):e12767. doi:10.1111/acel.12767, PMID:29706024.
[31] Kovooru L, Zhang J, Monni FG, Dutta T, Gongye X, Asiedu B, *et al*. MTARC1 p.A165 ablation reduces hepatocellular carcinoma aggressiveness in vitro and in vivo. Clin Mol Hepatol 2026;32(2):829–842. doi:10.3350/cmh.2025.1261, PMID:41644117.
[32] Yin X, Bickerton C, MacDonald B, Arduini A, Shi Y, Haas M, *et al*. Loss of Mtarc1 Protects Against Steatotic Liver Disease in Mice. Liver Int 2026;46(2):e70507. doi:10.1111/liv.70507, PMID:41527487.
[33] Baptista C, Esteves F, Fallowfield JA, Kendall TJ, Nelson LJ, Kranendonk M. Deciphering cytochrome P450 reductase role in MASLD: molecular mechanisms and pathophysiological implications. Nat Rev Gastroenterol Hepatol 2026;23(8):655–668. doi:10.1038/s41575-026-01202-y, PMID:42009939.
[34] Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, *et al*. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun 2016;7:11624. doi:10.1038/ncomms11624, PMID:27211851.
[35] Guo Y, Wang Y, Wu S, Wang Y, Liu X. NAD+ in fatty liver disease: mechanistic insights and associated targets. Cell Biol Toxicol 2025;41(1):156. doi:10.1007/s10565-025-10106-7, PMID:41291376.
[36] de Cabo R, Mattson MP. Effects of Intermittent Fasting on Health, Aging, and Disease. N Engl J Med 2019;381(26):2541–2551. doi:10.1056/NEJMra1905136, PMID:31881139.
[37] Friedman SL, Pinzani M. Hepatic fibrosis 2022: Unmet needs and a blueprint for the future. Hepatology 2022;75(2):473–488. doi:10.1002/hep.32285, PMID:34923653.
[38] Sanyal AJ, Van Natta ML, Clark J, Neuschwander-Tetri BA, Diehl A, Dasarathy S, *et al*. Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease. N Engl J Med 2021;385(17):1559–1569. doi:10.1056/NEJMoa2029349, PMID:34670043.
[39] Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, *et al*. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 2017;67(6):1265–1273. doi:10.1016/j.jhep.2017.07.027, PMID:28803953.
[40] Mantovani A, Morandin R, Fiorio V, Lando MG, Stefan N, Tilg H, *et al*. Glucagon-Like Peptide-1 Receptor Agonists Improve MASH and Liver Fibrosis: A Meta-Analysis of Randomised Controlled Trials. Liver Int 2025;45(9):e70256. doi:10.1111/liv.70256, PMID:40736113.