

LGALS3BP Links Centrosomes and Mitochondria to Maintain Energetic Fitness and Restrain Compensatory Lipid Catabolic Reprogramming in Hepatocellular Carcinoma

JUN EUL HWANG^{1*}, HYUN JEONG SHIM^{1*}, HYUN JIN BANG¹, HYEON-JONG KIM¹,
SUNG YUN JUNG², IK-JOO CHUNG^{1,3}, SANG-HEE CHO¹ and DAE-HWAN KIM^{1,3}

¹Department of Internal Medicine, Division of Hematology and Oncology, Chonnam National University Medical School and Hwasun Hospital, Hwasun, Republic of Korea;

²Department of Biochemistry and Molecular Pharmacology, Baylor College of Medicine, Houston, TX, U.S.A.;

³National Immunotherapy Innovation Center, Hwasun, Republic of Korea

Abstract

Background/Aim: Hepatocellular carcinoma (HCC) exhibits substantial metabolic plasticity that supports tumor progression and therapeutic resistance. LGALS3BP has been primarily characterized as a secreted immunomodulatory protein; however, its cell-intrinsic role in regulating subcellular organization and metabolism in HCC remains poorly understood.

Materials and Methods: Transcriptomic analyses of public HCC cohorts were performed to assess metabolic programs associated with LGALS3BP expression. Proteomic profiling was conducted to define the LGALS3BP interactome. Mitochondrial function was evaluated by measuring adenosine triphosphate (ATP) levels, mitochondrial membrane potential ($\Delta\Psi_m$), and AMP-activated protein kinase (AMPK) activation. Lipid metabolic programs were assessed by gene expression analyses and lipid accumulation assays. Clinical relevance was independently validated in an institutional HCC cohort.

Results: Low LGALS3BP expression was associated with activation of peroxisome proliferator-activated receptor alpha (PPAR α)-driven peroxisomal and lipid catabolic gene programs. Proteomic analyses revealed that LGALS3BP associates with centrosomal γ -tubulin ring complex components and mitochondrial proteins, suggesting a role in centrosome-mitochondria subcellular organization. Functionally, LGALS3BP deficiency resulted in impaired mitochondrial energetic

continued

*These Authors contributed equally to this work.



Dae-Hwan Kim, Department of Internal Medicine, Division of Hematology and Oncology, Chonnam National University Medical School and Hwasun Hospital, 264, Seoyang-ro, Hwasun-eup, Hwasun-gun, 58128, Republic of Korea. Tel: +82 613792580, e-mail: dhkim007@jnu.ac.kr and Sang-Hee Cho, Department of Internal Medicine, Division of Hematology and Oncology, Chonnam National University Medical School and Hwasun Hospital, 264, Seoyang-ro, Hwasun-eup, Hwasun-gun, 58128 Republic of Korea. Tel: +82 613797623, e-mail: shcho@jnu.ac.kr

Received February 20, 2026 | Revised March 31, 2026 | Accepted April 2, 2026



This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

©2026 The Author(s). Anticancer Research is published by the International Institute of Anticancer Research.

fitness, reduced ATP production, and activation of AMPK. This energetic stress was accompanied by induction of PPAR α and compensatory lipid catabolic transcriptional programs. Consistent inverse associations between LGALS3BP expression and PPAR α -peroxisome-related genes, including PPARG, ACOX1, EPHX2, and SCP2, were observed across public HCC datasets and were independently validated in an institutional HCC patient cohort.

Conclusion: LGALS3BP acts as a cell-intrinsic organizer that links centrosomal architecture to mitochondrial energetic homeostasis in HCC. Loss of this organizational axis induces mitochondrial energetic stress and promotes compensatory PPAR α -peroxisome-mediated lipid catabolic reprogramming, highlighting a previously unrecognized connection between subcellular organization and metabolic plasticity in liver cancer.

Keywords: LGALS3BP, hepatocellular carcinoma, centrosome, subcellular organization, PPAR α , metabolic plasticity.

Introduction

Cancer cells undergo extensive metabolic reprogramming to sustain proliferation and survive under stress (1, 2). Beyond the well-established Warburg effect, accumulating evidence highlights alterations in mitochondrial function, lipid utilization, and energy-sensing pathways as defining features of tumor metabolism (2-4). This metabolic rewiring not only fuels tumor growth but also actively contributes to immune evasion and therapeutic resistance, underscoring its role as a driver rather than a passive consequence of malignant transformation (5). Accordingly, elucidating the mechanisms that maintain metabolic homeostasis in cancer cells has emerged as an important area of cancer research (5, 6).

Among these mechanisms, mitochondria play a central role not only in adenosine triphosphate (ATP) production but also in coordinating overall metabolic balance (7, 8). In solid tumors, fluctuating oxygen and nutrient availability impose persistent metabolic stress, rendering mitochondrial energetic fitness critical for cancer cell survival (8, 9). AMP-activated protein kinase (AMPK) serves as a key cellular energy sensor that is activated upon ATP depletion and restores energetic balance by suppressing anabolic processes while promoting catabolic pathways (10, 11). Sustained AMPK activation can inhibit tumor growth or induce cell death; consequently, cancer cells evolve strategies to preserve mitochondrial function and restrain chronic AMPK signaling (12). One important downstream effector of AMPK is peroxisome proliferator-

activated receptor alpha (PPAR α), a transcriptional regulator that drives fatty acid oxidation and facilitates compensatory adaptation to energetic stress (13). These energy-sensing networks are particularly relevant in hepatocellular carcinoma (HCC), where lipid metabolism and oxidative energy production are highly active and intimately linked to disease progression (14, 15).

LGALS3BP (Glectin-3 binding protein, 90K, CyCAP, and Mac2BP) has been best characterized as a secreted glycoprotein involved in immune regulation and tumor-stroma communication (16-18). In cancer, and particularly in HCC, LGALS3BP has been implicated in the establishment of an immunosuppressive tumor microenvironment through extracellular signaling pathways (19, 20). As a result, most prior studies have focused on its secretory functions and receptor-mediated interactions with neighboring cells (18, 21). However, early reports describing LGALS3BP localization in proximity to intracellular structures such as the centrosome raise the possibility that it may also perform noncanonical cell-intrinsic functions (22). These observations suggest a potential role for LGALS3BP in intracellular organelle organization and metabolic regulation that remains largely unexplored.

In our previous work, we examined genes positively correlated with LGALS3BP expression and demonstrated its role in promoting immunosuppressive signaling *via* transforming growth factor- β 1 (TGF- β 1) (18). In contrast, the present study focuses on genes negatively correlated with LGALS3BP expression in HCC, which revealed distinct metabolic signatures. To test the hypothesis

that LGALS3BP regulates mitochondrial function and cellular energy homeostasis, we investigated its intracellular interaction partners and functional impact on mitochondrial fitness, fatty acid utilization, and AMPK-PPAR α signaling in liver cancer models.

Materials and Methods

Transcriptomic correlation and pathway enrichment analysis. RNA-sequencing data for HCC patients were obtained from The Cancer Genome Atlas (TCGA) database (<https://www.cancer.gov/ccg/research/genome-sequencing/tcga>), specifically the Liver Hepatocellular Carcinoma (LIHC) cohort. A total of 817 genes inversely correlated with LGALS3BP expression (Spearman's $\rho < -0.25$, FDR-adjusted $q < 0.05$) were selected for downstream analyses, as listed in Supplementary Table I. Pathway enrichment analysis was conducted using the Enrichr platform (<https://maayanlab.cloud/Enrichr/>) with Reactome, KEGG, and MSigDB Hallmark databases. To infer potential upstream transcriptional regulators, transcription factor (TF) enrichment analysis was performed using the TRRUST database with a curated subset of 138 genes related to lipid metabolism and peroxisomal function (Supplementary Table II). Using the same gene set, perturbation signatures from published transcriptomic datasets were queried to assess overlap with gene expression profiles from mouse models of hepatic metabolic regulation.

Immunoprecipitation-mass spectrometry (IP-MS) for LGALS3BP interactome profiling. For interactome analysis, Hepa1c1c7 murine hepatoma cells were transfected with either N-terminal Flag-tagged or C-terminal Myc-tagged LGALS3BP constructs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After 48 hours, cells were lysed in IP buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA) supplemented with protease inhibitors. Immunoprecipitation was performed using a c-Myc-Tag IP/Co-IP Kit (Thermo Fisher Scientific, Waltham,

MA, USA). Bound proteins were washed, eluted, and subjected to in-solution trypsin digestion. The resulting peptides were analyzed using LC-MS/MS on a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific). Raw files were processed using MaxQuant (v1.6.10.43) with the Andromeda search engine against the UniProt mouse database. Only proteins that were enriched by at least 10-fold compared to IgG controls and consistently detected across all IP-MS conditions (Myc1, Myc2, Flag1, Flag2) were defined as high-confidence LGALS3BP interactors. The complete interactome lists for each IP condition are provided in Supplementary Tables III to VI.

Mouse model and primary hepatocyte isolation. Whole-body Lgals3bp knockout (KO) mice were generated *via* conventional homologous recombination-based gene targeting, as previously described (18). Primary hepatocytes were isolated from adult whole-body KO and wild-type (WT) littermate controls by two-step collagenase perfusion and cultured in William's E medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin-streptomycin (Thermo Fisher Scientific). All procedures involving animals were performed in accordance with institutional guidelines.

siRNA transfection. For LGALS3BP knockdown, Hepa1c1c7 (mouse) and Hep3B (human) hepatoma cells were transfected with two independent siRNAs targeting LGALS3BP (Bioneer, Daejeon, Republic of Korea) using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A non-targeting siRNA served as a negative control. Knockdown efficiency was confirmed by quantitative real-time PCR (qRT-PCR) and western blotting. The siRNA sequences are listed in Supplementary Table VII.

MitoTracker staining and mitochondrial membrane potential. To assess mitochondrial content and membrane potential, cells were incubated with MitoTracker Green (MTG) FM (Invitrogen, Waltham, MA, USA) or

MitoTracker Red (MTR) CMXRos (Invitrogen) for 30 min at 37 °C. Fluorescence intensity was measured using flow cytometry (FACSCanto II, BD Biosciences, San Jose, CA, USA) and visualized by fluorescence microscopy (Axio Observer, Carl Zeiss AG, Oberkochen, Germany).

ATP and AMP measurement. Cellular ATP levels were measured using the ATP Assay Kit (Colorimetric/Fluorometric) (Abcam, Cambridge, UK; ab83355/ K354) according to the manufacturer's instructions. AMP levels were quantified using the AMP Assay Kit (Colorimetric) (Abcam; ab273275), and the AMP/ATP ratio was calculated after normalization to total protein concentration.

Western blotting. Total protein lysates were prepared using RIPA buffer supplemented with protease and phosphatase inhibitors, separated by SDS-PAGE, and transferred onto polyvinylidene difluoride (PVDF) membranes. Immunoblotting was performed using the following primary antibodies: anti-phospho-AMPK α (Thr172), anti-total AMPK α , and anti- β -actin (#2535, #5832, and #4970; all from Cell Signaling Technology, Danvers, MA, USA). Detection was carried out using HRP-conjugated secondary antibodies (Goat anti-Rabbit IgG H&L, HRP, Abcam, ab205718; Goat anti-Mouse IgG H&L, HRP, Abcam, ab205719) and enhanced chemiluminescence (ECL) reagents.

Fatty acid treatment and lipid accumulation assay. Hepa1c1c7 (mouse) and Hep3B (human) cells were transfected with LGALS3BP-targeting siRNAs and cultured in serum-free α -MEM (Gibco, Thermo Fisher Scientific) or Hank's Balanced Salt Solution (HBSS) (Gibco) for 6-12 h to induce metabolic stress. Cells were then treated with a palmitate/oleate (PO) mixture (2:1 molar ratio, 300 μ M total) conjugated to fatty acid-free BSA (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. Vehicle control cells were treated with BSA alone. Following treatment, cells were fixed with 4% paraformaldehyde for 10 min and stained with freshly prepared Oil Red O working solution for 20 min. Excess dye was removed by thorough washing, and lipid droplets were visualized under

a light microscope (CKX41, Olympus, Tokyo, Japan). For quantification, the retained dye was extracted with 100% isopropanol, and absorbance was measured at 500 nm using a microplate reader (SpectraMax, Molecular Devices, San Jose, CA, USA). Lipid accumulation was normalized to total protein content.

Quantitative real-time PCR (qRT-PCR). Total RNA was extracted using TRIzol Reagent (Invitrogen, Thermo Fisher Scientific) and reverse-transcribed with M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocols. Quantitative real-time PCR was performed using SYBR Green Master Mix (Thermo Fisher Scientific) on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Primer sequences used for gene expression analysis are provided in Supplementary Table VIII.

Public database analysis. TCGA-LIHC (cBioPortal PanCancer Atlas) transcriptomic data were analyzed to predict the potential roles of LGALS3BP in patients with liver cancer. Genes associated with LGALS3BP expression were identified and subjected to pathway enrichment analysis. Correlation analysis was also performed to assess the relationship between LGALS3BP and selected metabolism-related genes.

Clinical samples from Chonnam National University Hwasun Hospital. Tumoral and peri-tumoral tissues were collected from 83 patients with HCC (Date of visit: from 2017-01-25 to 2021-07-14) at Chonnam National University Hwasun Hospital (CNUHH, Hwasun, Jeollanam-do, Republic of Korea), as previously described (18). This study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital (IRB No. H-2021-07-032). Informed consent was obtained from all subjects. All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Chonnam National University Medical School. The study protocol was reviewed and approved by the Committee (approval number: CNU IACUC-H2021-7).

Statistical analysis. All data are presented as mean±standard error of the mean (SEM) from at least three independent biological replicates, unless otherwise indicated. Statistical significance between two groups was assessed using the unpaired two-tailed Student's *t*-test. For comparisons involving more than two groups, one-way or two-way ANOVA followed by Tukey's *post hoc* test was applied as appropriate. A *p*-value less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

Results

Low LGALS3BP expression is associated with lipid metabolic reprogramming. To identify biological processes inversely associated with LGALS3BP expression in HCC, we performed a transcriptome-wide correlation analysis using RNA-sequencing data from The Cancer Genome Atlas (TCGA) HCC cohort. Genes showing significant negative correlation with LGALS3BP (Spearman's $\rho < -0.25$, FDR-adjusted $q < 0.05$; $n=817$) were subjected to pathway enrichment analysis using Reactome, KEGG, and MSigDB Hallmark databases.

Across all three platforms, we observed consistent enrichment of pathways related to fatty acid metabolism, indicating a convergent metabolic signature associated with reduced LGALS3BP expression. Specifically, Reactome revealed enrichment in fatty acid metabolism, metabolism of lipids, peroxisomal protein import, and synthesis of bile acids *via* 7 α -hydroxycholesterol. KEGG analysis identified fatty acid degradation, peroxisome, primary bile acid biosynthesis, and PPAR signaling pathways. MSigDB Hallmark further supported these findings with enrichment in fatty acid metabolism, bile acid metabolism, adipogenesis, and peroxisome pathways (Figure 1A). Collectively, these results suggest that LGALS3BP downregulation is closely linked to enrichment of PPAR-driven lipid catabolic transcriptional programs (including peroxisomal pathways and bile acid metabolism), consistent with a coordinated stress-adaptive metabolic signature in HCC.

From the 817 anticorrelated genes, we selected 138 unique genes involved in lipid metabolic processes that were consistently ranked within the top enriched pathways across the three databases. These included key enzymes for fatty acid oxidation, bile acid biosynthesis, and peroxisomal function. Transcription factor (TF) enrichment analysis using TRRUST identified PPARA, NR1H4 (FXR), HNF4A, and RXRA as top regulators (Figure 1B). Analysis of TF perturbation datasets further showed that LGALS3BP-anticorrelated genes significantly overlapped with PPARA and NR1H3 knockout, as well as KLF15-low expression mouse models (Figure 1C). In particular, genes encoding mitochondrial β -oxidation enzymes (ACADL, CPT1A, HADH), bile acid processing enzymes, and TCA cycle components (IDH1, ACO2) were strongly anticorrelated with LGALS3BP and suppressed under these perturbations (Figure 1D). These findings indicate that LGALS3BP expression is inversely linked to a PPARA-driven catabolic program, suggesting that loss of LGALS3BP may trigger a broader shift toward lipid catabolic gene programs associated with oxidative metabolism in HCC.

Interactome profiling reveals dual centrosomal and mitochondrial associations of LGALS3BP. To investigate the mechanistic basis of LGALS3BP-mediated metabolic regulation, we performed interactome profiling using immunoprecipitation-mass spectrometry (IP-MS) in Hepa1c1c7 cells expressing either C-terminal Myc-tagged or N-terminal Flag-tagged LGALS3BP. Each tag construct was analyzed under two independent immunoprecipitation conditions, totaling four IP-MS datasets (Myc1, Myc2, Flag1, Flag2) compared to negative controls (Figure 2A).

Convergence across constructs enabled identification of a robust core interactome. Forty-two proteins were reproducibly enriched across all datasets, representing high-confidence, tag-independent LGALS3BP interactors. In agreement with earlier reports, several centrosome-associated proteins were identified (22), confirming previously suggested roles in cellular structural organization. Notably, we also observed a broad enrichment of mitochondrial proteins, strongly suggesting that LGALS3BP

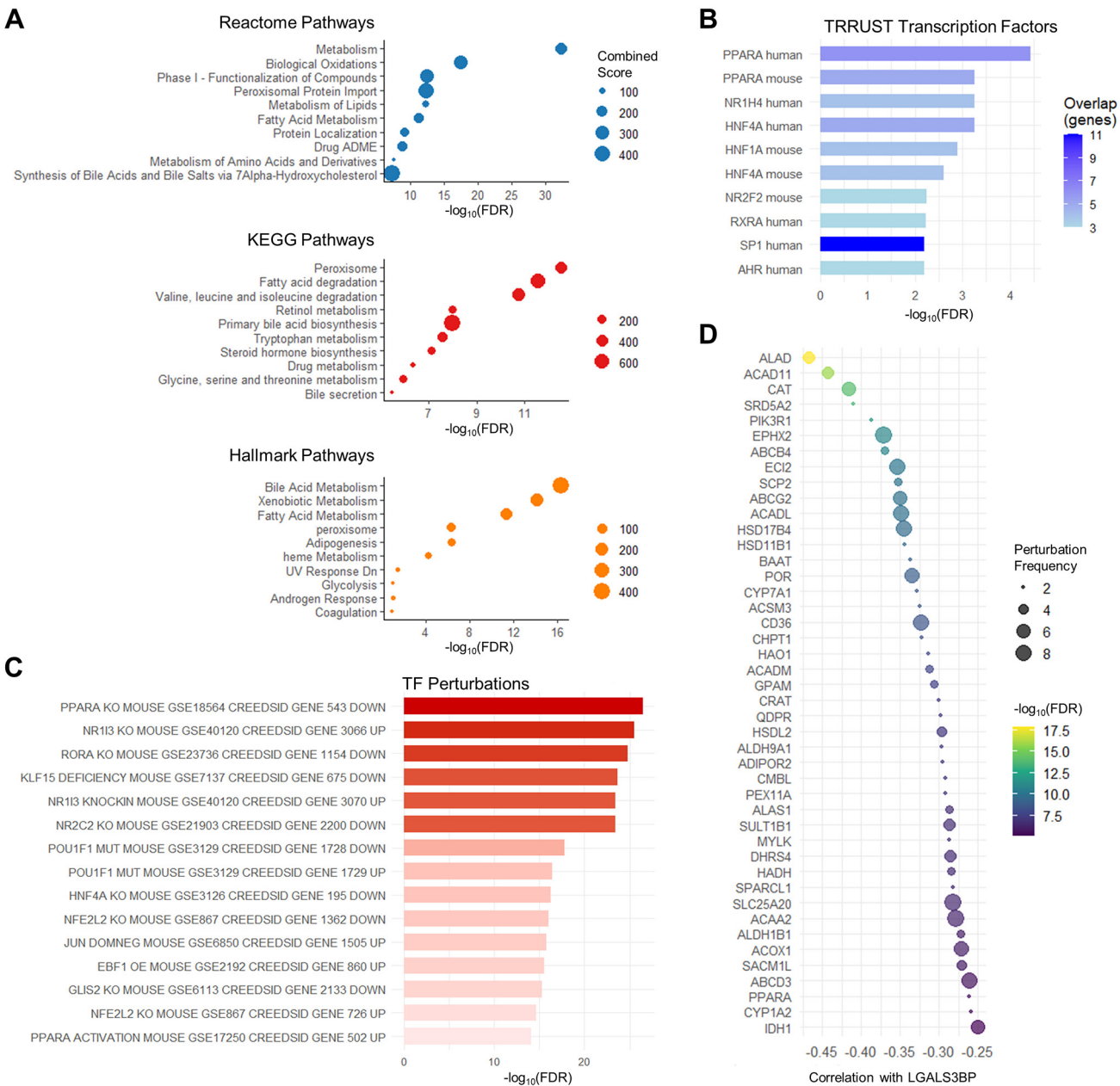


Figure 1. Transcriptomic programs inversely associated with LGALS3BP expression in HCC. (A) Pathway enrichment of 817 genes inversely correlated with LGALS3BP expression (Spearman $p < -0.25$, FDR $q < 0.05$) in TCGA-LIHC using Reactome, KEGG, and Hallmark databases. (B) Transcription factor enrichment based on the TRRUST database. (C) Enrichment of TF perturbation signatures from GEO-derived datasets. (D) Scatterplot showing correlation strength and perturbation frequency for individual anticorrelated genes. HCC: Hepatocellular carcinoma.

may serve as a cell-intrinsic scaffold that links centrosomal architecture, potentially involving microtubule-dependent positioning, with mitochondrial metabolic machinery.

Among the 42 interactors, multiple proteins were annotated as mitochondrial based on Gene Ontology and MitoCarta3.0 (Figure 2B). STRING network

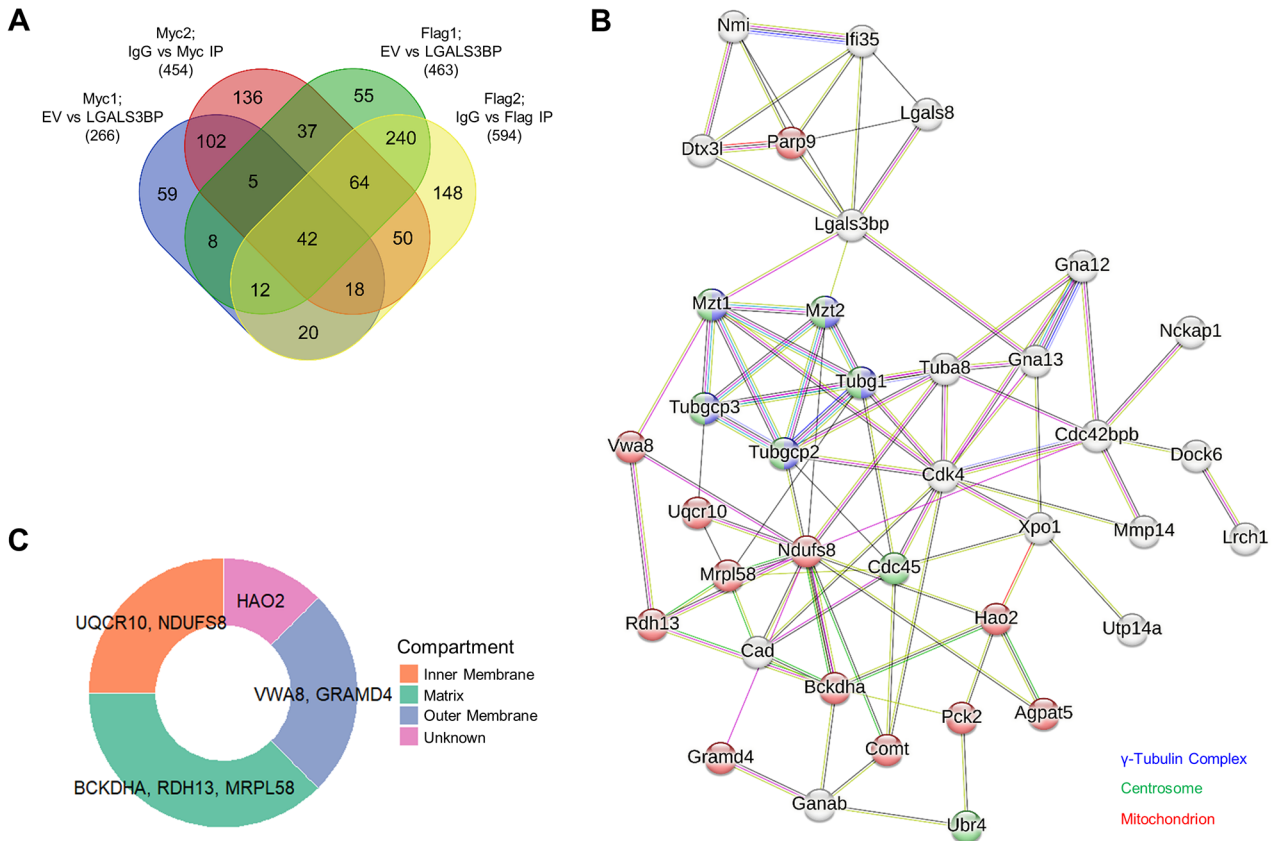


Figure 2. Proteomic profiling identifies centrosomal and mitochondrial associations of LGALS3BP. (A) Venn diagram showing overlap of LGALS3BP-interacting proteins from four IP-MS conditions (Myc1, Myc2, Flag1, Flag2) in Hepa1c7 cells. Proteins enriched by at least 10-fold were identified under each condition: Myc1 (266 proteins), Myc2 (454 proteins), Flag1 (463 proteins), and Flag2 (594 proteins). (B) STRING network visualization of the 42 proteins reproducibly enriched across all four IP-MS conditions. Proteins are grouped based on reported subcellular localization, with γ -tubulin complex components (blue), centrosomal proteins (green), and mitochondrial proteins (red) highlighted. (C) Donut chart showing sub-mitochondrial localization of eight MitoCarta-confirmed LGALS3BP interactors. IP-MS: Immunoprecipitation-mass spectrometry.

analysis revealed dense interconnectivity among these mitochondrial components, while sub-mitochondrial mapping indicated distribution across the outer membrane (VWA8, GRAMD4), inner membrane (UQCR10, NDUF8), matrix (BCKDHA, RDH13, MRPL58), and undefined compartments (HAO2) (Figure 2C). This widespread distribution implies that LGALS3BP does not bind a single metabolic complex but instead associates with proteins across different mitochondrial subdomains, consistent with a role in organizing and stabilizing mitochondrial fitness under metabolic stress. These findings expand the functional spectrum of LGALS3BP

from a secreted immunomodulator to an intracellular scaffold that supports energy metabolism.

LGALS3BP deficiency disrupts mitochondrial energetic fitness and activates AMPK signaling under nutrient stress.

To assess the functional consequences of LGALS3BP deficiency on mitochondrial homeostasis, we analyzed primary hepatocytes from Lgals3bp knockout (KO) mice, which we previously described in our recent study (18). Flow cytometry using MTG revealed a marked reduction in mitochondria-associated fluorescence intensity in KO hepatocytes compared with wild-type controls (Figure

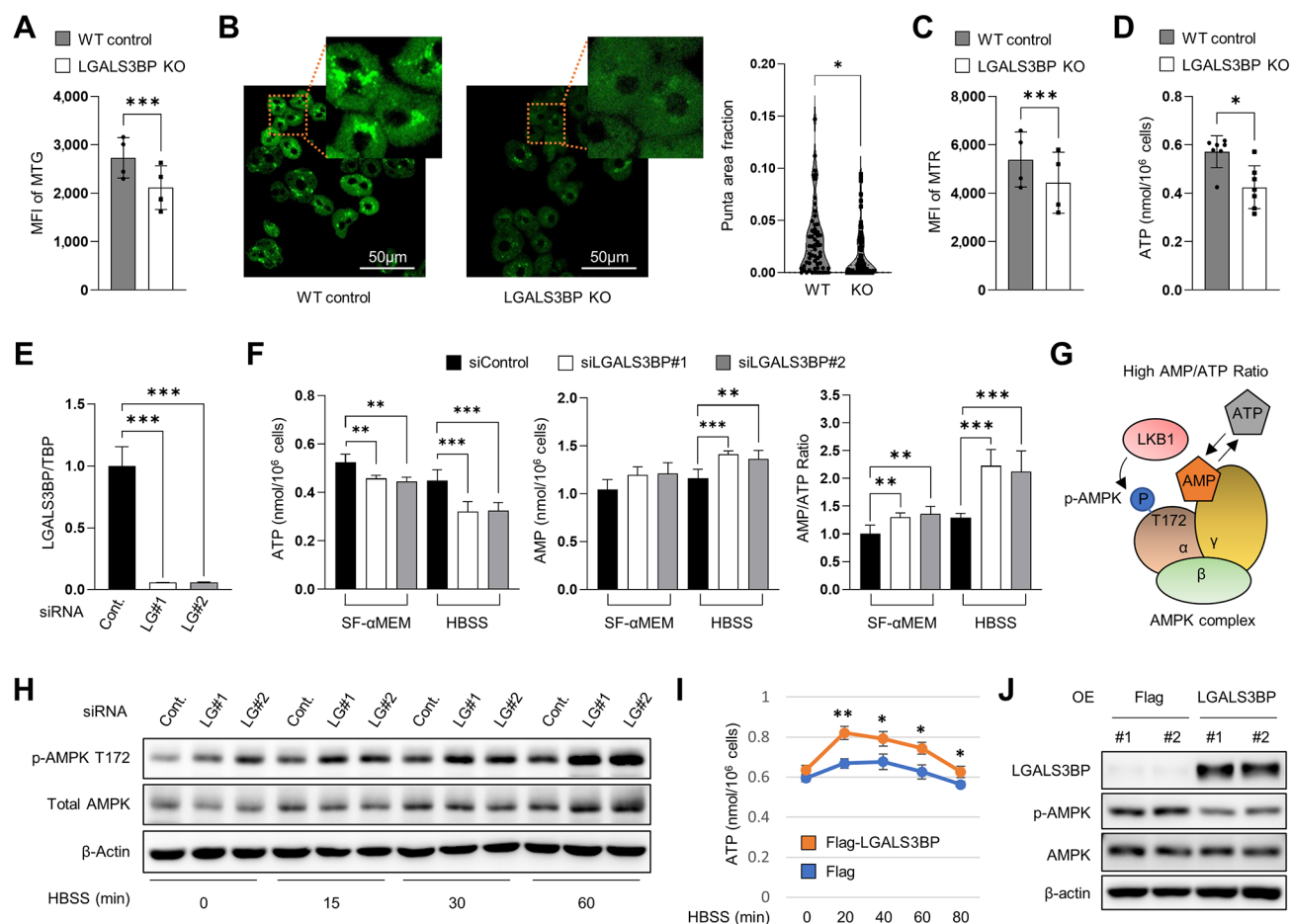


Figure 3. *LGALS3BP* knockdown or low expression compromises energetic fitness and activates AMPK signaling. (A) Flow cytometry analysis of mitochondria-associated fluorescence intensity (MitoTracker Green, 200 nM) in primary hepatocytes from *Lgals3bp*-KO vs. WT mice. (B) Fluorescence microscopy showing mitochondrial fragmentation in MTG-stained KO hepatocytes, with quantification of puncta area fraction shown in the graph on the right. (C) Mitochondrial membrane potential measured by MitoTracker Red. (D) ATP quantification in KO hepatocytes compared with wild-type primary hepatocytes. (E) qRT-PCR confirming *LGALS3BP* knockdown in Hepa1c17 using siRNAs. (F) ATP and AMP levels, and AMP/ATP ratio measured after 6 h of nutrient deprivation (α-MEM or HBSS). (G) Schematic showing AMP/ATP-mediated AMPK activation. (H) Western blot for phospho-AMPK (Thr172) in cells treated with HBSS for 0 to 60 min. (I) ATP levels in Flag-*LGALS3BP* vs. vector-expressing cells after exposure to HBSS stress conditions. (J) Western blot for each indicated protein after 6 h of HBSS treatment in Flag or Flag-tagged *LGALS3BP* overexpressing Hepa-1c17 cells. All quantitative data represent mean ± standard error of the mean (SEM) from ≥3 independent experiments. Statistical significance was assessed by Student's *t*-test for two-group comparisons and by one-way or two-way ANOVA followed by Tukey's post hoc test for multiple-group comparisons (E, F, and I). *p* < 0.05 was considered statistically significant. AMPK: AMP-activated protein kinase; WT: wild-type; MTG: MitoTracker Green; ATP: adenosine triphosphate; HBSS: Hank's Balanced Salt Solution.

3A). This decrease was accompanied by fragmented and spatially disorganized mitochondrial morphology observed by fluorescence microscopy (Figure 3B). In parallel, $\Delta\Psi_m$ assessed by MTR was significantly diminished in KO cells (Figure 3C), indicating impaired mitochondrial energetic fitness.

KO hepatocytes exhibited a significant reduction in intracellular ATP levels (Figure 3D), consistent with compromised oxidative phosphorylation. To validate these findings *in vitro*, we depleted *LGALS3BP* in Hepa1c17 hepatoma cells using two independent siRNAs (Figure 3E). Upon nutrient deprivation (serum-free

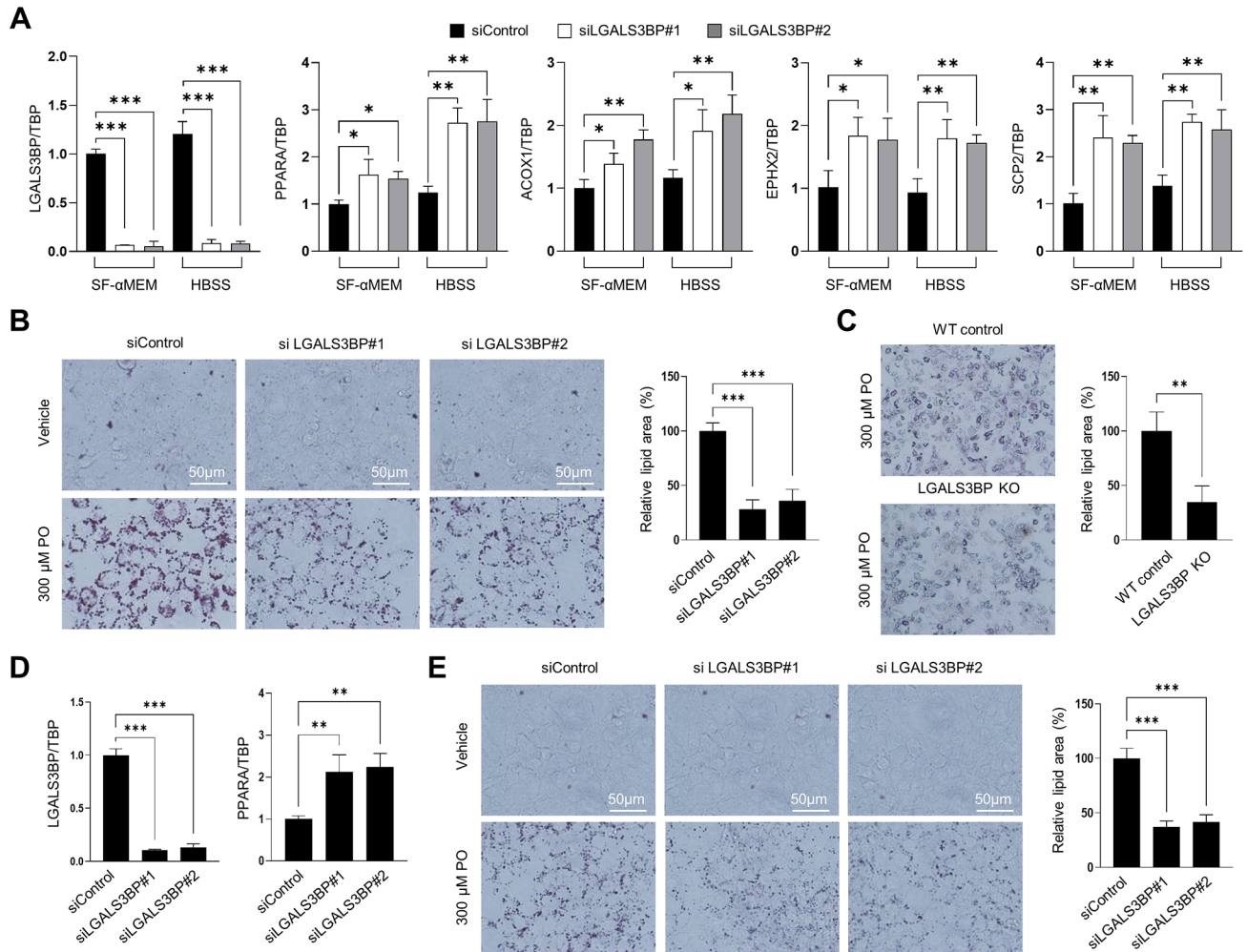


Figure 4. Low LGALS3BP expression promotes PPARA-dependent lipid catabolism and suppresses triglyceride accumulation. (A) Hepa1c1c7 cells were transfected with control or siLGALS3BP siRNAs and cultured in either serum-free α -MEM or HBSS for 6 h. Expression of each indicated gene was measured by qRT-PCR. (B) siLGALS3BP- or control-transfected Hepa1c1c7 cells were treated with 300 μ M palmitate/oleate mix (PO) for 24 h and subjected to Oil Red O staining. Left: representative images. Right: ImageJ quantification of lipid content. (C) Primary hepatocytes isolated from wild-type or *Lgals3bp*-KO mice were treated with 300 μ M PO for 24 h and analyzed by Oil Red O staining. (D) The human hepatic cancer cell line Hep3B was transfected with control or siLGALS3BP siRNAs and incubated in HBSS for 6 h. PPARA expression was assessed by qRT-PCR. (E) Lipid accumulation in Hep3B cells treated with 300 μ M PO for 24 h after siRNA transfection. Representative images and quantitative analysis are shown. All data represent mean $\pm$ standard error of the mean (SEM) from ≥ 3 independent experiments. Statistical significance was assessed by two-way ANOVA followed by Tukey's post hoc test for panel (A), one-way ANOVA followed by Tukey's post hoc test for panels (B), (D), and (E), and an unpaired two-tailed Student's *t*-test for panel (C). $p < 0.05$ was considered statistically significant. PPARA: Peroxisome proliferator-activated receptor alpha; HBSS: Hank's Balanced Salt Solution.

α -MEM or HBSS), LGALS3BP-low expression cells showed further ATP depletion, increased AMP accumulation, and an elevated AMP/ATP ratio (Figure 3F).

An increased AMP/ATP ratio can be sensed by the upstream kinase LKB1, leading to activation of AMPK

via phosphorylation at Thr172 (Figure 3G). Consistent with this model, siLGALS3BP-transfected cells exhibited earlier and more pronounced AMPK phosphorylation under HBSS-induced stress compared to control cells (Figure 3H). Conversely, overexpression of Flag-tagged

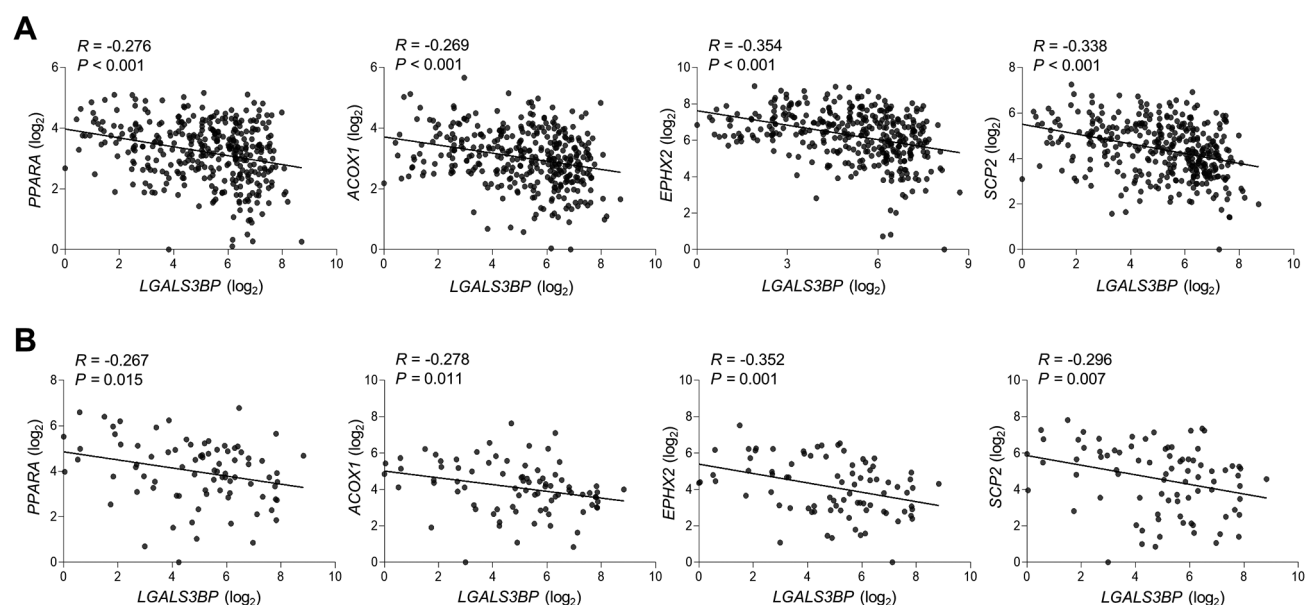


Figure 5. Validation of LGALS3BP-PPARA axis in HCC patient cohorts. (A) Scatter plots showing correlations between LGALS3BP and PPARA, ACOX1, EPHX2, and SCP2 expression in TCGA-LIHC tumors ($n=366$). (B) Scatter plots of the same correlations in tumoral tissues from an institutional HCC cohort (CNUHH, $n=83$). The Pearson's correlation coefficient (R) and two-tailed p -Values are indicated in each graph. HCC: Hepatocellular carcinoma; PPARA: peroxisome proliferator-activated receptor alpha.

LGALS3BP mitigated ATP depletion and suppressed AMPK phosphorylation (Figure 3I-J). Together, these results indicate that LGALS3BP is required to sustain mitochondrial energetic fitness and restrain excessive activation of AMPK during nutrient stress.

Low LGALS3BP expression promotes PPARA-dependent lipid catabolism and reduces lipid accumulation. Given the observed AMPK activation, we next examined whether LGALS3BP loss promotes PPARA-linked lipid catabolic transcriptional programs. Under nutrient stress, siLGALS3BP-transfected Hepa1c1c7 cells displayed robust transcriptional upregulation of PPARA and its canonical β -oxidation targets ACOX1, EPHX2, and SCP2 (Figure 4A). Induction of these β -oxidation genes was accompanied by a clear functional outcome, as siLGALS3BP-low expression cells exposed to palmitate/oleate (PO) exhibited significantly reduced intracellular lipid accumulation by Oil Red O staining (Figure 4B). Similar results were consistently observed in primary

hepatocytes from Lgals3bp-KO mice (Figure 4C) and in human Hep3B cells (Figure 4D-E).

These findings indicate that LGALS3BP normally maintains mitochondrial energetic fitness, thereby limiting the need for compensatory activation of PPAR α -linked lipid catabolic transcriptional programs under nutrient stress. LGALS3BP deficiency enforces a compensatory metabolic shift toward lipid catabolism through activation of the AMPK-PPARA axis. This adaptation prevents lipid storage and is consistent with a compensatory response to energetic stress under lipotoxic conditions, potentially influencing stress tolerance.

Validation of LGALS3BP-PPARA axis in HCC patient cohorts.

To evaluate whether the AMPK-PPARA axis identified in experimental models is also reflected in patient tumors, we analyzed transcriptomic correlations between LGALS3BP and key peroxisomal fatty acid oxidation genes in two independent cohorts (Figure 5A, B). In TCGA-LIHC tumors, reduced LGALS3BP expression correlated inversely with

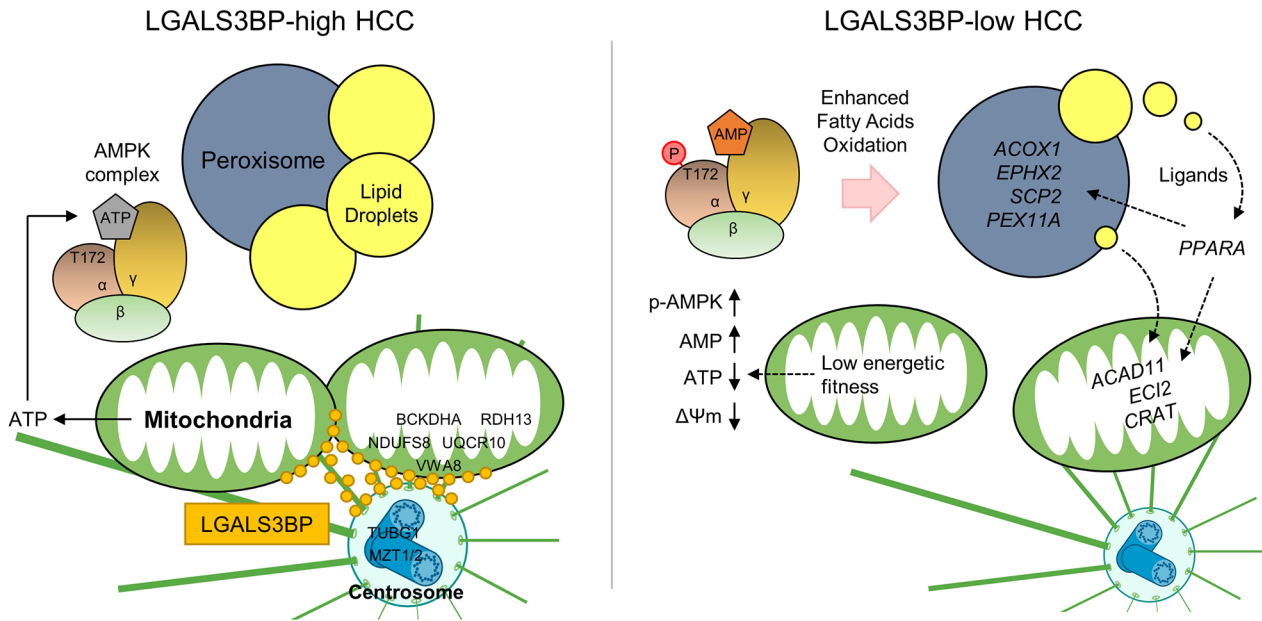


Figure 6. *LGALS3BP* links a centrosome-mitochondria interface that connects energetic fitness to compensatory PPAR α -driven lipid catabolic programs in hepatocellular carcinoma. *LGALS3BP* supports centrosome-proximal mitochondrial organization and energetic fitness, thereby restraining AMPK-PPAR α -linked lipid catabolic transcriptional programs. Loss of *LGALS3BP* disrupts this organizational axis, leading to mitochondrial dysfunction, ATP depletion, and AMPK activation. Activated AMPK is associated with the induction of PPAR α and its downstream peroxisomal lipid catabolic genes, including *ACOX1*, *EPHX2*, and *SCP2*, representing a compensatory metabolic response to mitochondrial energetic stress. PPAR α : Peroxisome proliferator-activated receptor alpha; AMPK: AMP-activated protein kinase; ATP: adenosine triphosphate.

PPAR α ($R=-0.276$, $p<0.001$), *ACOX1* ($R=-0.269$, $p<0.001$), *EPHX2* ($R=-0.354$, $p<0.001$), and *SCP2* ($R=-0.338$, $p<0.001$). Consistent results were obtained in resected HCC tissues from CNUHH, where *LGALS3BP* expression also negatively correlated with PPAR α ($R=-0.267$, $p=0.015$), *ACOX1* ($R=-0.278$, $p=0.011$), *EPHX2* ($R=-0.352$, $p=0.001$), and *SCP2* ($R=-0.296$, $p=0.007$). This reproducibility across both public and institutional datasets underscores the clinical relevance of *LGALS3BP* as a determinant of centrosome-mitochondria organizational state and PPAR α -linked lipid catabolic transcriptional programs in HCC.

Integrative model: *LGALS3BP* as a cell-intrinsic organizer of a centrosome-mitochondria interface and lipid catabolic reprogramming. Our integrated transcriptomic, proteomic, and functional analyses converge on a model in which *LGALS3BP* acts as a cell-intrinsic organizer that defines a centrosome-proximal mitochondrial interface

(Figure 6). By bridging centrosomal and mitochondrial components, *LGALS3BP* supports the centrosome-proximal mitochondrial organization and energetic fitness. This spatial organization safeguards ATP production and prevents hyperactivation of the AMPK-PPAR α lipid catabolic program.

Loss of *LGALS3BP* disrupts this coordination, resulting in mitochondrial fragmentation, ATP depletion, and sustained AMPK activation. As a consequence, hepatocytes compensate by activating PPAR α -dependent peroxisomal and mitochondrial fatty acid oxidation, which reduces lipid accumulation and enforces a catabolic state. These cellular events closely align with the transcriptomic signatures observed in *LGALS3BP*-low HCC tumors, underscoring the translational relevance of this pathway. Thus, *LGALS3BP* emerges as a key cell-intrinsic organizer of energy homeostasis that integrates mitochondrial fitness with lipid metabolic regulation in liver cancer.

Discussion

This study uncovers a previously unrecognized cell-intrinsic role of LGALS3BP in supporting a centrosome-mitochondria interface that stabilizes energetic fitness and restrains compensatory AMPK-PPAR α -linked lipid catabolic programs in HCC. Although LGALS3BP has been primarily described as a secreted immunomodulatory glycoprotein, our integrated transcriptomic, proteomic, and functional analyses reveal that it also functions within tumor cells to maintain metabolic homeostasis under nutrient stress.

Patient-derived transcriptomic data demonstrated that low LGALS3BP expression is strongly associated with enrichment of mitochondrial oxidative metabolism, peroxisomal function, and bile acid biosynthesis pathways, indicating an inverse relationship between LGALS3BP levels and activation of lipid catabolism. Consistent with these observations, LGALS3BP knockdown in hepatoma cells or genetic deletion in hepatocytes resulted in ATP depletion, AMPK activation, and reduced mitochondrial membrane potential, reflecting compromised mitochondrial energetic fitness. These alterations were accompanied by a compensatory increase in PPARA expression and induction of canonical β -oxidation genes, which translated into decreased triglyceride accumulation following palmitate/oleate challenge. Together, these findings establish LGALS3BP as a cell-intrinsic regulator that limits excessive reliance on lipid oxidation by preserving mitochondrial energetic capacity, in line with recent studies highlighting the importance of proteomic remodeling and growth-regulatory signaling in hepatocellular carcinoma progression and metabolic adaptation (23, 24).

Notably, in our previous work, *Lgals3bp*-low expression mice did not exhibit overt metabolic abnormalities under basal conditions (18). The present study highlights that the metabolic consequences of LGALS3BP loss become pronounced under stress conditions, particularly in HCC cells, manifesting as mitochondrial fragmentation, AMPK activation, and PPAR α -driven lipid catabolism.

This context dependency likely reflects the heightened metabolic demand and adaptive flexibility of tumor cells, which rely heavily on mitochondrial fitness to survive in nutrient-limited microenvironments. These observations align with emerging concepts of metabolic plasticity as a hallmark of cancer adaptation to stress (1, 2, 9, 12).

Our proteomic analyses further revealed that LGALS3BP interacts with both centrosomal and mitochondrial proteins, including components of oxidative phosphorylation (*e.g.*, NDUFS8, UQCRC1) and lipid remodeling enzymes (*e.g.*, AGPAT5). These data suggest that LGALS3BP may act as a structural scaffold linking centrosomal architecture (and potentially microtubule-dependent positioning) to mitochondrial and lipid metabolic domains. This spatial coordination offers a plausible mechanism by which LGALS3BP sustains mitochondrial function and protects against excessive activation of the AMPK-PPAR α catabolic program, as summarized in our working model (Figure 6).

LGALS3BP is frequently overexpressed in aggressive HCC (20), raising the possibility that tumor cells exploit this protein to enhance mitochondrial resilience and suppress chronic energy stress. By preserving ATP levels and restraining uncontrolled fatty acid oxidation, elevated LGALS3BP expression may confer metabolic stability that supports tumor progression and therapy resistance. In this context, LGALS3BP functions not only as a suppressor of energetic stress but also as a contributor to metabolic resilience in liver cancer cells.

In our transcription factor enrichment analysis, HNF4A was also identified, which may reflect not only its role in maintaining hepatocyte identity but also differences in hepatocyte differentiation state, metabolic zoning, and/or tumor purity in low-LGALS3BP tumors, as previously described in studies of liver-specific gene regulation (25, 26). Nevertheless, PPARA emerged as the functionally prioritized effector in our study because, beyond the transcriptomic enrichment results, LGALS3BP-deficient models consistently showed mitochondrial energetic stress, ATP depletion, increased AMP/ATP ratio, AMPK activation, and induction of canonical PPARA-associated lipid catabolic genes. Since

AMPK activation is known to promote fatty acid oxidation by increasing CPT1 expression and enhancing endogenous PPARA ligands, the observed AMPK-PPARA axis activation supports a model in which LGALS3BP functions upstream of both mitochondrial energetic fitness and transcriptional metabolic control (11).

Although the present data support activation of an AMPK-PPARA-associated compensatory program, we did not directly test whether PPARA is required for the reduced lipid accumulation phenotype or whether AMPK is causally required upstream of this transcriptional response in LGALS3BP-deficient cells. Moreover, it remains to be determined whether LGALS3BP regulates mitochondrial fitness through mitophagy, biogenesis, or organelle dynamics, and how its intracellular metabolic role intersects with its extracellular immunomodulatory functions. Future studies defining the LGALS3BP interactome *in vivo* and directly quantifying fatty acid oxidation flux (*e.g.*, palmitate-driven oxygen consumption) will be valuable to further clarify how scaffold-like associations influence organelle organization and metabolic plasticity.

Taken together, our findings identify LGALS3BP as a dual-function modulator that integrates metabolic and immunological regulation in HCC. By supporting mitochondrial energetic fitness and restraining compensatory AMPK-PPAR α -driven lipid catabolism, LGALS3BP contributes to the metabolic adaptability and survival of liver cancer cells.

Conclusion

In conclusion, tumors with low LGALS3BP expression exhibit impaired mitochondrial energetic fitness and enrichment of lipid catabolic transcriptional programs, whereas high LGALS3BP expression supports mitochondrial resilience by restraining chronic AMPK-PPAR α activation. This cell-intrinsic scaffold function complements the well-established secretory role of LGALS3BP and positions it as both a biomarker and a potential therapeutic target for metabolic stratification in hepatocellular carcinoma.

Supplementary Material

Supplementary tables associated with this study have been deposited in Zenodo and are available at the following DOI: 10.5281/zenodo.19326391

Conflicts of Interest

The Authors have declared that no competing interests exist.

Authors' Contributions

Conceptualization: JEH, HJS, IJC, SHC, DHK. Methodology & Investigation: HJB, HJK, SYJ, IJC, SHC, DHK. Data Curation & Formal Analysis: JEH, HJS. Proteomics and Bioinformatics Analysis: SYJ. Visualization: JEH, HJS. Writing - Original Draft Preparation: JEH, HJS. Writing - Review & Editing: SHC, DHK. Funding Acquisition and Supervision: SHC.

Acknowledgements

We thank the members of the Department of Internal Medicine, Division of Hematology and Oncology, Chonnam National University Hwasun Hospital, for their invaluable support and assistance throughout this study.

Funding

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (Grant number: RS-2025-19252970).

Artificial Intelligence (AI) Disclosure

Artificial intelligence-based tools (ChatGPT, OpenAI) were used to assist with language editing and improvement of clarity during manuscript preparation. All scientific content, experimental design, and interpretation were conceived and written independently by the authors.

References

- 1 Pavlova NN, Thompson CB: The emerging hallmarks of cancer metabolism. *Cell Metab* 23(1): 27-47, 2016. DOI: 10.1016/j.cmet.2015.12.006
- 2 Pavlova NN, Zhu J, Thompson CB: The hallmarks of cancer metabolism: Still emerging. *Cell Metab* 34(3): 355-377, 2022. DOI: 10.1016/j.cmet.2022.01.007
- 3 Vyas S, Zaganjor E, Haigis MC: Mitochondria and cancer. *Cell* 166(3): 555-566, 2016. DOI: 10.1016/j.cell.2016.07.002
- 4 Bergonzini C, Kroese K, Zweemer AJM, Danen EHJ: Targeting integrins for cancer therapy – disappointments and opportunities. *Front Cell Dev Biol* 10: 863850, 2022. DOI: 10.3389/fcell.2022.863850
- 5 Buck MD, Sowell RT, Kaech SM, Pearce EL: Metabolic instruction of immunity. *Cell* 169(4): 570-586, 2017. DOI: 10.1016/j.cell.2017.04.004
- 6 Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP: Cancer metabolism: a therapeutic perspective. *Nat Rev Clin Oncol* 14(1): 11-31, 2017. DOI: 10.1038/nrclinonc.2016.60
- 7 Nunnari J, Suomalainen A: Mitochondria: in sickness and in health. *Cell* 148(6): 1145-1159, 2012. DOI: 10.1016/j.cell.2012.02.035
- 8 Sainero-Alcolado L, Liaño-Pons J, Ruiz-Pérez MV, Arsenian-Henriksson M: Targeting mitochondrial metabolism for precision medicine in cancer. *Cell Death Differ* 29(7): 1304-1317, 2022. DOI: 10.1038/s41418-022-01022-y
- 9 Ward PS, Thompson CB: Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell* 21(3): 297-308, 2012. DOI: 10.1016/j.ccr.2012.02.014
- 10 Hardie DG, Ross FA, Hawley SA: AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 13(4): 251-262, 2012. DOI: 10.1038/nrm3311
- 11 Garcia D, Shaw RJ: AMPK: Mechanisms of cellular energy sensing and restoration of metabolic balance. *Mol Cell* 66(6): 789-800, 2017. DOI: 10.1016/j.molcel.2017.05.032
- 12 Faubert B, Solmonson A, DeBerardinis RJ: Metabolic reprogramming and cancer progression. *Science* 368(6487): eaaw5473, 2020. DOI: 10.1126/science.aaw5473
- 13 Kersten S: Integrated physiology and systems biology of PPAR α . *Mol Metab* 3(4): 354-371, 2014. DOI: 10.1016/j.molmet.2014.02.002
- 14 Schulze A, Harris AL: How cancer metabolism is tuned for proliferation and vulnerable to disruption. *Nature* 491(7424): 364-373, 2012. DOI: 10.1038/nature11706
- 15 Feng XC, Liu FC, Chen WY, Du J, Liu H: Lipid metabolism of hepatocellular carcinoma impacts targeted therapy and immunotherapy. *World J Gastrointest Oncol* 15(4): 617-631, 2023. DOI: 10.4251/wjgo.v15.i4.617
- 16 Capone E, Iacobelli S, Sala G: Role of galectin 3 binding protein in cancer progression: a potential novel therapeutic target. *J Transl Med* 19(1): 405, 2021. DOI: 10.1186/s12967-021-03085-w
- 17 Loimaranta V, Hepojoki J, Laaksoaho O, Pulliainen AT: Galectin-3-binding protein: A multitask glycoprotein with innate immunity functions in viral and bacterial infections. *J Leukoc Biol* 104(4): 777-786, 2018. DOI: 10.1002/JLB.3VMR0118-036R
- 18 Kim DH, Sung M, Park MS, Sun EG, Yoon S, Yoo KH, Radhakrishnan K, Jung SY, Bae WK, Cho SH, Chung IJ: Galectin 3-binding protein (LGALS3BP) depletion attenuates hepatic fibrosis by reducing transforming growth factor- β 1 (TGF- β 1) availability and inhibits hepatocarcinogenesis. *Cancer Commun (Lond)* 44(10): 1106-1129, 2024. DOI: 10.1002/cac2.12600
- 19 Yin Y, Feng W, Chen J, Chen X, Wang G, Wang S, Xu X, Nie Y, Fan D, Wu K, Xia L: Immunosuppressive tumor microenvironment in the progression, metastasis, and therapy of hepatocellular carcinoma: from bench to bedside. *Exp Hematol Oncol* 13(1): 72, 2024. DOI: 10.1186/s40164-024-00539-x
- 20 Liu Y, Liang L, Li J, Pang T, Zhang SH, Xia ZY: Aberrant expression of LGALS3BP drives an unfavorable prognosis and more aggressive in HCC via regulating PI3K/AKT signaling. *Tissue Cell* 89: 102471, 2024. DOI: 10.1016/j.tice.2024.102471
- 21 White MJ, Roife D, Gomer RH: Galectin-3 binding protein secreted by breast cancer cells inhibits monocyte-derived fibrocyte differentiation. *J Immunol* 195(4): 1858-1867, 2015. DOI: 10.4049/jimmunol.1500365
- 22 Fogeron ML, Müller H, Schade S, Dreher F, Lehmann V, Kühnel A, Scholz AK, Kashofer K, Zerck A, Fauler B, Lurz R, Herwig R, Zatloukal K, Lehrach H, Gobom J, Nordhoff E, Lange BM: LGALS3BP regulates centriole biogenesis and centrosome hypertrophy in cancer cells. *Nat Commun* 4: 1531, 2013. DOI: 10.1038/ncomms2517
- 23 Salihah SS, Tahir M, Bibi B, Sultan R, Larsen MR, Mirza MR, Mahmood S, Alam MR, Iqbal J, Cho WC, Gul A: Differential proteomic analysis of DEN-induced hepatocellular carcinoma in male and female Balb/c mice reveals novel sex-specific markers. *Cancer Genomics Proteomics* 22(6): 912-928, 2025. DOI: 10.21873/cgp.20540
- 24 Li LI, Cheng J, Guo HL, Liu QI, Wang NA, Wu CX, Sun H: Augmenter of liver regeneration enhances hepatocellular carcinoma cell growth through COX-2-associated signaling. *Cancer Genomics Proteomics* 23(1): 109-126, 2026. DOI: 10.21873/cgp.20564
- 25 Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA: Control of pancreas and liver gene expression by HNF transcription factors. *Science* 303(5662): 1378-1381, 2004. DOI: 10.1126/science.1089769
- 26 Ehle C, Iyer-Bierhoff A, Wu Y, Xing S, Kiehntopf M, Mosig AS, Godmann M, Heinzel T: Downregulation of HNF4A enables transcriptomic reprogramming during the hepatic acute-phase response. *Commun Biol* 7(1): 589, 2024. DOI: 10.1038/s42003-024-06288-1