Research Square

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YBX1 Drives SREBP2-Dependent Cholesterol Metabolic Reprogramming to Confer Drug Resistance in Hepatocellular Carcinoma

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Abstract

Background

Drug resistance remains a major challenge in the treatment of hepatocellular carcinoma (HCC). Dysregulated cholesterol metabolism contributes to tumor progression and therapeutic adaptation in multiple cancers, including HCC. Y-box binding protein 1 (YBX1) has been implicated in HCC progression and drug resistance; however, its relationship with sterol regulatory element-binding protein 2 (SREBP2)-associated cholesterol metabolism remains incompletely understood.

Methods

Clinical datasets and HCC cell models were analyzed to investigate the relationship between YBX1 expression, SREBP2, cholesterol metabolism, and sorafenib resistance.

Results

YBX1 expression was elevated in HCC and associated with unfavorable clinical outcomes. YBX1 overexpression was associated with increased SREBP2 expression, altered cholesterol metabolism, and enhanced drug resistance-associated phenotypes. Conversely, YBX1 knockdown reduced SREBP2 expression, lipid accumulation, and intracellular cholesterol levels. Sorafenib-resistant HCC cells exhibited elevated YBX1 and SREBP2 expression accompanied by increased intracellular cholesterol, supporting an association between this regulatory axis and acquired drug resistance. Pharmacological inhibition of YBX1 or SREBP2 reduced lipid accumulation and improved sensitivity to sorafenib.

Conclusions

These findings support an association between YBX1/SREBP2 signaling, cholesterol-associated metabolic changes, and drug resistance in HCC models. Targeting the YBX1/SREBP2/cholesterol axis may represent a potential strategy to improve therapeutic response in HCC.

1. INTRODUCTION

Cancer remains one of the leading causes of cancer-related mortality worldwide despite major advances in diagnosis and treatment. Drug resistance and metastasis continue to be major factors contributing to poor outcomes. Dysregulation of genes and signaling pathways can promote cancer progression and therapeutic resistance. Hepatocellular carcinoma (HCC) is among the most prevalent cancers and a leading cause of cancer-related deaths worldwide, with limited therapeutic options and a poor prognosis [1, 2]. The poor prognosis of HCC is largely attributed to late-stage diagnosis and early metastasis or

recurrence. Tyrosine kinase inhibitors (TKIs) offer significant survival benefits for patients with advanced HCC; however, acquired resistance is inevitable and limits efficacy.

Y-box binding protein 1 (YBX1) is a multifunctional DNA/RNA-binding protein involved in transcriptional regulation, mRNA stabilization, and stress-responsive pathways in cancers [3, 4]. Its versatile functions play a significant role in cancer metastasis and drug resistance [5, 6]. YBX1 exerts multiple biological functions and is positively correlated with stemness-related and multidrug resistance genes. Moreover, YBX1 promotes stemness and therapeutic resistance in several malignancies [7] .YBX1 augments sorafenib resistance by promoting EMT and the PI3K/Akt signaling pathway in HCC [8, 9]. Metabolic alterations or reprogramming of lipid metabolism are recognized as a hallmark of cancer progression and development [10]. Cholesterol is a crucial lipid component and is essential for cancer cell proliferation and survival [11].

Recent findings suggest that dysregulated cholesterol metabolism promotes oncogenic signaling and drug resistance [12–16]. Long-term exposure to Gefitinib or Osimertinib leads to cholesterol accumulation in lipid rafts, thereby reactivating the EGFR/Src/ERK signaling pathway and contributing to resistance to EGFR-TKIs [12]. Cholesterol activates the PI3K/Akt pathway, stimulates proliferation, inhibits apoptosis, and promotes colon cancer progression [17]. Excessive cholesterol accumulation within membrane lipid rafts activates several raft-associated pathways, promoting tumor cell proliferation and contributing to resistance to tumor therapies [18, 19]. Sterol regulatory element-binding proteins (SREBPs) are key transcription factors that regulate genes involved in lipid synthesis and play a central role in lipid metabolism under both physiological and pathological conditions. Dysregulation of SREBPs has been reported to cause aggressive pathological features of prostate cancer, and the blocking of SREBP-regulated metabolic pathways displays high antitumor activity [20]. Loss of SREBP activity inhibits cancer cell growth and viability by uncoupling fatty acid synthesis from desaturation [21]. While SREBP1 primarily regulates fatty acid synthesis, SREBP2 encodes the SREBP2 protein, which plays a crucial role in regulating cholesterol synthesis [22]. Accumulated evidence indicates that SREBP2 contributes to therapeutic resistance in several cancers, including cisplatin- resistant ovarian cancer [23]. SREBP2 activation drives drug resistance by promoting cholesterol biosynthesis in HCC [24]. Sorafenib- and lenvatinib-resistant cells showed increased SREBP2 nuclear translocation, cholesterol biosynthesis, and cancer stemness, thereby modulating the acquired drug resistance of cancer cells [24].

Recent studies have demonstrated that YBX1 regulates SREBP2 mRNA stability and promotes cholesterol biosynthesis in HCC [25]. YBX1 is highly expressed in MASLD liver tissue and significantly affects lipid accumulation in hepatocytes [26]. Li et al. showed that YBX1 binds to the 3’ UTR region of SREBP2, and overexpression of YBX1 upregulates the mRNA and protein levels of SREBP2 [25]. CXCL2 is a chemokine ligand that binds to YBX1 and prevents its nuclear translocation; consequently, this reduces SREBP2 transcription, suppresses cholesterol biosynthesis, and impedes HCC development [27]. In the present study, we show that YBX1 activity is significantly increased in HCC and activates SREBP2 and cholesterol metabolism. The YBX1-SREBP2 axis promotes cholesterol biosynthesis, stabilizes receptor kinases, and drives drug resistance. Targeting the cholesterol metabolic pathway restores drug

sensitivity and represents a promising therapeutic strategy for overcoming treatment resistance, with potential as an anticancer strategy in HCC.

2. MATERIALS AND METHODS

2.1 Cell culture

Human HCC cell lines SK-Hep1, HepG2, Huh7 luc, HepG2 luc, Hep3B, and C3A were obtained from ATCC (USA) and cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% antibiotics (Gibco, USA) at 37 ºC in a humidified atmosphere containing 5% CO_2. Cells were maintained at low passage numbers and routinely tested for mycoplasma contamination.

2.2 Cell transfection

We obtained siYBX1, the YBX1 expression vector, and the corresponding vector from Abcam (USA) and transfected them into SK-Hep1 cells using Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's instructions (Supplementary Table 3).

2.3 LipidSpot assay

Lipid droplet staining was performed using LipidSpot Lipid 488 Lipid Droplet stain (Biotium 70065-T and LipidSpot 610 Lipid Droplet stain (Biotium 70069-T) according to the manufacturer's protocol. Cells were fixed with 4% paraformaldehyde for 10 min at room temperature and washed twice with PBS. Cells were then permeabilized with 0.1% Triton X-100 for 5 minutes at room temperature, followed by 2 PBS washes. Cells were incubated with LipidSpot stain for 15 minutes, washed 3 times with PBS, mounted with EverBrite mounting medium (Biotium), and imaged on a Leica confocal microscope with 488 nm excitation. Staining quantification was performed in ImageJ.

2.4 Cholesterol assay

The total cholesterol was measured using the Amplex Red Cholesterol Assay Kit (Invitrogen, USA) according to the manufacturer's protocol. Fluorescence was measured using a Tecan Genex plate reader (Tecan, Italy; excitation 530nm, emission 610nm). Protein concentrations were determined using the Bradford protein assay according to the manufacturer's directions.

2.5. Oil Red O staining

SK Hep1 cells were stained with Oil Red O, washed 3 times with PBS, and fixed with 4% neutral formaldehyde for 10 min. Next, fixed cells were thoroughly washed with PBS and incubated in 60% isopropanol for 5 min. The cells were then stained with Oil Red and incubated for 30 min. Finally, after 3 washes, the cells were stained with hematoxylin for 10 min, and images were taken with an EVOS microscope.

2.6 RNA extraction and quantitative PCR

RNA was extracted from cells and tissues using the TRIzol isolation kit (Baltimore, USA). We synthesized complementary DNA (cDNA) using a reverse transcription kit (USA) according to the manufacturer's instructions. We calculated relative gene expression using the 2ΔΔCt method and normalized it to β-actin using the SYBR Green PCR kit (USA). Primer sequences are provided in Table S2.

2.7 Western blot

We lysed total cell protein in RIPA buffer containing protease and phosphatase inhibitors (Sandiago, USA) and determined protein concentrations by the Bradford method (Baltimore, USA). After protein denaturation, we detected expression levels by immunoblotting; the antibodies are listed in Table S3.

2.8 Cell viability assay

Cell viability was measured by MTT assay. We seeded 10,000 cells per well in 96-well plates (VWR, Cat# 10861-562). After 24 hours, cells received varying concentrations of the DMSO-dissolved test compound (final DMSO ≤ 0.1%). Following the standard protocol, absorbance was read at 570 nm with a Varioskan Lux plate reader (Thermo Fisher Scientific). Values were normalized to untreated controls and reported as percent viability. Each condition was tested in quadruplicate across at least three independent experiments.

2.9 In silico studies

To analyze localized binding conformations and estimate binding affinities, molecular docking was performed using BIOVIA Discovery Studio. The three-dimensional (3D) crystal structures of the target proteins were obtained from the RCSB Protein Data Bank (PDB), specifically PDB IDs 8UWC and 6KUG [28]. To evaluate molecular complexes and characterize their interaction profiles based on geometric complementarity, the protein structures and candidate molecules were imported into the BIOVIA Discovery Studio modeling environment. The structures were processed with the Prepare Protein and Prepare Ligands protocols to remove water molecules, assign appropriate protonation states, and optimize ionization states. Subsequently, energy minimization was performed using a two-step workflow. The Steepest Descent algorithm was first applied to resolve severe steric clashes, followed by the Conjugate Gradient algorithm to refine the structures into stable, low-energy conformations. Binding sites were identified from receptor cavities, and an SBD (Structure-Based Design) Site Sphere was generated and centered on the selected binding cavity to define the docking search space.

Molecular docking simulations were performed using the high-throughput LibDock protocol, following the HEX docking method. This method evaluates the geometric complementarity of candidate structures to polar and apolar interaction hotspots within the predefined receptor binding site. After docking, the relative energetic favorability of the resulting complexes was assessed using the Generalized Born with a Simple Switching (GBSW) implicit solvent model. We selected the highest-ranked complexes for further analysis based on geometric complementarity and predicted binding energetics. The selected docking poses were visualized to characterize interactions between the target proteins and their binding partners.

2.10 Statistical analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software, version 16.2). Data are presented as specified in each figure legend. Two-group comparisons were assessed using an unpaired two-sided Student's t-test, and one- or two-way ANOVA was used as appropriate, followed by Tukey's multiple-comparisons test. Data are presented as mean ± SD. Statistical significance was defined as (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

3. RESULTS

3.1. YBX1 and SREBP2 are overexpressed in HCC

To assess YBX1 and SREBP2 expression in HCC, we analyzed tissue microarrays, cell models, and publicly available datasets. Immunohistochemical (IHC) staining of tissue microarrays (LV8011a and b), along with mRNA and immunoblot analyses in HCC cell lines, showed expression of both proteins (Fig. 1A-F). These results are consistent with transcriptional profiling of the Cancer Genome Atlas (TCGA) HCC dataset; YBX1 and SREBP2 expression was markedly higher in HCC than in corresponding normal tissues (Fig. 1G). This pattern held for both YBX1 and SREBP2 protein expression and across tumor grades (Fig. 1H and 1I). Kaplan -Meier analysis showed poorer survival in the high YBX1 expression group than in the low- or medium-expression group, suggesting that YBX1 is a central factor in HCC (Fig. 1J). SREBP2 expression was higher at the transcriptional level, suggesting that elevated transcriptional activity, along with YBX1, might be involved in intracellular cholesterol synthesis. YBX1 and SREBP2 expression were elevated at both the mRNA and protein levels compared with normal liver cancer cells, and the TCGA correlation database also showed that YBX1 expression positively correlated with SREBP2 expression (R = 0.26), supporting coexpression of YBX1 and SREBP2 in HCC.

Furthermore, in a cohort of clinical samples (n = 42), YBX1 and SREBP2 expression increased with HCC progression (Fig. 2A). The accompanying schematic summarizes the progression from healthy liver through MASLD, MASH, cirrhosis, and HCC (Fig. 2B). Zhang Q et al. (2025) demonstrated that YBX1 is progressively elevated during the development of MASLD and associated liver diseases [26]. Our clinical data also showed that YBX1 and SREBP2 are sequentially upregulated. Subsequent computational and correlation analyses of publicly available transcriptomic data on YBX1 and SREBP2 mRNA expression in human HCC samples revealed a potential interaction between these two proteins (Fig. 2C and D). Collectively, these findings suggest a coordinated activation of YBX1 and SREBP2 during HCC progression, supporting a functional interaction between these regulators.

3.2. YBX1 overexpression increases SREBP2 signaling and cholesterol biosynthesis

YBX1 has been implicated in tumor progression and drug resistance through its transcriptional activity [3, 5, 16, 29]. YBX1 is a key HCC biomarker in the TCGA cohort presented. Liu Xin et al. (2025) showed

that YBX1 binds to the SREBP2 promoter and regulates cholesterol biosynthesis in HCC [22]. We therefore examined whether changes in YBX1 expression were associated with SREBP2 expression and cholesterol-related phenotypes. We observed that YBX1 overexpression was accompanied by increased expression of the resistance-associated marker genes MDR1, CD44, and PD-L1 (Fig. 3A-F). Immunoblotting showed increased SREBP2 protein expression (Fig. 3G). Further, RT-qPCR confirmed that YBX1 positively regulates SREBP2 and the cholesterol regulatory genes HMGCR, SERPIN1, and LDLR (Fig. 3H-K). These results indicate that YBX1 enhances expression of resistance-associated markers while activating SREBP2-mediated cholesterol-biosynthesis pathways in HCC cells. We also analyzed lipid droplets in YBX1-overexpressing cells and found a significant increase in lipid droplets in YBX1-overexpressing cell lines (Fig. 3L-M). We further evaluated resistance using sorafenib IC_{50} values measured by a cytotoxicity assay (MTT). YBX1 overexpression increased IC_{50} by nearly 40% (Fig. 3N and O). Collectively, these results show that YBX1 contributes to SREBP2 regulation and drug resistance by increasing resistance markers and requiring higher drug concentrations for inhibition.

3.3. YBX1 Enhances Cholesterol Biosynthesis and Lipid Accumulation in HCC cells

In the studies above, YBX1 expression was associated with increased lipid droplet content, as assessed by the LipidSpot assay, and with cholesterol levels (Fig. 3L&M). These positive correlations align with Western blot and quantitative PCR results, indicating increased SREBP2 in YBX1-overexpressing cells. These findings support a mechanistic role for YBX1 in regulating lipid accumulation by modulating SREBP2 signaling. To determine whether YBX1 drives lipid accumulation, we used YBX1-overexpressing cell lines treated with palmitic acid (PA). We incubated YBX1-overexpressing cells with PA at various concentrations. Oil Red O staining showed lipid accumulation, and PA-treated YBX1-overexpressing cells showed increased lipids, confirming that YBX1 is involved in lipid synthesis (Fig. 4A and 4B). The lipid droplet assay showed increased lipid droplet formation (Fig. 4C and 4D). Furthermore, PA treatment increased YBX1 and SREBP2 levels in YBX1-overexpressing cells at the mRNA level. Collectively, these results show that YBX1 expression positively correlates with increased lipid droplets and cholesterol levels in HCC cells. These findings are consistent with an association between YBX1 expression and lipid accumulation.

3.4. YBX1 Silencing Suppresses Cholesterol Biosynthesis and Lipid Droplet Formation

To evaluate YBX1 depletion, we transfected SK-Hep1 cells with YBX1-targeting siRNA and assessed knockdown by immunoblotting and qRT-PCR (Fig. 5A and 5B). Additional plasmid information, along with YBX1 overexpression and knockdown cell lines, is provided in Supplementary Figs. S1 and S2 (developed in our laboratory [30]). YBX1 knockdown was associated with reduced expression of resistance-associated genes (Fig. 5C-E). Furthermore, YBX1 knockdown markedly reduced SREBP2 and its downstream targets, HMGSR and SERPIN1 (Fig. 5F-J). We also tested whether YBX1 knockdown reduces cholesterol droplets. The lipid droplet assay showed decreased lipid droplet formation and

reduced cholesterol levels (Fig. 5K and 5L). We also performed phenotypic assays; YBX1 knockdown decreased HCC phenotypic characteristics (Fig. 5M). YBX1 knockdown decreased the sorafenib IC50 compared with scramble (Fig. 5N and 5O). Taken together, our data show that YBX1 is a positive transcriptional regulator of SREBP2 and that it regulates cholesterol metabolism and resistance.

3.5. YBX1 regulates SREBP2 and Lipid droplet formation

YBX1 is a DNA/RNA-binding protein implicated in transcription, RNA processing, RNA stability, pre-mRNA splicing, regulation, and cancer-associated stress response. Our findings support a potential link between YBX1 and SREBP2-associated cholesterol regulation, indicating that YBX1 influences the cholesterol-regulatory gene SREBP2 and leads to intracellular cholesterol accumulation. This study shows that changes in YBX1 expression are associated with SREBP2 expression and cholesterol-related phenotypes. Treatment with the YBX1 inhibitor SU056 reduces YBX1 levels, and subsequent SREBP2 inhibition reduces cholesterol droplets; the combination of sorafenib and the YBX1 inhibitor sensitizes the HCC cell lines (Fig. 6A and 6B). Further, the SREBP2 inhibitor betulin sensitizes the HCC cell lines (Fig. S5). The cell migration assay shows that SU056 and betulin efficiently inhibit HCC cell migration (Fig. 6J and 6K). A schematic representation of YBX1 and SREBP2 inhibitors in cholesterol biosynthesis is shown in Fig. 6L. The pronounced overexpression of YBX1 and SREBP2 in HCC, along with their contribution to intracellular cholesterol reprogramming and the subsequent increase in resistance markers, establishes this axis as an attractive therapeutic target. This supports the YBX1/SREBP2 axis as a promising therapeutic target in HCC.

3.6 Resistant cells exhibited elevated expression of YBX1 and SREBP2, along with an upregulation of cholesterol

To investigate acquired resistance, we established sorafenib-resistant HCC cell models (Fig. 7M-7N and S3). Resistant HepG2 luc and Huh7 luc cells showed increased YBX1, SREBP2, and the resistance-associated markers (Fig. 7A-7J). Consistent with this, we observed further increases in lipid droplets and cholesterol levels in HepG2 luc-resistant cells, indicating that lipid accumulation contributes to intracellular lipid levels and resistance (Fig. 7K and 7L). Increased cholesterol levels in drug-resistant cells also showed that higher YBX1 and SREBP2 levels influence intracellular cholesterol accumulation. We assessed the successful establishment of sorafenib-resistant cells using a sorafenib cytotoxicity assay, in which resistant cells showed increased sorafenib IC_{50} from 10.83 μM to 22.82 μM at 24 h and from 4.47 μM to 15.14 μM at 48 h (Fig. 7M & N). Western blot and RT-qPCR analyses confirmed that YBX1 was upregulated in resistant cells and, subsequently, in SREBP2- and drug-resistant cells. These results support that the resistant cells showed higher YBX1 and SREBP2-related cholesterol associated with resistance.

3.7 Inhibition of YBX1 sensitizes to sorafenib resistance

We next examined whether inhibiting YBX1 or SREBP2 altered phenotypes and sorafenib response in parental and resistant cells. Previous sections show that YBX1 drives drug resistance, increases resistance markers, and reprograms cholesterol metabolism. LipidSpot staining after sorafenib, SU056,

and betulin treatment is shown in Fig. 8A-8D. Interestingly, the YBX1 inhibitor SU056 produced fewer lipid droplets than sorafenib or the control. The positive-control SREBP2 inhibitor betulin reduced lipid droplet formation. In addition to the cholesterol assay, results clearly showed that resistant cells had reduced cholesterol compared with sorafenib treatment (Fig. 8E). Furthermore, cell proliferation and cytotoxicity assays showed decreased IC_{50} values with the combination of sorafenib and SU056 or sorafenib and betulin (Fig. 8F-G). Inhibition of YBX1 sensitizes HCC cells, as shown in HCC-resistant cells. These results indicate that, in combination with sorafenib, YBX1 inhibitors or SREBP2 inhibitors help improve sorafenib sensitivity and increase HCC survival. Collectively, these data suggest that inhibition of YBX1 or SREBP2 could reduce cholesterol accumulation and may enhance drug sensitivity in HCC cells.

4 DISCUSSION

Acquired resistance to systemic therapy remains a significant challenge in advanced HCC. In this study, we investigated the relationship between YBX1, SREBP2-associated cholesterol regulation, and response to sorafenib. Findings from clinical datasets and HCC cell models support an association among YBX1 expression, cholesterol-related phenotypes, and drug-resistance traits. Therapeutic resistance remains a principal barrier to durable clinical responses in advanced malignancies, including HCC. Improving therapeutic outcomes in advanced HCC remains a major unmet clinical need because treatment resistance frequently emerges. Both intrinsic and acquired resistance contribute to treatment failure in HCC; however, acquired resistance frequently emerges during prolonged systemic therapy. Resistance to systemic sorafenib therapy develops early in HCC, and to maintain sorafenib's efficacy, we need to examine combination therapies for initial or acquired resistance. Sterol regulatory element-binding proteins (SREBPs) are key transcription factors that regulate genes involved in lipid synthesis and play a central role in lipid metabolism under both physiological and pathological conditions. Previous studies have emphasized YBX1's crucial role in cancer progression and drug resistance [5, 9]. Our study builds on these findings by showing that YBX1 expression promotes drug resistance through resistance markers and reprogramming of cholesterol metabolism.

A recent study demonstrated that YBX1 binds to the SREBP2 promoter involved in cholesterol biosynthesis in HCC [27]. Higher expression of YBX1 was validated in MASLD patients using data from GEO datasets (GSE193084 and GSE135251) [26]. Our clinical samples showed that YBX1 and SREBP2 expression were positively correlated with liver disease progression and with HCC. Additionally, YBX1 overexpression increased SREBP2 expression and cholesterol levels, whereas YBX1 knockdown decreased both, indicating a direct association between YBX1 and SREBP2. Tyrosine kinase inhibitors (TKIs), including sorafenib and lenvatinib, are recommended as first-line treatments for HCC [30], [31]. Combination therapies that include immunotherapy and anti-angiogenic agents are increasingly favored as the preferred treatment for unresectable HCC; TKI drugs remain the primary option for those who cannot receive immunotherapy. Therefore, overcoming TKI resistance is clinically important. Although sorafenib is a first-line treatment for advanced HCC, long-term exposure can lead to adaptive resistance by increasing resistance genes and YBX1 expression. Sorafenib suppresses multiple oncogenic signaling pathways; however, prolonged treatment often results in adaptive resistance mechanisms that

limit durable responses. Novel combination strategies, including sorafenib with cytotoxic chemotherapeutic agents, help overcome sorafenib resistance in HCC.

Recent studies have shown that YBX1 is highly upregulated in HCC and may contribute to resistance. In the present study, docking analysis was used to investigate interactions between the structures represented by PDB IDs 8UWC and 6KUG. The analysis identified a potential hydrogen-bonding interaction between Tyrosine 99 in the SREBP2 structure and Serine 102 in the YBX1 structure, with a reported distance of 2.72 Å between the relevant functional groups. Analysis of TCGA-LIHC datasets revealed that both YBX1 and SREBP2 are significantly upregulated across tumor stages. Additionally, we observed a positive correlation between YBX1 and SREBP2 expression, suggesting coordinated activation of the cholesterol biosynthesis pathway during HCC progression (Fig. 2A and S4). Given SREBP2's central role in cholesterol biosynthesis, the positive expression association between YBX1 and SREBP2 supports a functional link between YBX1 and cholesterol metabolic regulation in HCC. Together with our loss-of-function data, these findings suggest that YBX1 may promote hepatocarcinogenesis by sustaining SREBP2-driven cholesterol biosynthesis programs.

Previous reports indicate that the transcription factor YBX1 is a key contributor to TKI resistance [32], [33]. However, TKI drugs such as sorafenib are unable to completely suppress the activation of YBX1. These observations suggest that YBX1 activity may be maintained or enhanced during sustained therapeutic stress. Recent studies show that YBX1 regulates lipid metabolism [34], [26]. Our findings identify the YBX1-SREBP2 axis as a potential regulator of cholesterol biosynthesis and therapeutic adaptation in HCC. Overexpression of YBX1 can simultaneously increase SREBP2 and resistance-associated genes PD-L1, MDR1, and CD44. These findings support further investigation of the relation between YBX1-associated cholesterol regulation and drug response in HCC. Our findings identify YBX1 as a positive regulator of SREBP2 signaling and cholesterol biosynthesis in HCC. However, the precise mechanism by which YBX1 enhances SREBP2 activity remains to be fully elucidated. Whether YBX1 directly regulates SREBP2 transcription, influences its post-transcriptional processing, or modulates upstream signaling pathways involved in SREBP2 activation warrants further investigation. Future studies exploring these possibilities will provide deeper insight into the YBX1-SREBP2 regulatory axis and its contribution to HCC progression and therapeutic resistance. Targeting the cholesterol metabolic pathway restores drug sensitivity and offers a promising therapeutic approach to overcome treatment resistance, with potential applications as an anticancer strategy in HCC.

5 CONCLUSION

Drug resistance represents a significant obstacle to effective anticancer therapy. Our findings support an association between YBX1/SREBP2-associated cholesterol regulation and sorafenib response in HCC cell models. Genetic or pharmacological perturbation of this pathway altered lipid-associated phenotypes and drug response under the conditions tested. Inhibition of YBX1 or SREBP2 decreases cholesterol levels and mitigates sorafenib resistance in HCC.

Collectively, our findings identify YBX1 as a critical regulator of SREBP2-dependent cholesterol biosynthesis that contributes to drug resistance in HCC. Given its crucial role in cancer progression and therapeutic resistance, YBX1, which increases cholesterol levels through SREBP2 activation, is a promising therapeutic target to overcome resistance. Further mechanistic validation and in vivo studies are required to determine whether targeting this axis can improve therapeutic outcomes. The data support continued investigation of YBX1 and SREBP2 as candidate targets for strategies to overcome HCC drug resistance.

Declarations

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

GENERATIVE AI DECLARATION

The authors state that they did not use generative AI to create this manuscript.

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Author Contribution

AUTHOR CONTRIBUTIONS Conceptualization, V.N. and M.T.; methodology, V.N. and M.T.; software, V.N., S.T. and M.S.; validation, V.N., M.S., and M.T.; formal analysis. V.N. and M.T.; investigation. V.N. and M.T.; resources, M.T.; data curation. V.N. and M.S., writing original draft preparation; V.N., writing review and editing; V.N., M.S., S.T. and M.T., visualization; V.N. and M.T., supervision; M.T., project administration; M.T., funding acquisition, M.T.; All authors have read and agreed to the published version of the manuscript.

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Figures

Figure 1. YBX1 and SREBP2 expression in HCC and their association with clinical outcomes.

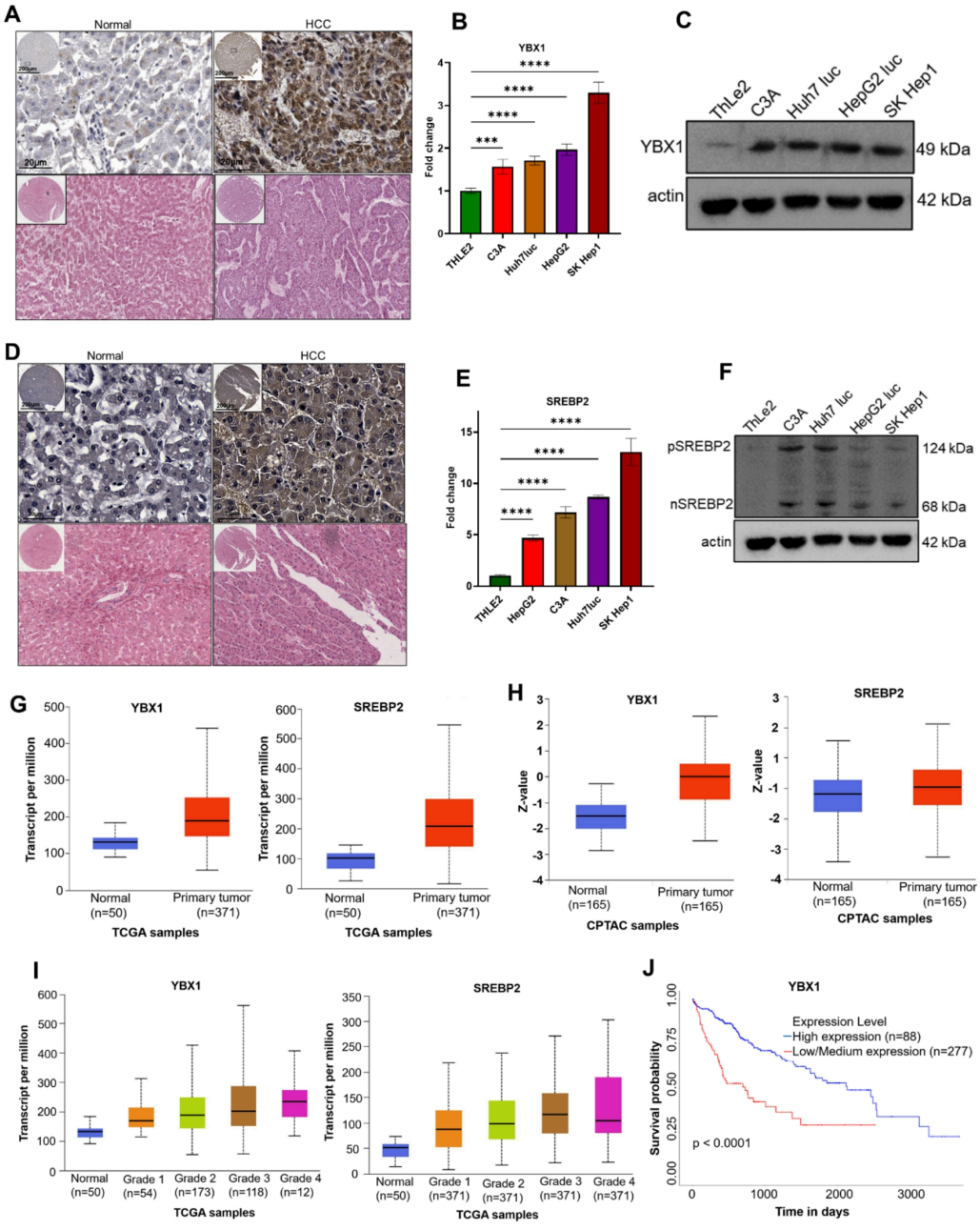

Figure 1

See image above for figure legend.

Figure 2. YBX1 and SREBP2 Exhibit Sequential Activation During Liver Disease Progression and Positively Correlate in Human HCC.

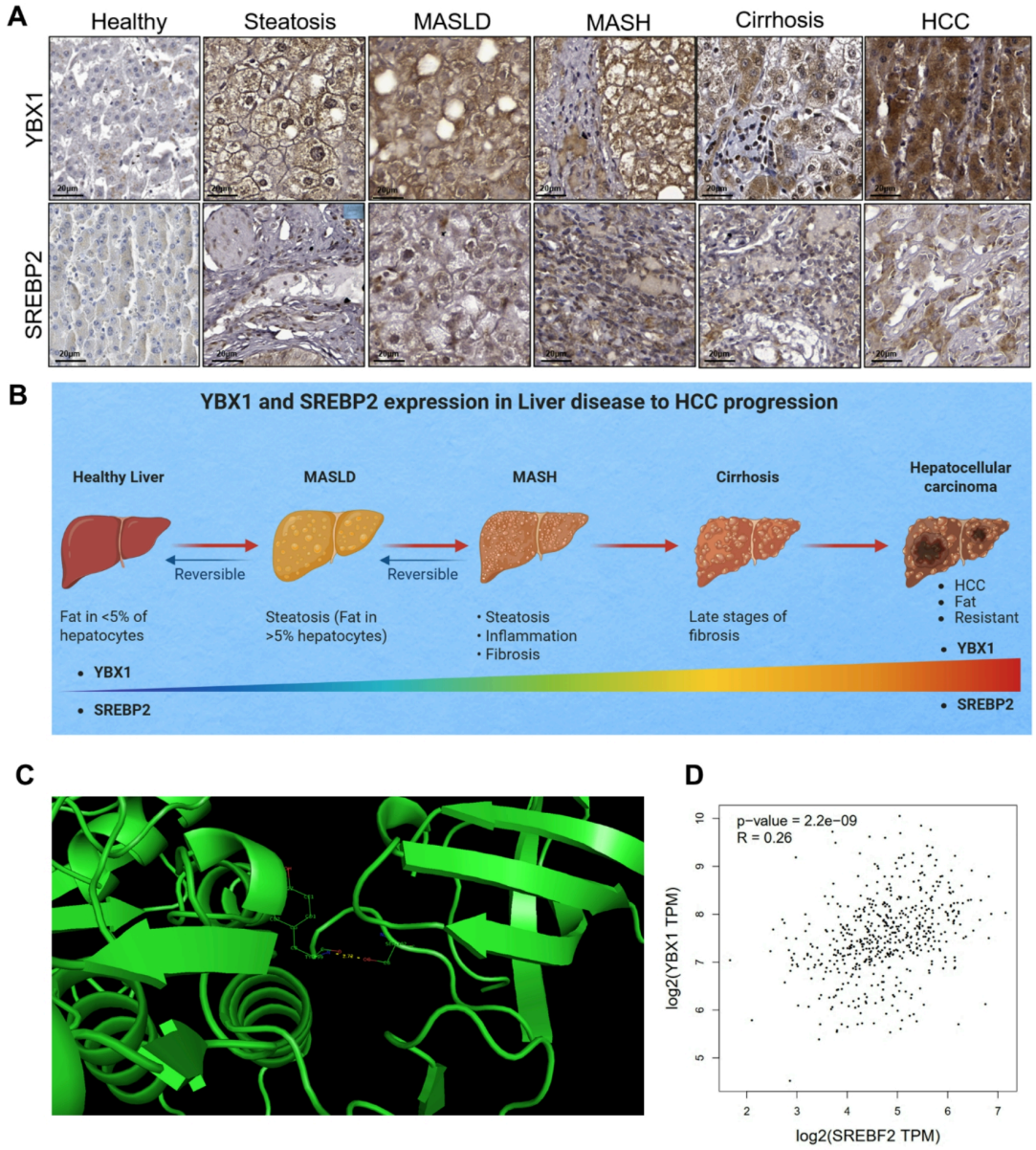

Figure 2

See image above for figure legend.

Figure 3. YBX1 overexpression increases SREBP2 signaling and cholesterol biosynthesis

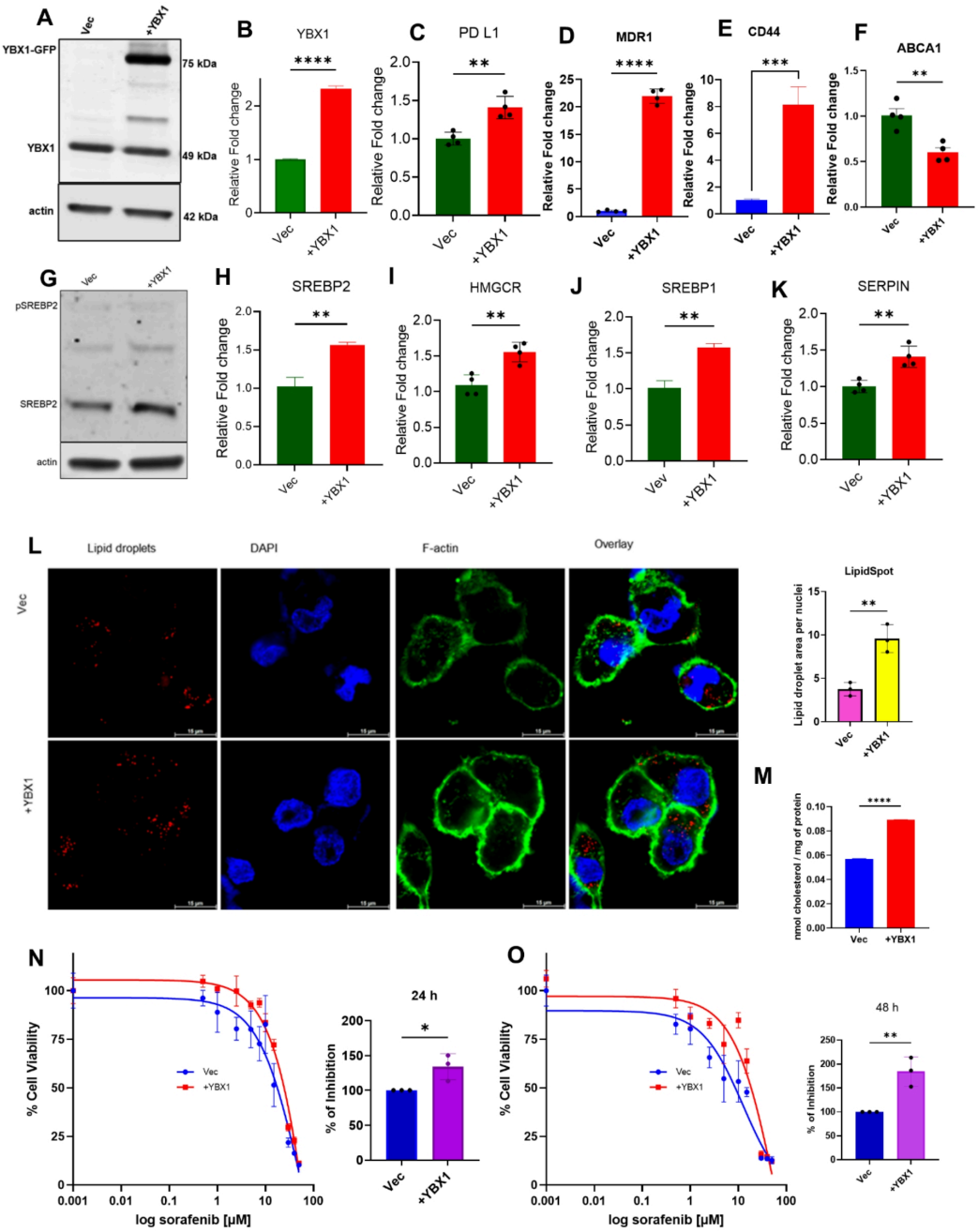

Figure 3

See image above for figure legend.

Figure 4. Overexpression of YBX1 increases the lipid droplets

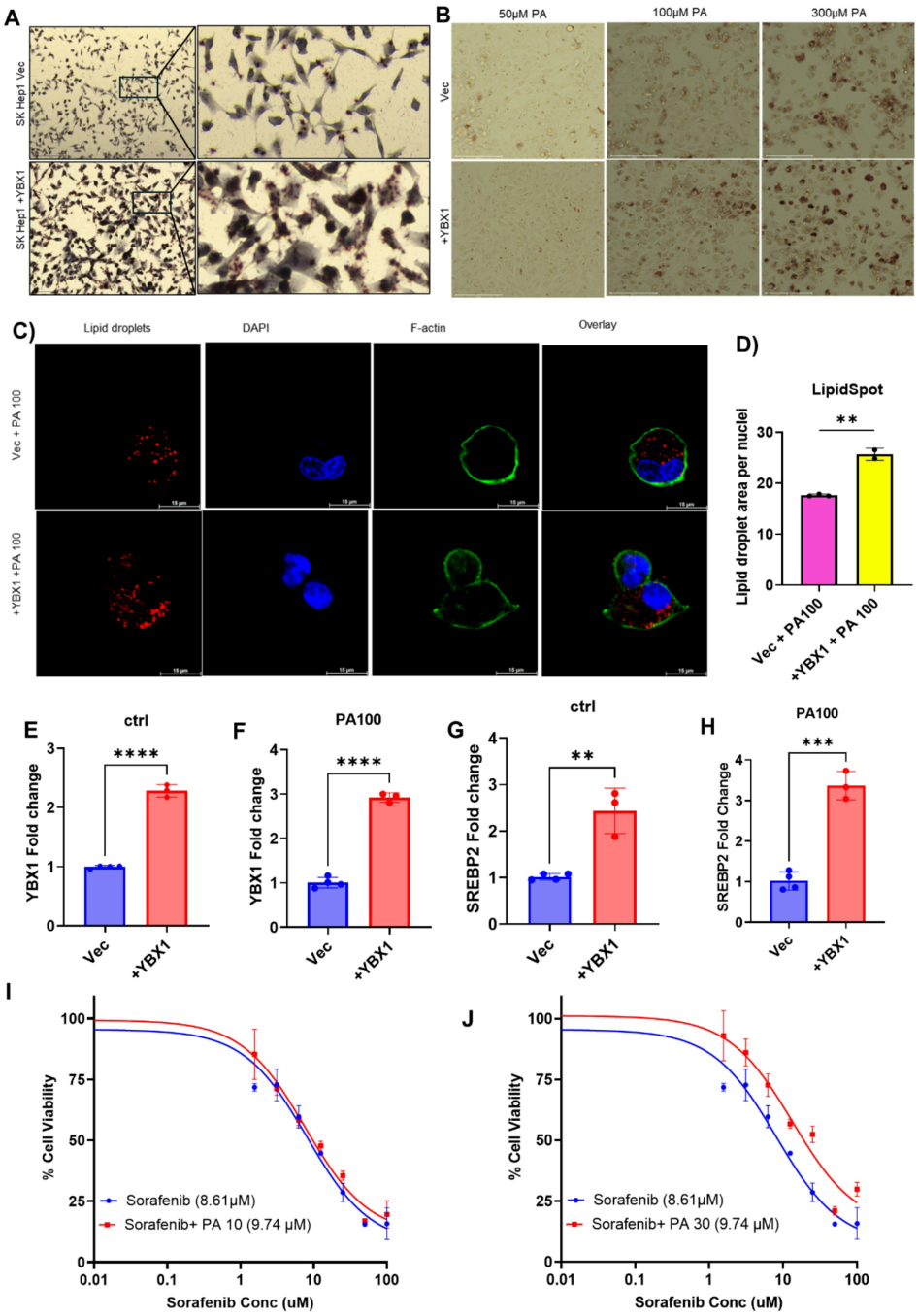

Figure 4

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Figure 5. YBX1 silencing suppresses cholesterol biosynthesis and attenuates malignant phenotypes in hepatocellular carcinoma cells.

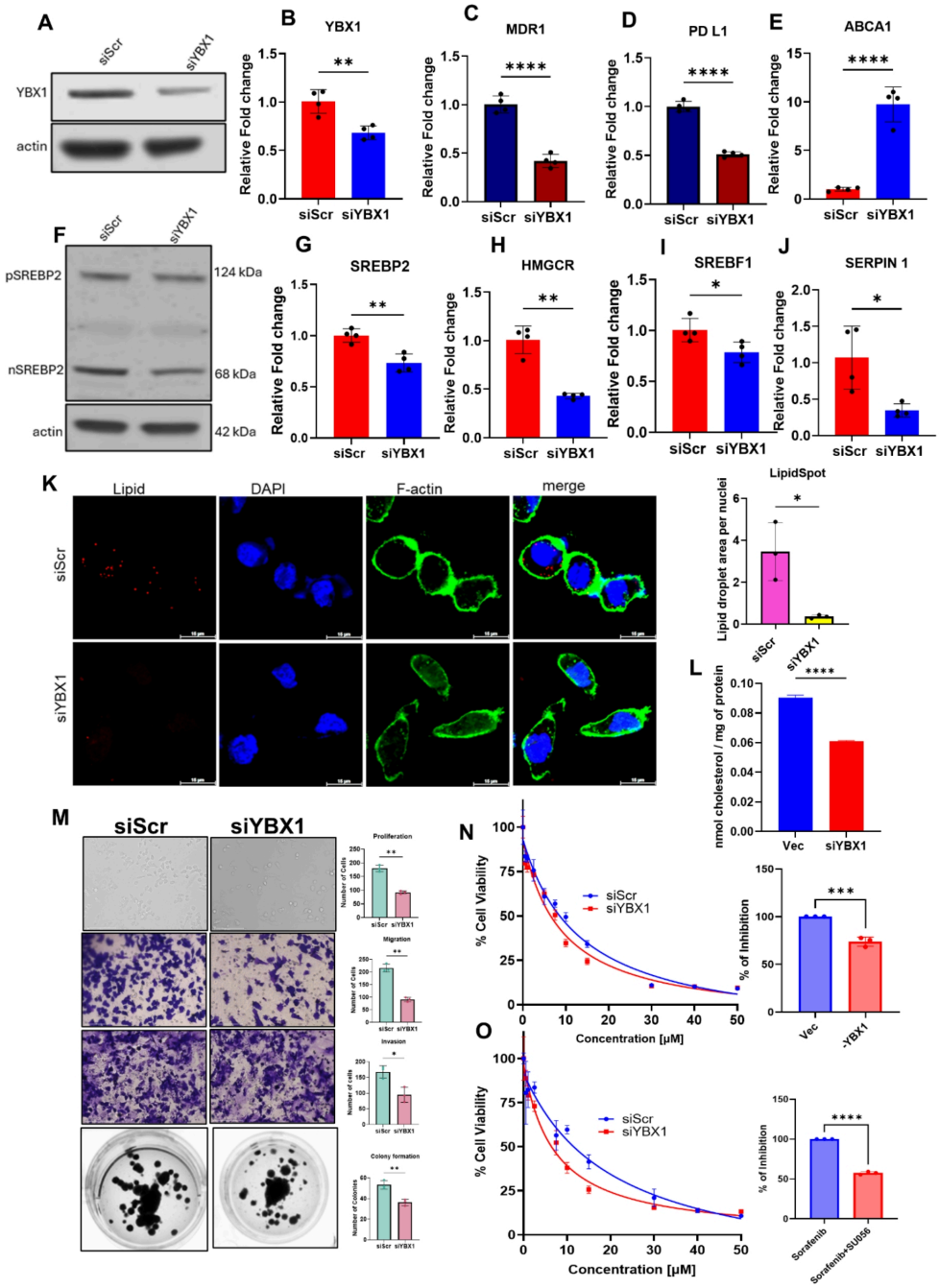

Figure 5

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Figure 6. Inhibition of the YBX1–SREBP2 axis suppresses cholesterol metabolism and overcomes therapeutic resistance in HCC cells.

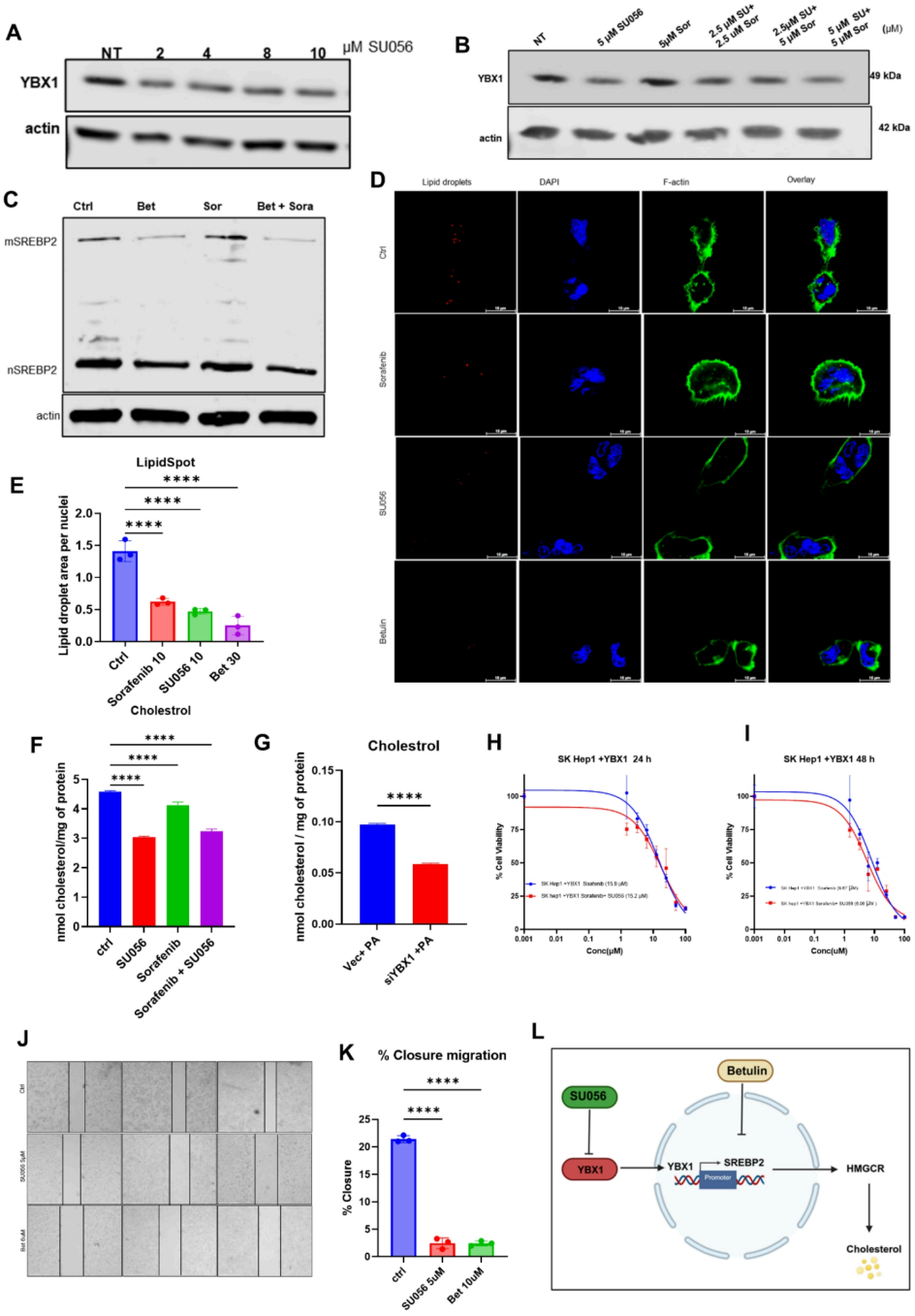

Figure 6

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Figure 7. Upregulation of the YBX1/SREBP2 pathway in resistant HCC cells.

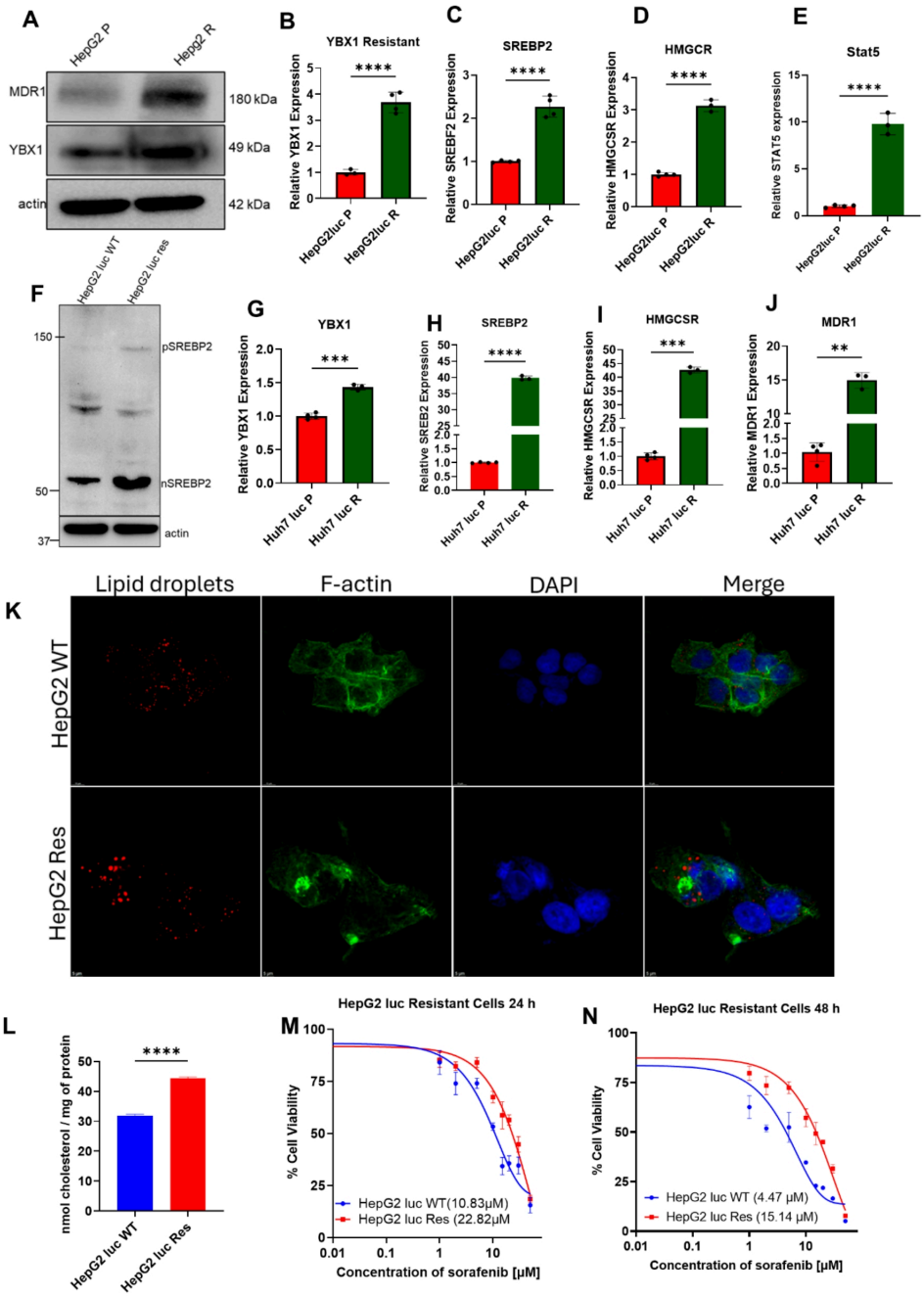

Figure 7

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Figure 8: Pharmacological inhibition of YBX1 or SREBP2 restores drug sensitivity in Resistant HCC cells.

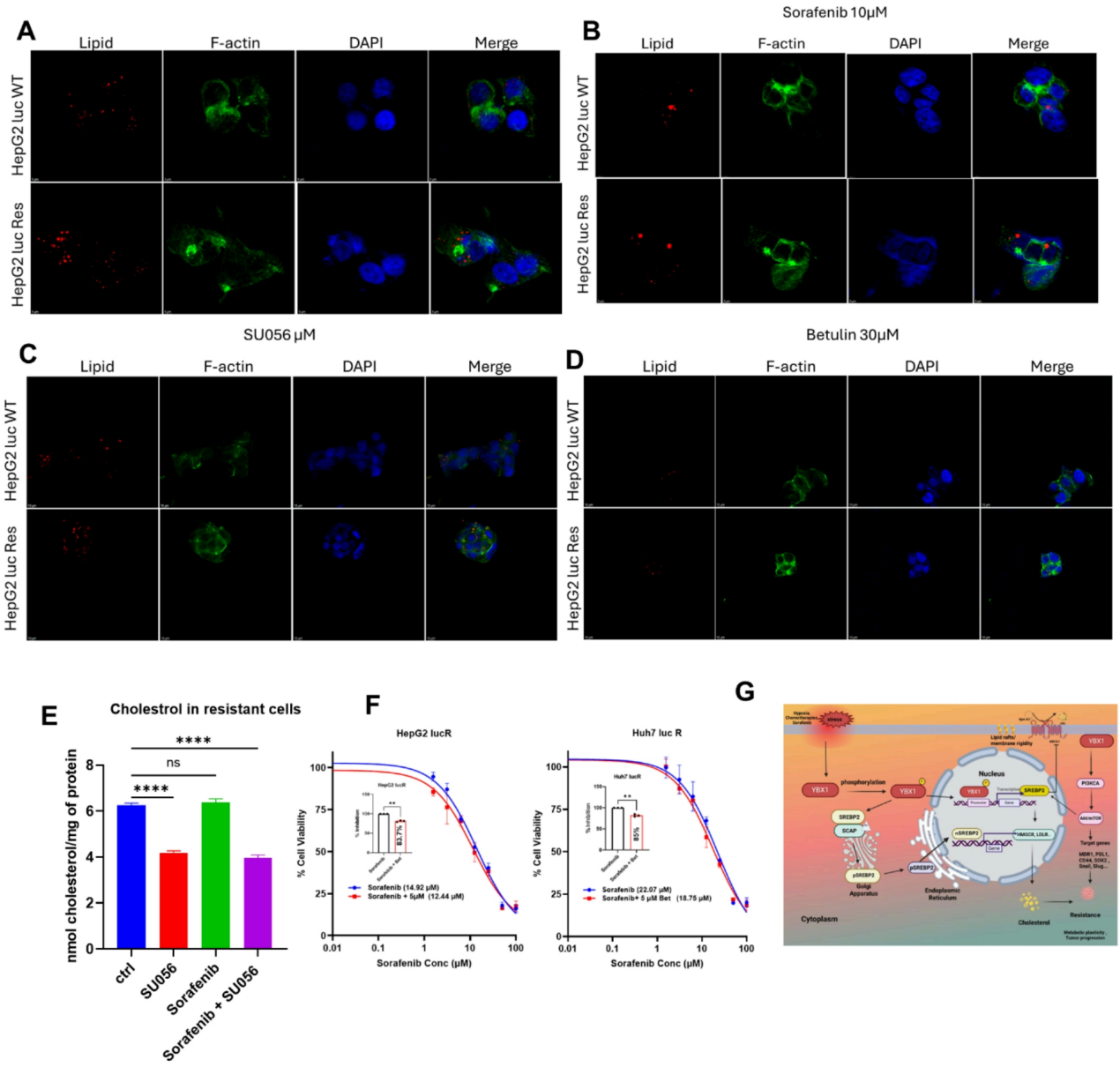

Figure 8

See image above for figure legend.

Supplementary Files

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