UTU Publishing Group

Bulletin of Biomedical Science

Journal Homepage: www.utu.com.sg

REVIEW

From Gene Identification to Drug Discovery: Unleashing the Power of Multum in Parvo

Jun Cheng[1,2], Song Yang[1]

[1]Liver Diseases Center, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China
[2]Hebei UTU Pharma Co. Ltd., Shijiazhuang, Hebei Province 052165, China

ARTICLE INFO

Received 15 May 2023
Accepted 12 July 2023
Online 01 August 2023

KEY WORDS:
HBV;
HCV;
New gene;
HBV cccDNA;
liver steatosis;
liver fibrosis/cirrhosis;
hepatocellular carcinoma;
drug;
therapeutic target;
database;
big data

Abstract

Understanding the intricate mechanisms and molecular landscape of hepatitis B virus (HBV) and hepatitis C virus (HCV) interactions with hepatocytes is crucial for elucidating their pathogenesis and identifying potential therapeutic interventions. In this study, we employed a range of advanced techniques, including yeast-two hybrid (Y2H), yeast-one hybrid (Y1H), phage display, suppression subtractive hybridization (SSH), and cDNA microarray, to comprehensively analyze the mechanisms and molecules involved in HBV/HCV-hepatocyte interactions. Each protein of HBV and HCV was utilized to screen a liver cDNA library, leading to the identification of 127 novel genes associated with HBV/HCV, which were meticulously cataloged and submitted to the GenBank with new names and accession numbers.

The investigation of the biological functions, regulatory mechanisms, and their correlation with liver diseases, such as HBV covalently closed circular (ccc) DNA formation, liver steatosis, liver fibrosis/cirrhosis, and hepatocellular carcinoma (HCC), required extensive time and effort. Within this extensive analysis, we successfully identified several genes as potential therapeutic targets for liver diseases.

Moreover, we employed database and big data techniques to explore small molecular chemical compounds. Through a structure-activity relationship (SAR) strategy, we identified and patented a repertoire of compounds with potential for therapeutic intervention. These compounds underwent rigorous evaluation, encompassing pharmacokinetics/pharmacodynamics (PK/PD) kinetics, tissue and blood half-life (T1/2), maximum toxic dosage (MTD) in rats, and efficacy assessments in cell and mouse models. The findings from these evaluations provide a strong foundation for the future development of innovative drugs targeting liver diseases. Notably, several promising pre-clinical compounds (PCC) have been identified, showcasing their potential for advancement in the drug development process.

In conclusion, our comprehensive study sheds light on 127 novel genes associated with HBV/HCV and reveals their significance in liver diseases. The identification of these genes, coupled with the exploration of small molecular chemical compounds, opens new avenues for developing innovative therapeutic strategies against liver diseases. This study contributes valuable insights into the field and sets the stage for further advancements in the treatment of HBV/HCV-related liver diseases.

Corresponding author: Song Yang, Liver Diseases Center, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China. sduyangsong@163.com

Introduction

In December 1997, culminating a rigorous 3-year postdoctoral training at the esteemed University of Texas Health Science Center at San Antonio (UTHSCSA), Texas, USA, Dr. Cheng returned to the 302 Hospital of PLA with a fervent passion for hepatology. The nascent phase of my independent research journey presented formidable challenges, encompassing not only a paucity of financial support but also the scarcity of time and qualified team members. The dual role of a clinical doctor engrossed in daily clinical practice rendered it arduous to dedicate ample time to laboratory research. Nevertheless, an imperative to chart a well-conceived research trajectory for effectively training master's and Ph.D. candidates as their mentor spurred his unwavering determination.

During this epoch, chronic hepatitis B (CHB) and chronic hepatitis C (CHC) prevailed as the predominant ailments observed in clinical practice, substantiated by a substantial body of literature (Cheng et al., 1991; Cheng et al., 1999; Cheng et al., 2000; Cheng et al., 2001; Dong et al., 2001; Zhao et al., 2001; Cheng et al., 2002; Deng et al., 2002; Dong et al., 2002; Zeng et al., 2005; Wang et al., 2007; Chainovati et al., 2009; Liu et al., 2009; Ahn et al., 2010; Yan et al., 2011; Xie et al., 2012; Sun et al., 2014; Sun et al., 2014; Yang et al., 2014; Ju et al., 2015; Liang et al., 2015; Zhang et al., 2015; Chen et al., 2016; Fan et al., 2016; Hou et al., 2016; Li et al., 2016; Sun et al., 2016; Sun et al., 2016; Yang et al., 2016; Hou et al., 2017; Li et al., 2018; Liang et al., 2018; Wang et al., 2018; Yang et al., 2018; Zhang et al., 2018; Liang et al., 2019; Liang et al., 2019; Shan et al., 2019; Chen et al., 2021; Fan et al., 2021; Li et al., 2021; Cai et al., 2023). Conceiving a novel direction of exploration, we strategically focused on comprehending the molecular mechanisms governing HBV/HCV-hepatocyte interactions. A comprehensive analysis involving the identification and systematic characterization of 127 new genes was diligently pursued over the course of the last three decades. Through relentless endeavors, the structural and functional attributes, expression and regulation profiles, as well as the biological and medical significance of these genes were elucidated.

In consequence, several promising therapeutic targets have been unearthed, while the identification of numerous chemical compounds has further expanded our potential repertoire. Preliminary investigations into the characteristics, efficacy, and safety profiles of these compounds now propel us towards the cusp of pre-IND (Investigational New Drug) phase initiation. With a clear focus on combating HBV cccDNA formation, liver steatosis, liver fibrosis/cirrhosis, and hepatocellular carcinoma (HCC), our research endeavors are poised to deliver innovative therapeutic interventions to address these pressing hepatic pathologies. Through a comprehensive understanding of the molecular mechanisms underlying HBV/HCV infection and the identification of novel genes and therapeutic targets, we aim to revolutionize the management and treatment of these liver diseases.

Identification of HBV/HCV-related genes

Viruses, the smallest entities in the realm of biology, wield substantial influence over human health. Among them, hepatitis B virus (HBV) and hepatitis C virus (HCV) emerge as major pathogens implicated in a spectrum of liver diseases, including acute viral hepatitis (AVH), chronic viral hepatitis (CVH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC). Notably, HBV and HCV exhibit a restricted host range, exclusively infecting humans and chimpanzees while sparing monkeys, canines, and mice. While these viruses demonstrate a capacity to infect various tissues and organs, it is the liver that bears the brunt of their devastating impact. Consequently, the hepatic molecular milieu provides an ideal environment for HBV/HCV replication and expression. Yet, the precise repertoire of proteins and RNAs involved in the HBV/HCV life cycle remains largely elusive. Host factors that dictate the HBV/HCV life cycle hold the key to potential therapeutic targets for combatting HBV/HCV infection and related liver diseases.

Unraveling the complex interplay between HBV/HCV and their host necessitates the identification of DNA/RNA-binding proteins associated with these viruses. HBV/HCV proteins are known to interact with host proteins, exerting profound effects on signal transduction pathways—a pivotal and life cycle-dependent step in the pathogenesis of HBV/HCV infection. Notably, viral proteins exert transactivation effects on human gene expression. The yeast-two hybrid technique has proven efficacious in identifying binding proteins for known proteins (Cheng et al., 2002; Lu et al., 2002; Cheng et al., 2003; Bai et al., 2005; Huang et al., 2005; Huang et al., 2005; Lu et al., 2005; Yang et al., 2005; Lin et al., 2006; Zhang et al., 2006; Li et al., 2014). Similarly, the yeast-one hybrid technique enables the identification of DNA-binding proteins, exemplified by the discovery of HBV S promoter I DNA-binding protein 1 (SBP1) (Zhong et al., 2002; Zhong et al., 2011). Moreover, employing a biotinylated single primer amplification strategy facilitates the preparation of copious amounts of potential promoter DNA fragments, which can subsequently be screened using the phage display technique in a cDNA library, employing an avidin-coated plate for immobilization through biotin-avidin complex formation (Cheng et al., 2000). By transfecting the hepatoblastoma cell line HepG2 with plasmid DNA expressing diverse HBV/HCV DNA/cDNA fragments, differentially expressed genes can be discerned via RNAseq and suppressive subtraction hybridization (SSH) methods (Qu et al., 2007; Yu et al., 2018). Through these comprehensive methodologies,

host factors linked to HBV/HCV infection were systematically screened using cDNA libraries derived from liver tissue and various human and mouse tissues. Subsequent cloning and sequencing efforts revealed that most of the identified candidates corresponded to known genes cataloged in the GenBank database. Nonetheless, our exhaustive screening efforts have yielded an impressive collection of 127 new genes (Table 1). Each newly discovered gene has been meticulously

Table 1. Data list for 127 new genes

S/N	Names	Other names	Accession No.	Amino Acid	Chromosome location
001	NS5ATP1	HIBADH	AF529362	336	7p15.2
002	NS5ATP2	HDDC2 C6orf74 CGI-130	AF529363	204	6q13-q24.3
003	NS5ATP3	SLC35F5	AF529364	523	2q14.1
004	NS5ATP4	C1orf43 NICE-3 S863-3 HSPC012	AF529365	253	1q21.2
005	NS5ATP5	TFB2M mtTFB2 hTFB2M Hkp1	AF529366	396	1q44
006	NS5ATP6	FAM174A TMEM157	AF529367	190	5q21.1
007	NS5ATP7	MEMO1 C2orf4	AF529368	297	2p22.3
008	NS5ATP8	BTBD1 C15orf1	AF529369	482	15q24
009	NS5ATP9	PAF15 KIAA0101 OEATC-1 PCLAF	AF529370	111	15q22.31
010	NS5ATP10	TMEM127	AK000514	238	2q11.2
011	NS5ATP11	SLC25A46	AK091427	418	5q22.1
012	DNAPTP1TPA	SWI5 C9orf119 SAE3	DQ414820	235	9q34.11
013	NS5ATP13	NOLC1 NOPP140 KIAA0035	AY820769	699	10q24.32
014	NS5BBP1	CYP2C8	BC020596	388	10q23.33
015	MBP1	CREBL2-SEP	DQ307498	66	12p13.1
016	XTP1	DEPDC1B XTP8 BRCC3	AF488828	380	5q12.1
017	XTP2	PRRC2C BAT2D1	AF488829	2702	1q24.3
018	XTP3	SARAF FOAP-7 TMEM66	AF490252	339	8p12
019	XTP4	MIEN1 ORB3 RDX12 C17orf37	AF490253	115	17q12
020	XTP5	PUM3 HA-8 KIAA0020 PUF6	AF490254	508	9p24.2
021	XTP6	DLEU1 Leu1	AF490255	78	13q14.2-q14.3
022	XTP7	EXOC3L2	AF490256	195	19q13.32
023	XTP8	DEPDC1B XTP1 BRCC3	AF490257	529	5q12.1

Table 1. Continued

S/N	Names	Other names	Accession No.	Amino Acid	Chromosome location
024	XTP9	FBRSL1 AUTS2L AF490258	AF490258	667	12q24.33
025	XTP10	ZC3H14 dNab2 MSUT2 MRT56	NM_001326303	401	14q31.3
026	X-30	ZNF230-SEP	AY280722	104	19q13.31
027	C1	GNAI2-SEP GIP	AY555145	121	3p21.31
028	C2	KIBRA WWC1 PPP1R168 KIAA0869 MEMRYQTL HBEBP3 HBEBP36	AF530058	912	5q34
029	HBCBP	HBCBP C12 GPR182-SEP	AF529371	196	12q13.3
030	E2BP1	ENTPD6-SEP	AY459290	61	20p11.21
031	E2BP2	PFKL-SEP	AF529373	146	21q22.3
032	E2BP3	SLC23A3 SVCT3 Yspl1	DQ294736	157	2q35
033	E2BP4	LIR5-SEP LILRB4-SEP	AF189768	73	19q13.42
034	EBP1	TMEM203-SEP	AF529372	123	9q34.3
035	EBP2	GLYCTK-SEP	AF529373	116	3p21.2
036	EBP3	KIBRA WWC1 PPP1R168 C2 HBEBP3 KIAA0869 MEMRYQTL	AF530058	912	5q34
037	EBP4	GLYCTK	AY134474	457	3p21.2
038	EBP19	GLTCTK-SEP	AF529373	116	3p21.2
039	EBP36	KIBRA WWC1 PPP1R168 C2 HBEBP3 KIAA0869 MEMRYQTL	AY189820	912	5q34
040	HCBP1	ACY3 AA3 ASPA	AF359506	280	11q13.2
041	HCBP6	FUNDC2 DC44 HCC3	AY032594	189	Xq28
042	HCBP12	RDH11 MDT1 CGI82 PSDR1 RALR1 SCALD ARSDR1	AF395068	331	14q24.1
043	HCTP4	TPX2 C20orf1 DIL-2 FLS353 HCA519 REPP86 p100	AY734680	747	20q11.21
044	NS3BP	LOC171391-SEP	AF435951	212	11p15.5

Table 1. Continued

S/N	Names	Other names	Accession No.	Amino Acid	Chromosome location
045	NS3TP1	ASNSD1	AY116969	643	2q32.2
046	NS3TP2	GRAMD3 GRAMD2B	AY116970	432	5q23.2
047	NS3TP6	KIAA1641 ANKRD36B	XM_017004595	137	2q11.2
048	PreS1BP	GLTSCR2 PICT1 p60 NOP53	AY535000	478	19q13.33
049	PS2BP1	MEGF8-SEP	AF497566	113	19q13.2
050	SBP1	IP3R3-SEP ITPR3-SEP	AY281252	92	6p21.31
051	TAHCCP1	SPG20 Spartin KIAA0610	AY038359	666	13q12.3
052	TAHCCP2	PKND1 MR1 PDC DYT8 FPD1 BRP17 FKSG19 KIPP1184 KIAA1184 MGC31943	AY039043	142	2q35
053	TTP1	COPRS COPR5 C17orf79 HSA272196	AF407672	184	17q11.2
054	HBXBP	ASGR2 CLEC4H2 HL-2	AF529374	306	17p
055	LRRP1	PGL CBT1 CWS3 PGL1 QPs3 SDH4 cybS CII-4	AY358788	159	11q23.1
056	PS1TP1	MXRA7 TMAP1	AY646229	213	17q25.1
057	PS1TP2	ADGRG6 DREG VIGR GPR126 APG1 LCCS9 STQTL1	AY426673	169	6q24.2
058	PS1TP3	CEP44 KIAA1712	AY426674	390	4q34.1
059	PS1TP4	SLC35F5-SEP NS5ATP3-SEP	AY427952	93	2q14.1
060	PS1TP5TP1	BRAP-SEP C6orf89-SEP	ABF61801	78	6p21.2
061	PS1TP6	NEAT1-SEP	AY444749	93	7p15.1
062	PS2TP1	LPGAT1 FAM34A KIAA9295 NET8	AY561706	370	1q32.3
063	PS2TP2	MRPP1 TRMT10C RG9MTD1 HNYA	AY561707	364	3q12.3

Table 1. Continued

S/N	Names	Other names	Accession No.	Amino Acid	Chromosome location
064	PS2TP3	NOP16 HSPC111 HSPC185	AY561704	178	5q35.2
065	PS2TP4	HINT1-SEP	AY561705	83	5q23.3
066	CSTP1	CPPED1	AY553877	314	16p13.12
067	PS1TP3BP1	PIK3CA-SEP	DQ910907	74	3q26.32
068	NS5ATP13TP1	MLLT6-SEP	AY459295	121	17q12
069	NS5ATP13TP2	OAF	AY459296	273	11q23.3
070	HBeAgTP	PPP2R2B-SEP	AY423624	107	10p15.1
071	PFAAP1	ARL6IP6 AIP6 AIP-6 MGC33864	AF530059	226	2q23.3
072	PFAAP2	NAA38 LSMD1	AF530060	125	17p13.1
073	PFAAP3	LPCAT1 AYTL2 AGPAT9 AGPAT10	AF530061	234	5p15.33
074	PFAAP4	PSMD6 S10 Rpn7 p42A p44S10 SGA-113M	AF530062	389	3p14.1
075	PFAAP5	N4BP2L2 CG005 CG016 92M18.3	AF530063	583	13q13.1
076	FBP2	PCNX4 C14orf135	AY553876	279	14q23.1
077	FBP1	RAB14	AY553875	215	9q33.2
078	NS5ATP5BP1	TBL1X-SEP	AY459291	79	Xp22.31-p22.2
079	HCTP4BP	CLDN9-SEP	AY390431	116	16p13.3
080	NS5ATP1BP16	CBX4 PC2 NBP16	AY390430	190	17q25.3
081	NS5ABP37	FNDC3B FAD104 PRO4979 YVTM2421 KIAA4164	AF543840	495	3q26.31
082	XTP3TPB	ERLEC1 CIM HEL117 C2orf30	AY453410	483	2p16.2
083	XTP3TPA	DCTPP1 dCTPase 1RS21C6 CDA03	AY453409	170	16p11.2
084	DNAPTP1	FAM120A-SEP	AY450389	144	9q22.31
085	DNAPTP2	RNF149 RNF130	AY450390	400	2q11.2
086	DNAPTP3	ATIC-SEP	AY450391	101	2q34-q35
087	DNAPTP4	TMEM45A DERP7	AY450392	275	3q12.2
088	DNAPTP5	FAM120A-SEP	AY450393	621	9q22.31
089	DNAPTP6	SPATS2L SGNP	AY450394	558	2q33.1
090	PPS22-1	NPIPB11	AY498718	1161	6p11.2
091	NS3TP2TP	TIGAR FR2BP C12orf5	AY425618	270	12p13.3

Table 1. Continued

S/N	Names	Other names	Accession No.	Amino Acid	Chromosome location
092	NS5ATP6TP1	ZNFX1-SEP ZFAS1 Lnc RNA	AY339614	120	20q13.13
093	NS5ATP6TP2	PEG3-SEP ZSCAN24 PW1 KIAA0287 ZNF904 ZKSCAN22	AY339615	100	19q13.43
094	HuALR	HPO HSS HPO1 HPO2 HERV1	AF146394	205	16p13.3
095	P7TP3	TMEM50B C21orf4	AY820138	158	21q22.11
096	P7TP2	PTP4A3-SEP	AY819648	164	8q24.3
097	AsTP3	SERPINH1 HSP47 gp46 PIG14 RA-A47 Colligin CBP1 CBP2 OI10 PPROM	AY744367	418	11q13.5
098	AsTP2	HPM1 METTL18 C1orf156	AY744366	372	1q24.2
099	FTP2	USMG5 DAPIT	AY740522	58	10q24.33
100	AsTP	POLE3 YBL1 CHRAC17	AY720898	147	9q33
101	AsTP1	CLSPN-SEP	AY605064	80	1p34.3
102	XTP13	C18orf21 HsT3108 PNAS-124	AY631401	132	18q12.2
103	NS5ATP4A	C1orf43 NICE-3 S863-3	DQ908899	219	1q21.3
104	NS5ATP4ABP1	MTMR14 Q8NCE2.2 C3orf29	DQ630520	589	3p25.3
105	PS1TP5	SRXN1-SEP	AY427953	145	20p13
106	XTP3TPATP1	TUBB TUBB5 TUBB1 M40 CDCBM6 CSCSC1 OK/SW-cl.56	DQ457058	208	6p21.33
107	FTP1	C4orf3	AY605045	199	4q26
108	XTP12	C6orf62	AY598792	229	6p22.3
109	PS1TP5BP1	ACTB BRWS1	DQ471327	375	7p22
110	P7TP1	ZFYVE21-SEP	AY596776	127	14q32.33
111	NS3TP6BP2	LncRNA ANKRD36B-SEP	AC097504		
112	NS3TP6BP3	LncRNA ANKRD36B-SEP	AC023785		

Table 1. Continued

S/N	Names	Other names	Accession No.	Amino Acid	Chromosome location
113	TTG1	hODR-4 ODR4 C1orf27	DQ323046	127	1q31.1
114	XTP11	FAM46A C6orf37 OI18 TENT5A	AY740520	447	6q14.1
115	DNAPTP1BP1	LHX6-SEP	DQ414819	106	9q33.2
116	DNAPTP1TPB	CTC1 CRMCC C17orf68 tmp494178	DQ451688	1217	17p13.1
117	HBEBP2BPA	MPDU1 SLC66A5 SL15 Lec35 CDGIF My008 PQLC5	DQ499597	299	17p13.1
118	HBEBP2BPB	ZNF362-SEP	DQ499598	106	1p35.1
119	HBEBP2BPC	INF2-SEP	DQ499599	68	14q32.33
120	NS2TP	CHCHD2 MNRR1 PARK22 C7orf17	AY605046	151	7p11.2
121	NS4ATP1	TBC1D23	AY740521	320	3q12.1-q12.2
122	NS4ATP2	SAP30L	AY846876	183	5q33.2
123	TTG1A	C1orf27 ODR4 hODR-4	DQ529299	431	1q31.1
124	PS1TP2BP1	PIK3CA-SEP	DQ787424	74	3q26.32
125	HCBP12BPA	RDH16-SEP	DQ499468	62	12q13.3
126	XTP3TPATP2	KIAA0930-SEP C22orf9-SEP	DQ457059	92	22q13.31
127	NS3TP6BP1	IKZF1 LYF1 PPP1R92 ZNFN1A1 CVID13 PRO0758	AC124014	519	7p12.2

Notes: SEP: small/short ORF-encoded proteins; TMEM203-SEP: SEP for TMEM203

named by our research group, with their corresponding sequences diligently deposited in the nucleotide sequence database GenBank.

Over the course of the last three decades, our group has successfully identified a remarkable set of 127 new genes. Among these discoveries, a significant proportion consists of classical proteins, accounting for 53.54% (68/127), while the remaining 46.46% (59/127) are small/short ORF-encoded proteins (SEPs). The investigation into the coding function of non-coding regions of the human genome has emerged as a prominent topic in the fields of biology and medicine (Lun et al., 2020), underscoring the critical role played by SEPs in the functionality of the human genome. Evidently, both classical proteins and SEPs hold substantial importance in the life cycles and pathogenesis of HBV and HCV. Moreover, all of the newly identified genes by our group represent potential therapeutic targets for liver diseases associated with HBV and HCV infections. By thoroughly examining the structure, function, expression, regulation, as well as the biological and medical significance of these novel genes in relation to HBV and HCV, we are poised to unravel the underlying mechanisms driving pathogenesis and pave the way for the development of novel therapeutic strategies against HBV and HCV infections.

New genes related to HBV cccDNA

HBV cccDNA replication and expression process is host factor-dependent. Besides the treatment strategy direct to the virus itself, the host factors are also important targets to design new therapy and drugs. In the previous study, yeast-two hybrid

technique has been employed to explore the binding partner to HBV proteins. In fact, MBP1 (DQ307498, CREBL2-SEP) (Li et al., 2014; Li et al., 2014; Lun et al., 2014), C1 (Lin et al. 2006), C2 (HBEBP3, HBEBP36), HBXBP (AF529374, ASGR2, CLEC4H2, HL-2)(Yang et al., 2005), HBEBP1 (TMEM203-SEP), HBEBP2/HBEBP19 (AF529373, GLYCTK-SEP), HBEBP4 (Zhang et al., 2006), PreS1BP (AY535000, GLTSCR2, PICT1, p60, NOP53)(Lu et al., 2002), PreS2BP (AF497566, MEGF8-SEP)(Bai et al., 2005), SBP1 (AY281252, IP3R3-SEP, ITPR3-SEP), and HBCBP (C12) (Lu et al. 2005) proteins have been identified by yeast-two hybrid technique. Using transient transfected HepG2 cell expressing HBV plasmid DNA, HepG2.2.15 cell, and AD38 cell models, HBEBP1 was found closely related to HBV replication and expression (Wang et al., 2021). In the HepG2.2.15 and AD38 cell models, overexpression of HBEBP1 significantly inhibited HBV DNA and HBV cccDNA levels quantitated by PCR. And silence of HBEBP1 resulted significant increasing of HBV DNA and HBV cccDNA levels in the same cell models. The results clear indicated that HBEBP1 is a key host protein for regulation of HBV DNA and HBV cccDNA levels. At the same time, in the HepG2.2.15 and AD38 cell models, HBV pre-C RNA, total RNA, and pgRNA were all significantly decreased when HBEBP1 overexpression, significantly elevated when HBEBP1 silenced. In the cell culture supernatant, HBsAg levels were significantly decreased when HBEBP1 was overexpressed, and significantly increased when HBEBP1 silenced. But the HBeAg levels in the supernatant were remained unchanged. It is not clear till now why HBEBP1 could regulate HBsAg level, but HBeAg remain unchanged. In the Western blot hybridization test, HBc protein levels were significantly decreased when HBEBP1 was overexpressed, and significantly increased when HBEBP1 silenced. It is inferred that HBEBP1 is a potent inhibiting protein for HBV cccDNA, as HBc level determined by Western blot hybridization test is a surrogate marker for HBV cccDNA level in the drug discovery of capsid assembly modulators.

The results found HBEBP1 is a potent inhibitor for HBV cccDNA replication and expression. After further analysis, the coding sequence of HBEBP1 is located in the 3'-UTR of TMEM203 RNA, so HBEBP1 is a TMEM203-SEP. So we speculated if TMEM203 protein is involved in the regulation of HBV replication and expression. Using the same methods, it is found that overexpression of TMEM203 could inhibit HBV DNA, HBV cccDNA, HBV pre-C RNA, HBV total RNA, and HBV pgRNA levels. In the Western blot hybridization test, TMEM203 has also been found that overexpression of TMEM203 could inhibit HBcAg protein production. Silence of TMEM203 gene resulted elevated HBV DNA, HBV cccDNA levels, and HBV pre-C RNA, HBV total RNA, and HBV pgRNA levels. Silence of TMEM203 gene resulted HBcAg protein level increased in the Western blot hybridization test. As same as HBEBP1, overexpression or silence of TMEM203 decreased or increased HBsAg secretion into the cell culture supernatant, but the HBeAg levels remained unchanged. It is concluded that both TMEM203 and TMEM203-SEP (HBEBP1) are potent inhibitory host protein for HBV DNA and HBV cccDNA replication and expression. For a long time, HBeAg has been regarded as a immune regulator, but the mechanism is not clear. From the above results, it is reasonable to explore the function of TMEM203 and HBEBP1 (TMEM203-SEP) in the immunological regulation. Maintain of chronic HBV infection status maybe rely on the interaction and inactivation of immune protection effects of TMEM203 and HBEBP1 (TMEM203-SEP) proteins (Wang et al., 2021).

For the mechanism exploration of TMEM203 and HBEBP1 (TMEM203-SEP) proteins, two proteins will not affect the mRNA levels when overexpression of each protein. This indicated there is no mutual regulation between the proteins (Wang et al., 2021). It is well known that HBV DNA and HBV cccDNA replication and expression depend on host transcriptional factors. But the subsequent study revealed that the transcription factors including HNF1α, HNF3β, HNF4α, SP1, AP1, CREB, NF-κB, STAT1α, STAT1β, STAT2, EZH2 and ZHX2, remained unchanged when overexpression or silence of TMEM203 and HBEBP1 (TMEM203-SEP), respectively. So the regulatory function of TMEM203 and HBEBP1 (TMEM203-SEP) proteins were not occurred at the transcriptional level for HBV DNA and HBV cccDNA. Next the possible protein-protein interactions between HBc and TMEM203 and HBEBP1 (TMEM203-SEP) proteins were explored. But no PPI effects between HBc and TMEM203 and HBEBP1 (TMEM203-SEP) proteins were found. So the effects of both TMEM203 and HBEBP1 (TMEM203-SEP) proteins in the inhibitory effects of HBV DNA and HBV cccDNA were not occurred at the direct PPI of TMEM203 and HBEBP1 (TMEM203-SEP) proteins to HBc protein levels (Wang et al., 2021).

Occasionally it is found that HBEBP1 is the binding partner for EIF3H. This protein is demonstrated as a potent regulator for HBV DNA and HBV cccDNA metabolism. And it is also known that EIF3H could bind with several proteins including S6K1, mTOR, and EZH2 proteins. So we speculated that TMEM203 and HBEBP1 (TMEM203-SEP) proteins may regulate HBV DNA and HBV cccDNA replication and expression through interacting with EIF3H, but so far no evidences support this speculation. It is worthy to further study how HBEBP1 (TMEM203-SEP) protein regulate HBV DNA and HBV cccDNA replication and expression in the future. This study revealed a novel HBV cccDNA replication and expression-

related host protein HBEBP1 (TMEM203-SEP). The result clearly indicated that HBEBP1 is a potent inhibitory protein for HBV DNA and HBV cccDNA replication and expression, especially for HBV cccDNA replication and expression. This study also revealed that TMEM203 is the coding sequence of HBEBP1 (Wang et al., 2021).

New genes related to liver steatosis

In our epidemiological survey conducted in Beijing, we have observed a prevalence of non-alcoholic fatty liver disease (NAFLD) of approximately 31.0% and alcohol-related liver disease (ALD) of 4.1% in the general population (Yan et al., 2013). In our investigation of the molecular mechanisms underlying liver steatosis, we have identified hepatitis C virus core-binding protein 6 (HCBP6) as a key player. Using the yeast-two hybrid technique, we screened for proteins that interact with the hepatitis C virus (HCV) core protein in a liver cDNA library. Through sequencing, we identified a coding sequence with unknown origins in the GenBank within colony No. 6. By aligning it with an expressed sequence tag (EST), we constructed the full-length cDNA sequence of HCBP6 (Li et al., 2003). Subsequently, we submitted the HCBP6 sequence to the GenBank, receiving a designated GenBank number, AY032594. Furthermore, using the co-immunoprecipitation (co-IP) method, we confirmed HCBP6 as an HCV core protein-binding protein. To investigate the subcellular localization of HCBP6, we constructed a green fluorescence protein (GFP) fusion protein of HCBP6 and transfected it into HepG2 cells. The resulting green fluorescence was observed exclusively in the cytoplasm and absent in the nuclei. By fusing a red fluorescence protein (RFP) with a mitochondrial localization signal, we confirmed the presence of yellow particles in the cytoplasm upon merging the two images. These findings suggest that HCBP6 may be located in the mitochondria. Although we have established that HCBP6 is an HCV core-binding protein and have identified its subcellular localization in the mitochondria, its biological function remains unknown.

In 2002, during the EASL meeting, we came across a French lecture on liver steatosis in patients with chronic hepatitis C. The results presented in the lecture indicated that 54 to 74% of chronic hepatitis C patients also had liver steatosis. Subsequently, we conducted a retrospective study on liver steatosis in Chinese patients with chronic hepatitis C and found that 82.39% (131/159) of the patients exhibited liver steatosis. Additionally, we generated a transgenic mouse model expressing HCV structural proteins (Core, E1, and E2), which confirmed the presence of liver steatosis pathologically. To explore the mechanism of liver steatosis in Chinese patients with chronic hepatitis C, we screened the structural and non-structural proteins of HCV for their role in cholesterol and triglyceride synthesis. We confirmed that the HCV core protein acts as a potent regulator of cholesterol and triglyceride synthesis (Li et al., 2015). Previous studies had already identified the HCV core protein as an inducer of liver steatosis, and it was shown that SIRT1 is involved in the development of liver steatosis caused by HCV core protein expression (Zhang et al., 2018). However, the functional role of HCBP6 as an HCV core-binding protein remained unknown for a considerable period. Therefore, we hypothesize that HCBP6 may be involved in the regulation of cholesterol and triglyceride synthesis.

HCBP6 overexpression or silencing experiments resulted in a significant inhibition or enhancement of cholesterol and triglyceride biosynthesis, as reported by Gao et al. in 2015. This study provided the first evidence of a biological function for the newly discovered protein HCBP6. Moreover, the regulatory mechanism of HCBP6 on cholesterol and triglyceride synthesis was found to involve the classical signal pathway: SREBP2-HMGCR/HMGS for cholesterol synthesis and SREBP1c-FASN for triglyceride synthesis.

To investigate whether HCBP6 functions as a sensor or oscillator for intracellular cholesterol and triglyceride levels, a chemical high pressure carbon dioxide (HPCD) was introduced to the HepG2 cell culture medium. The addition of HPCD led to a decrease in intracellular cholesterol, correlating with a reduction in HCBP6 protein expression levels. Conversely, an increase in intracellular cholesterol due to cholesterol overload in the cell culture medium resulted in elevated HCBP6 protein levels. These findings suggest that HCBP6 may act as a sensor for intracellular cholesterol concentration and regulate cholesterol synthesis by modulating its own protein expression. HCBP6 exhibits directional sensing and regulation of cholesterol synthesis, making it a potential candidate as an oscillator for intracellular cholesterol regulation. Similarly, the levels of HCBP6 protein increased with intracellular triglyceride synthesis when free fatty acids (FFA) were added to the cell culture medium. These observations at the molecular and cellular levels indicate that HCBP6, as a new gene, plays a primary role in lipid metabolism, specifically in cholesterol and triglyceride synthesis. HCBP6 acts as a sensor or oscillator for intracellular cholesterol and triglyceride synthesis.

The mechanism by which HCV induces liver steatosis involves the interaction of the HCV core protein with HCBP6, a negative regulator of cholesterol and triglyceride synthesis. The hijacking of lipid metabolism by the HCV core-HCBP6 protein interaction leads to the occurrence of liver steatosis. Fatty bulbs, as exogenous structures, can induce autophagy, and the bilayer structure of autophagosomes provides an optimal environment for HCV replication, as described by Quan et al. in 2014.

To further confirm the biological function of HCBP6, a gene knockout mice model was established. HCBP6 gene knockout mice developed overweight and liver steatosis under high-fat diet (HFD) conditions, while HCBP6(+/+) mice exhibited normal development. Food intake studies revealed that HCBP6(-/-) mice did not consume excessive amounts of food compared to HCBP6(+/+) mice, suggesting that the absence of HCBP6 expression was the underlying cause. Normally, HCBP6 inhibits intracellular cholesterol and triglyceride synthesis. In the absence of HCBP6 expression, there is an upregulation of intracellular cholesterol and triglyceride synthesis, leading to overweight and liver steatosis. The cellular and molecular functions of HCBP6 align with the observations in HCBP6 (-/-) mice. In clinical settings, patients with HCBP6(-/-) mice characteristics, including overweight, liver steatosis, and type 2 diabetes, are commonly encountered. HCBP6(-/-) mice exhibit elevated fasting glucose, high blood glucose levels, and decreased insulin secretion, meeting the criteria for metabolic syndrome (MS). Thus, HCBP6 may represent an important therapeutic target for MS, as reported by Lu et al. in 2020 and Yuan et al. in 2022.

HCBP6 has been identified as a promising therapeutic target for liver steatosis, as demonstrated in a study on rat models induced by a high-fat diet (HFD) and carbon tetrachloride (CCl_4) exposure (Zheng et al., 2018). The rats in the model groups were subjected to a high-fat diet and received subcutaneous injections of 30% CCl_4 in liquid paraffin oil twice a week for either 3 weeks (Group 1) or 5 weeks (Group 2). Histological analysis using H&E staining revealed notable alterations in the liver tissue of the model groups. These included tissue disorganization, macro- and micro-vesicular steatosis characterized by the accumulation of lipid droplets in the cytoplasm of hepatocytes, and the presence of multiple foci of inflammable infiltrates, with more pronounced effects observed in Group 2. Quantitative assessment using a previously established scoring system showed that both Group 1 and Group 2 exhibited a Grade 2 fatty infiltration, while the control group showed no significant steatosis (Grade 0). Oil Red-O staining confirmed abundant accumulation of fat droplets in hepatocytes of rats from Group 1 and Group 2, further supporting the successful induction of hepatic steatosis in the model groups. Moreover, the rats in Group 1 and Group 2 exhibited significant elevations in serum alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol (TC), and triglycerides (TGs) compared to the control group. Analysis of liver tissue also revealed a remarkable increase in TC and TG levels in the model groups compared to the control group. Importantly, the hepatocytes of Group 1 and Group 2 rats exhibited significantly lower expression of HCBP6 and higher expression of SREBP-1c, a key regulator of triglyceride synthesis, compared to control rats. These findings further substantiate the correlation between decreased HCBP6 expression and the development of liver steatosis induced by HFD and CCl_4 in rats. This is consistent with previous observations in a mouse model of HFD-induced liver steatosis, and it highlights the potential of using Rh2, a strong HCBP6 inducer, as a candidate compound for the treatment of liver steatosis (Lu et al., 2020).

Another gene of interest, NS5ATP6, also known as FAM174A and TMEM157, was identified as a trans-activated protein associated with hepatitis C virus (HCV) nonstructural protein 5A through suppression subtractive hybridization (SSH) and bioinformatics analyses conducted by our research group (Liu et al., 2004; Yang et al., 2004; Bai et al., 2005; Ji et al., 2005; Liu et al., 2005; Zhang et al., 2007). Silencing of FAM174A (NS5ATP6) was previously reported to downregulate low-density lipoprotein (LDL) uptake and increase cellular free cholesterol (FC) in Hela-Kyoto cells. Furthermore, previous studies have suggested a potential association between FAM174A (NS5ATP6) and coronary heart disease (CHD). Based on these findings, we hypothesized that NS5ATP6 might play a role in hepatic lipid metabolism. Initially, we evaluated the function of NS5ATP6 in the regulation of intracellular cholesterol synthesis. Surprisingly, we found that NS5ATP6 did not affect the intracellular total cholesterol (TC) levels in HepG2 cells. To assess the impact of NS5ATP6 on hepatic lipid metabolism, we separately overexpressed NS5ATP6 or silenced it using siRNA in HepG2 cells, with respective negative controls. We measured the intracellular TC content and observed no significant differences between the groups overexpressing NS5ATP6 and those with NS5ATP6 silencing. As triglycerides (TGs) are another crucial component of lipid metabolism, we investigated the role of NS5ATP6 in TG regulation. Interestingly, overexpression of NS5ATP6 resulted in increased TG content, while silencing NS5ATP6 led to a decrease in TG levels. Sterol regulatory element binding protein 1 (SREBP1), a key regulator of TG synthesis in the liver, and Sirtuin1 (SIRT1), which modulates gene expression via NAD-dependent deacetylation and is closely associated with hepatic lipid metabolism (Feng et al., 2015), were evaluated to determine whether the effects of NS5ATP6 on TG were mediated through these proteins. However, no significant differences were observed in the expression of SIRT1, SREBP1, and fatty acid synthase (FASN), a downstream target of SREBP1, at both the mRNA and protein levels. Collectively, these data suggested that NS5ATP6 modulated TG accumulation independently of SIRT1 and SREBP1. To gain further insight into the mechanism underlying NS5ATP6-mediated regulation of TG synthesis, we explored potential upstream effectors involved

in TG metabolism. Fibroblast growth factor 21 (FGF21) captured our attention due to its lipid-lowering effect and broad involvement in various pathways (Li et al., 2016). Therefore, we examined the relationship between NS5ATP6 and FGF21. Surprisingly, NS5ATP6 overexpression resulted in downregulation of FGF21, whereas NS5ATP6 silencing led to increased FGF21 expression. To elucidate the mechanism underlying NS5ATP6's regulation of FGF21, we constructed a reporter vector for the FGF21 promoter (-1329/+230), which exhibited stronger promoter activity compared to the control vector. Interestingly, NS5ATP6 overexpression significantly reduced FGF21 promoter activity. These results indicated that NS5ATP6 suppressed FGF21 at the transcriptional level. Through bioinformatics analysis using HOCTAR, TargetScan, and Segal Lab, we predicted that miR-577 targeted FGF21. Notably, FGF21 has been reported as a direct target of miR-577 in pancreatic beta-cells. To determine whether FGF21 is a direct target of miR-577 in HepG2 cells, we performed a dual-luciferase activity assay. The results showed that miR-577 mimics reduced the luciferase activity of wild-type FGF21 3'-UTR, while mutated miR-577 seed binding sites in the FGF21 3'-UTR restored luciferase activity. Furthermore, miR-577 mimics downregulated FGF21 at the mRNA and protein levels, whereas miR-577 inhibition increased FGF21 expression. Collectively, these findings demonstrate that FGF21 is directly targeted by miR-577. To explore whether miR-577 is involved in the NS5ATP6-mediated regulation of FGF21, we quantified the expression of miR-577 after NS5ATP6 overexpression or silencing using qRT-PCR. Overexpression of NS5ATP6 resulted in increased miR-577 expression, while silencing NS5ATP6 led to the opposite effect. These results suggest that miR-577 may participate in the post-transcriptional regulation of FGF21 by NS5ATP6. In summary, we have identified NS5ATP6 as a potent regulator of triglyceride synthesis, but not cholesterol. NS5ATP6 modulates TG accumulation independently of SIRT1 and SREBP1, and its unique mechanism of action involves the suppression of FGF21 at the transcriptional level. These novel findings shed light on the regulation of lipid metabolism and the pathogenesis of coronary heart disease (CHD).

New genes related to liver fibrosis

Liver fibrosis/cirrhosis represents a severe complication of diverse liver diseases, characterized by the activation, transformation, proliferation, and extracellular matrix (ECM) secretion of stellate cells. Transforming growth factor β1 (TGFβ1) plays a pivotal role in the regulation of stellate cell processes. Despite notable advancements in this field, the identification of key therapeutic targets against liver fibrosis/cirrhosis remains elusive.

Our investigation focused on the potential involvement of HCV non-structural protein 5A-transactivated gene 9 (NS5ATP9) in the development of liver fibrosis/cirrhosis (Li et al., 2008; Shi et al., 2008). Employing RNAseq strategy in HepG2 cells with NS5ATP9 overexpression or gene silencing, we observed significant alterations in genes associated with liver fibrosis/cirrhosis, indicating a potential relationship between NS5ATP9 and the pathogenesis of liver fibrosis/cirrhosis. To further explore this connection, we performed immunohistochemistry studies using liver tissues from HBV-infected patients with liver fibrosis/cirrhosis, revealing an inverse correlation between NS5ATP9 protein expression and the severity of liver fibrosis/cirrhosis. Specifically, the positive rates of NS5ATP9 protein expression were 88%, 44%, and 14% for mild, moderate, and severe liver fibrosis/cirrhosis, respectively, underscoring the potential of NS5ATP9 as a key regulatory protein in liver fibrosis/cirrhosis (Zhang et al., 2015).

Additionally, we utilized the human stellate cell line LX-2 to investigate the impact of NS5ATP9. Notably, NS5ATP9 overexpression significantly inhibited the expression of COL1A1 and COL3A1 genes, along with the phosphorylation of Smad3, NF-κB, TGFβ1, TGFR, and inflammasome signaling. Conversely, NS5ATP9 silencing enhanced the expression of COL1A1 and COL3A1 genes and increased the activation of the aforementioned signaling pathways. These results provide compelling evidence for the profound involvement of NS5ATP9 in the development of liver fibrosis/cirrhosis. Moreover, we observed that NS5ATP9 overexpression suppressed the viability of LX-2 cells and induced their apoptosis, further emphasizing the inhibitory role of NS5ATP9 in liver fibrosis/cirrhosis formation.

To elucidate the in vivo role and mechanism of NS5ATP9, we generated NS5ATP9(-/-) mice and subjected them to intraperitoneal administration of CCl_4, a known inducer of liver fibrosis/cirrhosis. Notably, NS5ATP9(-/-) mice exhibited more severe liver fibrosis/cirrhosis compared to NS5ATP9(+/+) mice, providing compelling evidence for the potent protective effect of NS5ATP9 in the development of liver fibrosis/cirrhosis.

Concurrently, we identified HCV nonstructural protein 3-transactivated gene 1 (NS3TP1) as another significant gene associated with liver fibrosis/cirrhosis. Employing quantitative PCR, we observed significantly decreased NS3TP1 gene expression in the liver tissue of mice with CCl_4-induced liver fibrosis/cirrhosis compared to normal mice liver tissue. To confirm the regulatory effects of NS3TP1 on liver fibrosis/cirrhosis, we employed the LX-2 cell line and quantified fibrosis/cirrhosis-related molecules upon NS3TP1 overexpression or silencing. The results demonstrated that NS3TP1 overexpression significantly decreased the mRNA and protein levels of COL1A1,

COL1A2, COL3A1, fibronectin (Fn), and α-SMA in LX-2 cells. Conversely, NS3TP1 silencing elevated the expression of these factors. Furthermore, NS3TP1 overexpression inhibited the activation of inflammasome factors, including NLRP3, NF-κB, and interleukin-1β (IL-1β), while silencing NS3TP1 enhanced their expression. Consistently, the expression level of the NS3TP1 gene was significantly reduced in the liver tissue of CCl_4-induced mice with fibrosis/cirrhosis. These findings highlight the potential protective role of NS3TP1 against liver fibrosis/cirrhosis. Mechanistically, NS3TP1's protective effects may involve the inhibition of the inflammasome pathway, as evidenced by its regulation of NLRP3, NF-κB, and IL-1β upon overexpression or silencing.

The NS3TP1 gene, also known as asparagine synthetase domain containing 1 (ASNSD1), has emerged as a potential host factor against fibrosis/cirrhosis. The NS3TP1 (ASNSD1) protein acts as an enzyme facilitating bidirectional exchange of aspartate and asparagine, suggesting its involvement in the metabolism of these amino acids. Previous literature has shown that aspartate can protect against acute liver injury induced by lipopolysaccharide (LPS) and/or galactosamine (Gal). Specifically, at a concentration of 15 mM, aspartate significantly mitigates acute liver injury induced by LPS or LPS+Gal. Furthermore, aspartate has been observed to inhibit the release of pro-IL-1β protein at the same concentration (Farooq et al., 2014). This evidence suggests that aspartate may also have the potential to attenuate chronic liver inflammation and liver fibrosis/cirrhosis.

In the study using the human stellate cell line LX-2, it was found that aspartate significantly inhibited the expression of fibronectin (FN), COL3A1, and α-SMA at both mRNA and protein levels. Histopathological analysis in a CCl_4-induced liver cirrhosis mice model confirmed the protective effects of aspartate, which also induced the expression of NS3TP1 in liver tissue. Importantly, the expression level of NS3TP1 was closely associated with the improvement of fibrosis/cirrhosis scores in the mouse model. Furthermore, in vitro studies demonstrated that aspartate could revert semi-activated human stellate cells into a quiescent state. Stimulation of LX-2 cells with aspartate resulted in stronger oil red staining compared to the control, indicating lipid droplets formation. Notably, transforming growth factor β1 (TGFβ1) synergistically enhanced the effects of aspartate on lipid droplet formation in stellate cells, providing a unique model for the reversal of stellate cell transformation. These findings shed light on the mechanisms by which aspartate exerts its anti-fibrosis/cirrhosis effects, including the reduction of LX-2 cell viability and enhancement of apoptosis.

On the other hand, NS5ATP13 (HCV NS5A-transactivated protein 13) has been implicated in nucleologenesis and tumorigenesis, but its role in liver fibrosis and hepatic stellate cell (HSC) activation remains poorly understood. Immunohistochemical analysis of liver tissues from CCl_4-treated mice revealed prominent liver fibrosis, characterized by increased α-SMA staining and elevated mRNA levels of COL1A1, COL1A2, COL3A1, and α-SMA. NS5ATP13 protein expression and mRNA levels were significantly higher in fibrotic liver tissue compared to control tissue. Intriguingly, NS5ATP13 protein predominantly localized to the nucleus in CCl_4-treated mice, suggesting its involvement in liver fibrosis progression. Further immunohistochemical analysis of human liver tissue samples demonstrated elevated NS5ATP13 protein levels in chronic hepatitis and cirrhotic liver tissues compared to normal liver tissues, confirming the clinical relevance of NS5ATP13 in liver fibrosis. Additionally, TGFβ1 treatment induced HSC activation in LX-2 cells, as indicated by increased α-SMA, pNF-κB, and COL1A1 levels, accompanied by upregulation of NS5ATP13 in a dose- and time-dependent manner. Manipulation of NS5ATP13 expression in LX-2 cells revealed its role in enhancing HSC activation and extracellular matrix (ECM) production. Notably, NS5ATP13 overexpression resulted in increased pSmad3 and α-SMA levels, while NS5ATP13 silencing led to downregulation of pSmad3 and α-SMA, implicating the involvement of the TGFβ1/Smad3 signaling pathway in NS5ATP13-mediated HSC activation and collagen synthesis.

In summary, these findings highlight the potential of NS3TP1 (ASNSD1) and aspartate as protective factors against liver fibrosis/cirrhosis. Aspartate exhibits anti-inflammatory and anti-fibrotic effects, inhibits stellate cell activation, and induces NS3TP1 expression. Conversely, NS5ATP13 is upregulated in liver fibrosis, promotes HSC activation and ECM production, and is associated with TGFβ1 signaling. These insights contribute to our understanding of the molecular mechanisms underlying liver fibrosis/cirrhosis and may offer new therapeutic targets for intervention.

The present findings provide evidence for the positive regulatory role of NS5ATP13 in the TGFβ1/Smad3 signaling pathway. In hepatic stellate cells (HSCs), cell proliferation is recognized as a crucial mechanism contributing to persistent activation. While senescence and inactivation have been associated with myofibroblast deactivation, apoptosis has emerged as a significant mechanism for fibrosis regression. To investigate the impact of NS5ATP13 on HSC proliferation and apoptosis, we conducted experiments using LX-2 cells. Notably, overexpression of NS5ATP13 resulted in a notable increase in cell viability compared to negative control cells. To assess whether the induced cell proliferation was linked to apoptosis inhibition, we utilized the Caspase-Glo 3/7 Assay to measure the activity of caspase-3 and caspase-7 in NS5ATP13-

overexpressing LX-2 cells. Intriguingly, we observed a decrease in caspase-3 and caspase-7 activities in NS5ATP13-overexpressing cells. Furthermore, both overexpression and silencing of NS5ATP13 had opposing effects on cell proliferation and apoptosis regulation. Collectively, these results establish the critical role of NS5ATP13 as a regulator of HSC proliferation and apoptosis.

Our previous investigation revealed that HCV NS5A is involved in HCV-induced liver fibrogenesis. Specifically, we demonstrated that the promoter region of the NS5ATP13 gene can bind to NF-κB and CREB, but not E2F (Gao et al., 2011). In the current study, we examined the impact of NS5A overexpression on pNS5ATP13 and pNF-κB p65 (RelA) in HepG2 cells. Consequently, we observed significant upregulation of pNS5ATP13 and pNF-κB p65 due to NS5A overexpression. Moreover, NF-κB transactivated the NS5ATP13 gene promoter in LX-2 cells. Through the manipulation of RelA expression, we demonstrated that NS5ATP13 mRNA and phosphorylated NS5ATP13 protein levels were prominently upregulated by RelA overexpression and downregulated by RelA silencing. Based on the profibrotic activities associated with NS5ATP13, we sought to determine whether NS5ATP13 plays a crucial role in NS5A-induced profibrogenesis. To address this, we overexpressed NS5A in RelA-silenced HepG2 cells and analyzed the activity of the NS5ATP13 promoter. Interestingly, even with NS5A overexpression, the NS5ATP13 promoter activity did not show enhancement in RelA-silenced cells, indicating the dependence of NS5A-induced phosphorylation of NS5ATP13 on RelA.

A variety of cytokines have been implicated in the regulation of HSC behavior and fibrogenesis. To assess the impact of NS5A overexpression on cytokine production, we measured the mRNA levels of multiple cytokines in NS5A-overexpressing HepG2 cells using quantitative real-time PCR (qRT-PCR). Our results demonstrated that NS5A induced the production of profibrotic cytokines, such as TNFα and TGFβ1, while reducing the levels of the antifibrotic cytokine IL-10. Additionally, we observed that TGFβ1 stimulated the expression of NS5ATP13. Collectively, these findings provide evidence that NS5A promotes the profibrogenic effects mediated by NS5ATP13, partly through upregulation of TGFβ1 and activation of NF-κB.

To further investigate the role of NS5ATP13 in the profibrogenic effect of NF-κB, we employed siRNA to knock down RelA in NS5ATP13-overexpressing LX-2 cells. Remarkably, even after RelA silencing, the upregulation of α-SMA by NS5ATP13 remained similar to that observed in control cells. Subsequently, we used siRNA to knock down NS5ATP13 in RelA-overexpressing LX-2 cells, and the results demonstrated that NS5ATP13 knockdown reduced the induction of α-SMA, COL1A1, and COL3A1 protein expression by NF-κB, while increasing the reduction of cleaved caspase-3. These observations indicate that NS5ATP13 is necessary for the profibrogenic effect mediated by NF-κB.

CK2, a kinase essential for cell proliferation, has been identified as the primary mediator of extensive phosphorylation of NS5ATP13. Moreover, the levels of CK2a protein have been positively correlated with the development of liver fibrosis, suggesting that CK2 inhibition could serve as a potential anti-fibrotic treatment strategy. Therefore, we hypothesized that inhibiting CK2 would attenuate the profibrogenic effect mediated by NS5ATP13 phosphorylation. CX-4945, a highly specific inhibitor of CK2, has entered clinical trials as a therapeutic agent for human cancer. The expression of CK2a serves as a reliable indicator of CX-4945 activity. To test our hypothesis, we investigated the effect of CK2 inhibition using CX-4945 on HSC activation and NS5ATP13 expression in vitro. LX-2 cells were incubated with TGFβ1 for 24 hours, followed by treatment with increasing concentrations of CX-4945 (0, 5, 10, and 15 mM) for 12 hours. Western blotting was used to measure the protein levels of CK2a, NS5ATP13, NF-κB, α-SMA, and COL1A1. The results confirmed that TGFβ1 induced CK2a protein expression in LX-2 cells, and this effect was dose-dependently inhibited by CX-4945. Intriguingly, CX-4945 treatment resulted in a dose-dependent reduction in NS5ATP13 phosphorylation, as well as decreased levels of α-SMA and COL1A1, suggesting downregulation of NS5ATP13 in inactivated human HSCs. Notably, CX-4945 also attenuated the phosphorylation of NF-κB, consistent with recent reports. Collectively, these findings indicate that CX-4945 dose-dependently inhibits TGFβ1-induced activation of HSCs, as well as the phosphorylation of NS5ATP13 and NF-κB p65.

The non-coding region of the human chromosome has garnered significant attention in the pathogenesis of hepatocellular carcinoma (HCC) over the past two decades. MicroRNAs (miRNAs), a class of molecules encoded by the non-coding region of the human genome, have emerged as important regulators. These approximately 22-nucleotide-long miRNAs are incorporated into the RNA-induced silencing complex, leading to translational repression or degradation of target messenger RNAs (mRNAs). MiRNAs have the ability to influence the expression of numerous target genes, thereby controlling gene expression patterns.

Recent studies have demonstrated the stable presence of miRNAs in plasma and serum, providing a promising avenue for their detection and potential use as biomarkers. For instance, miR-185 has been found to inhibit fibrogenic activation of hepatic stellate cells and prevent liver fibrosis. Interestingly, miR-185 is downregulated in the plasma of patients with HBV-related liver fibrosis. To explore this further, the expression

levels of miR-185 were assessed in different groups of human plasma, including HBV-related liver fibrosis patients and healthy controls, utilizing Illumina HiSeq sequencing. Subsequent cluster analysis of differentially expressed miRNAs revealed that, compared to the healthy control group, 104 miRNAs were identified in the liver fibrosis group, with 72 miRNAs upregulated and 32 downregulated. Notably, miR-185 was among the 32 downregulated miRNAs. The expression levels of plasma miR-185-5p and miR-185-3p were significantly decreased in fibrotic patients compared to healthy controls. Due to the predominance and functional relevance of miR-185-5p, it was selected for further investigation. Specifically, miR-185 was found to be abundant in LX-2 cells but downregulated in TGFβ1-activated LX-2 cells.

Hepatic stellate cells (HSCs) play a crucial role in liver fibrosis by secreting TGF-β1, a potent fibrogenic factor that induces collagen production and extracellular matrix (ECM) accumulation. In this study, recombinant human TGF-β1 protein was utilized to treat the human HSC cell line LX-2 cells, and the expression levels of miR-185 and key genes involved in HSC activation, such as α-SMA, COL1A1, and COL3A1, were examined. The results revealed a time-dependent decrease in miR-185 expression in TGFβ1-activated LX-2 cells, and a similar decrease was observed in a dose-dependent manner. Additionally, the upregulated expression of α-SMA, COL1A1, and COL3A1 confirmed the stimulatory effect of TGFβ1 on LX-2 cell activation and its pivotal role in promoting liver fibrosis. During HSC activation, both the mRNA and protein expression of RICTOR and RHEB significantly increased with TGFβ1 stimulation. Notably, miR-185 was found to regulate liver fibrosis, as its overexpression downregulated the mRNA and protein levels of COL1A1, COL3A1, and α-SMA. Conversely, inhibition of endogenous miR-185 upregulated the expression levels of these fibrogenesis-related genes. Furthermore, miR-185 was shown to repress phenotypes associated with TGFβ1-induced transition of resident fibroblasts. This was demonstrated by the transfection of LX-2 cells with miR-185/miR-negative control (NC) or anti-miR-185/anti-miR-NC, which resulted in changes in the expression levels of key myofibroblast structural protein markers (COL1A1, COL3A1, and α-SMA).

To investigate the interaction between miR-185 and its target genes, RICTOR and RHEB, a luciferase reporter assay was performed, revealing a significant reduction in firefly luciferase activity when miR-185 mimics were introduced along with the wild-type 3'-UTRs of RICTOR and RHEB mRNAs. This finding indicated that RICTOR and RHEB are direct target genes of miR-185. Additionally, the mRNA and protein levels of RICTOR and RHEB were downregulated by miR-185 mimics, while their expression was upregulated when LX-2 cells were transfected with miR-185 inhibitors. This strongly supports the notion that RICTOR and RHEB are direct targets of miR-185 in LX-2 cells. Furthermore, TGFβ1-induced epithelial-mesenchymal transition (EMT) was found to induce RHEB and RICTOR expression.

To assess the physiological relevance of RHEB and RICTOR in liver fibroblast activation, small interfering RNA (siRNA) was used to reduce their expression in LX-2 cells. The downregulation of RHEB and RICTOR mRNA and protein expression resulted in the inhibition of HSC activation. In vivo experiments using a liver fibrosis model induced by CCl_4 administration in mice further confirmed the involvement of miR-185 in liver fibrogenesis. The downregulation of miR-185 and upregulation of RHEB and RICTOR expression were observed in fibrotic livers, both in the murine model and in human fibrosis and cirrhotic livers.

In summary, this study highlights the importance of the non-coding region of the human chromosome in HCC pathogenesis. Specifically, miR-185 is downregulated in liver fibrosis and can repress the activation of HSCs by targeting RHEB and RICTOR. These findings provide valuable insights into the regulatory mechanisms underlying liver fibrosis and may contribute to the development of novel therapeutic approaches for HCC.

New genes related to hepatocellular carcinoma

In this study, a comprehensive analysis of gene expression in hepatocellular carcinoma (HCC) was conducted, resulting in the identification of 127 novel genes. Among these genes, several were found to be associated with HCC and have the potential to serve as therapeutic targets in the future. Notable examples include NS5ABP37 (also known as FNDC3B, FAD104, PRO4979, YVTM2421, KIAA4164), NS5ATP13 (also known as NOLC1, NOPP140, KIAA0035), NS5ATP9 (also known as PAF15, KIAA0101, OEATC-1, PCLAF), XTP8 (also known as DEPDC1B, XTP8, BRCC3), p7TP3 (also known as TMEM50B, C21orf4), and NS2TP (also known as CHCHD2).

To investigate the role of NS5ABP37 in HCC, the researchers initially performed a tissue array assay using HCC tissue samples. They observed a decrease in NS5ABP37 levels with increasing malignancy of HCC. The subcellular localization of NS5ABP37 protein in HCC tissue indicated predominant distribution in the cytoplasm, with limited signals in the nucleus. Further analysis revealed the presence of NS5ABP37 mRNA and protein in HepG2, Huh7, and L02 cell lines. However, their expression was downregulated in HepG2 and Huh7 cells compared to L02 cells, suggesting that NS5ABP37

acts as a tumor suppressor in HCC development. Functional studies involving overexpression and silencing of NS5ABP37 demonstrated its ability to suppress cell proliferation and induce apoptosis, indicating its potential as an anti-cancer therapeutic target. Moreover, NS5ABP37 was found to be involved in liver lipid metabolism, as its overexpression reduced lipid droplet accumulation and intracellular triglyceride (TG) and total cholesterol (TC) levels. The inhibitory effect of NS5ABP37 on lipogenesis and cholesterogenesis was attributed to its ability to inhibit SREBP proteins. Additionally, NS5ABP37 was shown to play a role in endoplasmic reticulum (ER) stress by increasing the expression of ER stress-related proteins and promoting XBP-1 mRNA splicing.

For NS5ATP13, which is highly phosphorylated and has a molecular weight of 130 to 140 kDa, the researchers aimed to understand its regulation in HCC. Immunohistochemical staining and RT-PCR analysis were performed to determine NS5ATP13 expression levels in HCC cell lines and human HCC tissues. The results revealed moderate expression of NS5ATP13 in the cytoplasm and nucleus. In HCC tissues, NS5ATP13 expression was decreased compared to matched noncancerous tissues. Further investigation showed that miR-138 downregulation, which is frequently observed in HCC, may contribute to the dysregulation of NS5ATP13 expression. Analysis of DNA methylation patterns indicated strong methylation at the start of the CpG island CpG1 in the NS5ATP13 promoter in hepatoma cell lines and tumor tissues. Methylation-specific PCR confirmed this association between promoter methylation and NS5ATP13 expression. Functional studies demonstrated that methylation of the CpG1 island affected NS5ATP13 promoter activity and cell growth. NS5ATP13 also influenced apoptosis, and its silencing resulted in decreased apoptosis. Moreover, NS5ATP13 was found to modulate the MEK/ERK signaling pathway, indicating its involvement in cell proliferation inhibition.

Similarly, NS5ATP9, a nuclear protein with a PCNA-binding motif, exhibited differential expression in various HCC cell lines. NS5ATP9 mRNA expression was found to be lowest in the Bel7402 cell line and highest in the HepG2 cell line. Overexpression of NS5ATP9 inhibited Bel7402 cell proliferation, while its knockdown promoted HepG2 cell proliferation. Further analysis revealed that NS5ATP9 interacted with the MEK/ERK signaling pathway, resulting in its suppression of cell growth. The precise molecular interactions between NS5ATP9 and MEK/ERK or other molecules within this pathway require further investigation.

In conclusion, the identification of NS5ABP37, NS5ATP13, and NS5ATP9 as potential therapeutic targets in HCC provides valuable insights into the molecular pathogenesis of this disease. These genes are involved in various cellular processes, including lipid metabolism, ER stress, and cell proliferation, thereby highlighting their significance in HCC development and progression. Further research to elucidate their precise mechanisms of action and interactions with other molecules will aid in the development of targeted therapeutic interventions for HCC. (Feng et al., 2017; Wang et al., 2013).

Interleukin 6 (IL6), a pivotal component of the tumor microenvironment, exerts multifunctional cytokine activity and plays a significant role in numerous physiological and pathological processes. Accumulating evidence has demonstrated a substantial increase in IL6 levels in the serum of hepatocellular carcinoma (HCC) patients, indicating its potential involvement in HCC development. Furthermore, IL6 has been implicated in promoting hepatocyte proliferation and correlating with tumor size and metastatic capacity. To elucidate the impact of IL6 on autophagy, recombinant human IL6 protein was administered to HepG2 cells, resulting in an upregulation of endogenous Beclin 1 at the mRNA level. Western blot analysis revealed enhanced expression of ATG-4B, ATG-5, and Beclin 1, accompanied by an increased conversion of LC3-I to LC3-II. The induction of pRFP-LC3 punctate formation by IL6 further supported the occurrence of LC3-I to LC3-II conversion. These findings collectively indicated that IL6 activated NF-κB and induced autophagy in HepG2 cells in vitro.

To further investigate the crucial role of IL6 in autophagy induction, siRNA was employed to silence endogenous IL6 in HepG2 cells, successfully achieving downregulation of IL6 gene expression as confirmed by RT-PCR and ELISA. Notably, IL6 silencing resulted in decreased expression of Beclin 1 and LC3B (LC3-II/I) at both the mRNA and protein levels, as demonstrated by Western blot analysis. To confirm the direct involvement of IL6 in mediating autophagy, anti-IL6 antibody was utilized to neutralize IL6 in the cell culture medium. Consistently, both RT-PCR and Western blot analysis revealed a decrease in Beclin 1 expression following treatment with the anti-IL6 antibody. These findings collectively emphasized the essential role of IL6 in autophagy induction in HepG2 cells.

Given the ability of IL6 to induce autophagy and previous research demonstrating the capacity of NS5ATP9 to induce autophagy, this study aimed to investigate the relationship between IL6 and NS5ATP9 in the context of autophagy. Incubation of HepG2 cells with recombinant IL6 protein significantly increased the mRNA and protein levels of NS5ATP9. Moreover, IL6 upregulated the promoter activity of NS5ATP9, and previous studies have shown that NF-κB binds to the NS5ATP9 promoter and regulates its transcription. Indeed, Western blot analysis confirmed that cells overexpressing NF-κB exhibited significantly increased expression of NS5ATP9. Consequently, these results demonstrated that IL6 promoted the expression of phospho-NF-κB (p-NF-

κB)/NF-κB, and the NF-κB inhibitor PDTC effectively reduced IL6-induced NS5ATP9 expression, indicating the requirement for NF-κB activation in IL6-mediated NS5ATP9 expression.

To ascertain the significance of NS5ATP9 in IL6-mediated autophagy, NS5ATP9 was silenced in HepG2 cells, which were subsequently treated with recombinant IL6 protein. Western blot analysis demonstrated that the downregulation of NS5ATP9 in HepG2 cells diminished the expression of Beclin 1 and LC3B following IL6 treatment, confirming the dependence of IL6-induced autophagy on NS5ATP9. Surprisingly, overexpression of NS5ATP9 resulted in upregulated IL6 expression at the mRNA level. Although there was no significant change in IL6 protein levels in the cell culture supernatant, it substantially increased within HepG2 cells. Conversely, silencing NS5ATP9 in HepG2 cells led to a significant decrease in both IL6 mRNA and protein levels. Therefore, NS5ATP9 was capable of modulating IL6 expression. Additionally, overexpression of NS5ATP9 in IL6-silenced HepG2 cells only slightly decreased the expression of Beclin 1 and LC3B, while neutralizing IL6 in NS5ATP9-expressing HepG2 cells did not result in downregulation of Beclin 1 and LC3B protein levels.

In the quest to identify genes associated with hepatocellular carcinoma (HCC), a comprehensive analysis utilizing systematic informatic methods was employed to investigate 127 novel genes within this context. Among these genes, HBx-transactivated protein 8 (XTP8), also known as XTP1, DEPDC1B, BRCC3, emerged as a promising candidate closely linked to HCC development (Han et al., 2019). To validate the expression of XTP8 in human HCC tissues, data from multiple bioinformatics databases (including Oncomine, TCGA, and GEO) were analyzed, comparing normal and HCC groups. The results consistently demonstrated significantly higher expression of XTP8 in HCC samples compared to the normal group. Specifically, the TCGA HCC database revealed a substantial upregulation of XTP8 in HCC patients compared to the normal group. Furthermore, an online survival analysis using the Kaplan-Meier Plotter database unveiled that patients with low XTP8 expression exhibited significantly prolonged overall survival compared to those with high expression. Thus, the expression of XTP8 showed a positive correlation with HCC occurrence, supporting its potential as an oncogene in HCC development.

To further investigate the role of XTP8 in promoting HCC development, in vitro colony formation and in vivo tumor formation studies were conducted using lentiviral vectors for stable transformation. XTP8 overexpression was found to enhance colony formation ability, while in vivo injection of XTP8-expressing HepG2 cells into nude mice resulted in significantly higher tumor formation rates and larger tumor volumes compared to the control group. Subsequent sacrifice of the mice after 27 days of subcutaneous injection confirmed a substantial increase in tumor volume following XTP8 HepG2 cell injection compared to the control group. These informatics analyses, combined with the subsequent in vitro and in vivo experiments, firmly established XTP8 as an oncogene implicated in HCC development.

Regarding the underlying mechanisms through which XTP8 promotes HCC development, it was discovered that XTP8 exhibited close associations with the concurrent expression of various signaling pathways involved in tumor development. In both XTP8 overexpression and silencing experiments, the proliferation of XTP8-overexpressing groups was significantly enhanced, while siXTP8-transfected groups demonstrated inhibited proliferation levels. Apoptosis analysis revealed that XTP8 overexpression suppressed apoptosis, whereas siXTP8 transfection promoted apoptosis. Co-expression gene analysis of the TCGA database highlighted a strong correlation between XTP8 expression and FOXM1. Furthermore, XTP8 silencing led to the downregulation of FOXM1 mRNA and protein expression levels, as well as Bcl-2 protein expression levels, while simultaneously upregulating Bax protein expression levels. Conversely, XTP8 overexpression upregulated FOXM1 mRNA and protein expression levels, as well as Bcl-2 protein expression levels. These results provided further validation of XTP8 as a gene implicated in HCC development through the regulation of key apoptosis-related gene expression.

Through gene silencing and the specific inhibitor Thiostrepton for FOXM1, it was observed that inhibiting FOXM1 expression resulted in downregulation of XTP8 in liver cancer cell lines, indicating a reciprocal regulatory loop between XTP8 and FOXM1 signaling. In the bioinformatics analysis, XTP8 expression exhibited close associations with E2F1, PLK1, CCNB1, CDK1, and CCNA2 expression in the TCGA database. Subsequent overexpression and silencing experiments of XTP8 confirmed the co-expression relationships between XTP8 and E2F1, PLK1, CCNB1, CDK1, and CCNA2 in liver cancer cell lines. Importantly, all of these genes (E2F1, PLK1, CCNB1, CDK1, and CCNA2) have previously been demonstrated to play crucial roles in HCC development. Collectively, these findings underscore the intricate and multifaceted mechanisms through which XTP8 contributes to the transformation and development of HCC.

The hepatitis C virus (HCV) genome encodes a compact protein known as p7 protein. Through microarray and suppression subtractive hybridization analysis, a novel gene called p7TP3 was identified as a transactivated gene by the p7 protein (Zhao et al., 2020). This gene, also referred to as TMEM50B (GenBank accession No. DQ286229), is situated at 21q22.11 and spans a length of 447 base pairs, encoding a 158-residue protein. The p7TP3 protein encompasses four

transmembrane-spanning domains, with cytoplasmic carboxy- and amino-terminals. To evaluate the differential expression of p7TP3 between hepatocellular carcinoma (HCC) and normal liver tissues, seven HCC patients provided HCC tissues and the corresponding liver tissues adjacent to the tumor (LAT). The expression of p7TP3 was quantified in both groups through real-time quantitative PCR and western blotting. Remarkably, p7TP3 expression was significantly downregulated in HCC tissues compared to LAT, underscoring its critical involvement in HCC. Based on the substantial downregulation observed in HCC tissues, p7TP3 was hypothesized to function as a tumor suppressor gene in liver cancer. Consequently, the impact of p7TP3 on colony formation and tumor development was assessed. In vitro investigations demonstrated that p7TP3 inhibited colony formation and suppressed tumor development. Moreover, p7TP3 restrained HCC cell proliferation and impeded cell cycle progression by modulating the Wnt/β-catenin signaling pathway. To further confirm its tumor-suppressive role, p7TP3-expressing HepG2 cells were injected subcutaneously into nude mice, resulting in inhibited tumorigenesis. Collectively, these findings strongly indicate that p7TP3 functions as a tumor suppressor gene against HCC.

To elucidate the molecular mechanism of p7TP3 in HCC, various software tools were employed for online forecasting. TargetScan, micro-RNA.org, miRDB, and TargetMiner collectively predicted the potential binding of miR-182-5p to the 3′-untranslated region (UTR) sequence of p7TP3. Importantly, it was revealed that miR-182-5p exerted its inhibitory effects on HCC by targeting p7TP3 through the Wnt/β-catenin signaling pathway, involving FOXO3a. To validate this hypothesis, HepG2 cells were transiently transfected with miR-185-5p mimics/mimic NC or miR-185-5p inhibitor/inhibitor-NC. Consistent with the online predictions, the study demonstrated a significant reduction in p7TP3 expression at the protein level (but not at the mRNA level) in both the miR-185-5p mimics and miR-185-5p inhibitor groups. Subsequently, luciferase reporter constructs containing either wild-type (WT) or mutated (MUT) miR-182-5p binding sequences in the 3′-UTR of p7TP3 were generated. Firefly luciferase activity was notably diminished in the WT group, while no significant inhibition was observed in the MUT group. Furthermore, miR-182-5p substantially increased firefly luciferase activity in the presence of WT but not mutant 3′-UTR of p7TP3. These results strongly support p7TP3 as a direct target gene of miR-182-5p.

To investigate the relationship between miR-182-5p and the Wnt signaling pathway, the activity of the pathway was assessed through Western blot hybridization, RT-qPCR, and dual-luciferase reporter assays. Consistent with previous findings, miR-182-5p mimics significantly upregulated Wnt signaling activity, whereas miR-182-5p inhibitors dramatically inhibited its activation. Furthermore, p7TP3 was identified as a direct target of miR-182-5p, acting as a potent tumor suppressor by targeting the Wnt/β-catenin signaling pathway. This, in turn, impedes tumor development, migration, invasion, proliferation, and cell cycle progression in HCC by regulating the expression of c-myc, MMP-7, and cyclin D1 proteins.

NS2TP (also known as CHCHD2) belongs to a protein family characterized by the presence of a (coiled-coil 1)-(helix 1)-(coiled-coil 2)-(helix 2) (CHCH) domain (Song et al., 2015). The CHCH domain comprises two coiled-coil regions, with the first region having a fixed length of 10 amino acids (aa), while the second region exhibits variable lengths ranging from 5 to 10 aa. The second coiled-coil region is believed to act as a bridge, facilitating the folding of the two helices toward each other. Within the CHCH domain, each α-helix contains two cysteine amino acids separated either by nine residues (CX9C motif), as observed in mitochondrial intermembrane space protein Mia40, cytochrome C oxidase copper chaperone, cyclooxygenase 1, and cytochrome C oxidase subunit VIIa, or by three residues (CX3C motif), as observed in the translocase of inner membrane proteins. The formation and stabilization of the protein's tertiary structure are facilitated by two interhelical disulfide bonds.

The cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB), a leucine zipper transcription factor, recognizes the cAMP-response element (CRE) promoter site. CREB is a target of various signaling pathways and can be activated by multiple stimuli, including peptide hormones, growth factors, and neuronal activity. Activation occurs through various protein kinases such as protein kinase A (PKA), PKC, mitogen-activated protein kinases (MAPKs), and Ca^{2+}/calmodulin-dependent protein kinases. Previous studies have indicated that phosphorylation of Ser133 is necessary for CREB-induced gene transcription. Phosphorylation at Ser133 triggers the translocation of cytoplasmic CREB to the nucleus, where it interacts with components of the basal transcription machinery, including transcription factor II D and RNA polymerase II, promoting transcriptional activation. Additionally, CREB undergoes post-translational modifications and contains residues other than Ser133. The regulatory role of CREB extends to various cellular functions, including inflammation, cell proliferation, differentiation, adaptation, and survival.

To investigate the function of NS2TP protein in hepatocellular carcinoma (HCC) development, immunohistochemistry was employed to examine NS2TP protein expression in HCC tissues. The results revealed highly positive rates for moderate- and low-grade HCC in the staining of gene expression markers such as CD34, GPC-3, GS, HSP70, Ki-67, PEG10, and CHCHD2. However, only CD34, CHCHD2, and PEG10 exhibited high

rates for high-grade HCC. Notably, immunohistochemical staining for the CHCHD2 biomarker showed strong positive and diffuse expression throughout the tumor tissue, while being absent in the adjacent normal liver tissue (Song et al., 2015). Subsequently, the transactivating effects of HCV NS2 protein on NS2TP gene expression were confirmed using qPCR and Western blot hybridization techniques. To further understand the underlying regulatory mechanism, a NS2TP promoter-luciferase construct was generated, and the transactivating effects of HCV NS2 protein on NS2TP gene expression were reconfirmed. A putative CREB binding site was predicted in the NS2TP gene promoter sequence. To investigate the binding ability of CREB to the proximal promoter of CHCHD2 in vitro, chromatin immunoprecipitation (ChIP) assays followed by qPCR were performed, revealing a specific binding of CREB to the CHCHD2 promoter. Overexpression of CREB significantly increased NS2TP gene promoter activity. These findings strongly indicate that HCV NS2 is a potential viral protein promoting carcinogenesis. The mechanism underlying the involvement of HCV NS2 protein in HCC development involves the upregulation of NS2TP gene expression, potentially mediated by CREB regulation (Song et al., 2015).

MicroRNA 185 (miR-185), located at 22q11.21, was initially characterized as a regulator of cancer progression. However, its role in hepatocellular carcinoma (HCC) development remained elusive. To investigate the impact of miR-185 on cell growth, we conducted a CCK-8 assay, enabling sensitive colorimetric assessment of viable cell numbers for cell proliferation and cytotoxicity. HepG2 cells were transfected with miR-185 mimics, resulting in a significant reduction in cellular proliferation compared to control cells. Our findings demonstrated that miR-185 mimics strongly suppressed HepG2 cell proliferation. Subsequently, we examined the effect of miR-185 mimics on cell-cycle progression. HepG2 cells were synchronized at the G1/S boundary by 12-hour starvation, followed by transfection with miR-185 mimics. Notably, miR-185 mimics induced significant G1 phase arrest, a critical step in the multistep progression of malignant tumors. We analyzed the expression of cell-cycle-related proteins using western blot analysis and observed an increase in cyclin D1 and a significant reduction in cyclin A and cyclin B1 in cells transfected with miR-185 mimics. Annexin V staining allows the identification of apoptotic cells. Thus, we employed flow cytometry to quantify apoptosis by measuring Annexin V-positive cells. While untreated HepG2 cells exhibited low rates of apoptosis, transfection of miR-185 mimics significantly increased the percentage of Annexin V-positive cells compared to control-transfected HepG2 cells, indicating enhanced apoptosis. The Bax and Bcl-2 proteins mediate hepatocellular carcinoma (HCC) carcinogenesis, and the ratio of Bax to Bcl-2 serves as a hallmark of cellular apoptosis. In our study, HepG2 cells transfected with miR-185 mimics showed significant downregulation of Bcl-2 expression. These results suggest that miR-185 induces apoptosis in HepG2 cells. Autophagy and miRNAs play crucial roles in regulating cancer cell tumorigenesis. Impaired autophagy and altered expression of oncogenic miR-185 are observed in HCC. However, the relationship between these phenomena remains enigmatic. We hypothesized that miR-185 may play an essential role in cellular autophagy, which we validated through an RFP-LC3 punctate formation assay using confocal microscopy and an LC3 conversion assay using western blot analysis. Co-transfection of miR-185 mimics and pRFP-LC3 in HepG2 cells led to increased punctate formation of pRFP-LC3, indicative of autophagosome accumulation. Quantification of RFP-LC3 dots confirmed that miR-185 mimics induced autophagosome accumulation in HepG2 cells. Furthermore, western blot analysis revealed the conversion of endogenous LC3-I to LC3-II, consistent with our earlier observations of RFP-LC3 punctate formation. The levels of SQSTM1/p62 protein indicate whether autophagosome accumulation is related to autophagy induction or inhibition of downstream pathways. Western blot analysis showed that transfection of miR-185 mimics resulted in approximately 40% reduction in SQSTM1 protein expression in HepG2 cells. We examined several autophagy-related genes (PI3KIII, ATG5, ATG3, and beclin 1) using western blot analysis and observed upregulation of their protein levels after transfection with miR-185 mimics, consistent with the LC3-I to LC3-II conversion, a critical autophagy marker. While the concept of miR-185 involvement in cell apoptosis is not entirely novel, we aimed to determine whether miR-185-mediated cell autophagy has a relationship with cell apoptosis. To inhibit autophagy, we used two autophagy inhibitors: 3-methyladenine (3-MA) and chloroquine (CQ). 3-MA blocks class III phosphatidylinositol 3-kinase-dependent autophagosome formation and suppresses starvation-induced autophagy. CQ, on the other hand, inhibits autophagic protein degradation through autophagosome acidification and subsequent proteolytic digestion. Western blot analysis demonstrated increased LC3-II expression after CQ treatment, while 3-MA treatment inhibited the conversion of endogenous LC3-I to LC3-II in HepG2 cells. Remarkably, both inhibitors exhibited autophagy inhibition-independent effects, indicating miR-185-induced autophagy. To investigate whether miR-185-mediated autophagy is a cause or consequence of apoptosis, we examined the contribution of miR-185-mediated autophagy to HepG2 cell apoptosis using 3-MA and CQ to block autophagy. The inhibition of autophagy by these agents increased cell apoptosis, suggesting that autophagy inhibits cell apoptosis and may contribute to tumor cell survival.

To validate these findings, we used small interfering RNA (siRNA) targeting beclin 1, a critical autophagy regulator. Silencing beclin 1 attenuated autophagy in HepG2 cells and reduced the expression of Bcl-2. Consequently, cell apoptosis in beclin 1-siRNA-treated cells resembled that of cells treated with 3-MA and CQ. Collectively, these data indicate that miR-185-mediated autophagy suppresses HepG2 cell apoptosis. To identify potential targets of miR-185 involved in preventing malignant tumor formation, we utilized FindTar, a prediction algorithm developed by Tsinghua University. FindTar predicted several regulators of proliferation and apoptosis signaling, such as AKT1, CDC42, CDK6, RHEB, RICTOR, and IGF2A, as potential miR-185 targets. Additionally, RhoA, SIX1, CCNE1, and RAB5A were also predicted targets of miR-185. We performed quantitative real-time polymerase chain reaction (qRT-PCR) to examine the mRNA levels of these putative miR-185 targets. MiR-185 mimics significantly attenuated the mRNA levels of AKT1, RICTOR, and RHEB in HepG2 cells, as determined by qRT-PCR. Western blot analysis confirmed decreased cellular levels of AKT1, RICTOR, and RHEB in cells transfected with miR-185 mimics. To assess the role of miR-185 in AKT signal transduction, we employed a phospho-Ser473 antibody to directly measure AKT1 activation. Phosphorylation of AKT1 proteins was significantly reduced upon transfection with miR-185 mimics, along with decreased mechanistic target of rapamycin (mTOR) protein levels and mTOR phosphorylation. Overexpression of miR-185 did not alter the expression levels of PTEN or phosphatidylinositol 3-kinase (PI3K), upstream regulators of the AKT pathway. In summary, miR-185 acts as a potent cancer suppressor by targeting multiple components of the AKT pathway, which regulate various cellular functions, including cell proliferation, apoptosis, cell-cycle progression, and autophagy. The relationship between apoptosis and autophagy is complex, as autophagy can serve as a stress adaptation that prevents cell death and suppresses apoptosis, or as an alternative cell-death pathway, depending on the cellular context. Our findings indicate that inhibiting miR-185-mediated cell autophagy promotes cell apoptosis. MiR-185 targets several genes in the AKT pathway to regulate cell proliferation, apoptosis, cell-cycle progression, and autophagy. When miR-185-mediated cell autophagy is suppressed, cell apoptosis is promoted.

Drug development against liver diseases

From our investigation into the host factors associated with HBV cccDNA formation, it is evident that EIF3H-binding proteins and certain calcium homeostasis-related proteins hold promise as therapeutic targets for the eradication of HBV cccDNA from the liver. In the clinical setting, a compound with dual antiviral and immune modulating effects appears to be the future of chronic hepatitis B treatment, as chronic HBV infection induces immune tolerance against the virus. Therefore, an ideal new drug against HBV infection should possess both antiviral and immune modulating properties.

Building upon our foundational research on host factors involved in HBV cccDNA formation, we identified a novel compound called UTU001 and conducted further analysis using structure-activity relationship (SAR) techniques. This preclinical compound (referred to as PCC) demonstrated potent inhibitory effects on HBV replication and expression, significantly reducing HBsAg secretion in cell culture ($EC_{50} \leq$ 100 nM). Moreover, the compound effectively inhibited HBV DNA and HBV cccDNA in various cell models transfected with 1.3-fold HBV genome, including HepG2, HepaG2.2.15, and HepGAD38 cells. Additionally, the PCC inhibited HBV total RNA, pregenomic RNA (pgRNA), and pre-Core RNA transcription. Notably, Southern blot hybridization analysis confirmed significant inhibition of HBV cccDNA by the compound. Western blot hybridization analysis of HBc protein expression, often used as a surrogate marker for HBV cccDNA in studies on capsid assembly inhibitors (CpAMs), revealed a significant decrease upon treatment with the PCC. Surprisingly, we discovered that the PCC also acts as a potent activator or inducer of the innate immune signals cGAS/STING. The induction of cGAS/STING expression by this compound was found to be both time- and dose-dependent. Consequently, UTU001 emerges as a promising candidate for the development of a new drug targeting HBV cccDNA and potentially leading to HBV eradication from the liver.

Furthermore, we identified the HCBP6 gene as a key player in liver steatosis and demonstrated its protective role in its development. Considering HCBP6 as a potential therapeutic target for liver steatosis, it is reasonable to explore strong inducers of HCBP6 expression. Interestingly, the water extract of Korea red ginseng (KRG) was found to induce the expression of HCBP6 (Fundc2) in rat testes, marking the first report of an inducer for HCBP6 expression. Although the study employed a mixture (water extract of KRG) without providing information on its chemical structure (Kim et al., 2011), the significant induction of HCBP6 suggests the presence of abundant compounds within the mixture. Over the past two decades, 48 compounds have been identified from the KRG mixture. Consequently, each of these compounds underwent testing to assess its ability to induce HCBP6 expression. Ultimately, ginsenoside Rh2 emerged as a potent inducer of HCBP6 gene expression. In a mouse model of steatosis induced by a high-fat diet (HFD), ginsenoside Rh2 exhibited a significant reduction in liver steatosis. Notably, ginsenoside Rh2 achieved this effect without discontinuing the

HFD. These in vivo results corroborated our in vitro studies, where ginsenoside Rh2 was confirmed to induce HCBP6 gene expression and reduce cholesterol and triglyceride synthesis in HepG2 and Huh7 cells. Additionally, ginsenoside Rh2 demonstrated the ability to decrease liver steatosis and lower blood glucose levels in overweight mice with type 2 diabetes. Taken together, these findings position ginsenoside Rh2 as a critical therapeutic drug for both liver steatosis and metabolic syndrome.

Furthermore, we identified the NS5ATP9 gene as a protective protein against liver fibrosis/cirrhosis, making the inducer of NS5ATP9 gene expression a potential therapeutic target for these conditions. Notably, our literature review revealed that the antiviral nucleotide analogue tenofovir (TDF) is a potent inducer of NS5ATP9 gene expression. A study assessing the safety profile of a 1% gel formulation of TDF on normal rectal and vaginal mucosa demonstrated a significant increase in NS5ATP9 (KIAA0101) expression after 7 days of application in rectal biopsies, while primary vaginal epithelial cell culture exhibited over a 10-fold increase in NS5ATP9 gene expression at a concentration of 500 μM tenofovir. Given NS5ATP9's inhibitory role in liver fibrosis/cirrhosis, it is reasonable to consider TDF as a potential candidate for combating these conditions. Subsequently, we evaluated the anti-fibrosis/cirrhosis effects of TDF and observed its profound inhibition of COL1A1 expression, with effects dependent on both time and dose. In an in vivo study, TDF significantly attenuated CCl_4-induced liver fibrosis/cirrhosis in mice. Mechanistically, TDF was found to inhibit the viability of the human stellate cell line LX-2 and enhance apoptosis in a time- and dose-dependent manner. Moreover, TDF inhibited Bcl-2 expression and increased Bax expression, thereby promoting apoptosis in the LX-2 cell line. The anti-fibrosis/cirrhosis effects of TDF were mediated by the inhibition of inflammasome signaling pathways, including NLRP3, NF-κB, and IL-1β expression. To expand our understanding of TDF's effects on fibrosis/cirrhosis, we employed a pulmonary fibrosis mouse model induced by bleomycin inhalation. Our results demonstrated that both TDF and tenofovir alafenamide (TAF) exhibited potent protective effects against pulmonary fibrosis in mice. The findings from both liver and pulmonary fibrosis/cirrhosis models highlight TDF/TAF as potential therapeutic drugs for these conditions.

Similarly, we identified NS3TP1 as a protective protein against liver fibrosis/cirrhosis, with its anti-fibrosis/cirrhosis mechanism involving aspartate-mediated regulation of inflammasome signaling pathways, including NLRP3, NF-κB, and IL-1β expression. Aspartate, an essential amino acid with a proven safety profile in clinical use, served as the basis for designing a prodrug containing two aspartate molecules. This compound releases aspartate in vivo, which effectively induces NS3TP1 gene expression and exhibits protective effects against liver fibrosis/cirrhosis. In vitro studies revealed that the compound inhibits COL1A1, COL3A1, and Fn expression while significantly suppressing NLRP3, NF-κB, and IL-1β expression. Moreover, in an in vivo study using a CCl_4-induced liver fibrosis/cirrhosis mouse model, the compound alleviated fibrosis/cirrhosis. These findings underscore NS3TP1 as a potent therapeutic target for liver fibrosis/cirrhosis, and aspartate and its derivatives as potential novel drugs for the treatment of these conditions. Preclinical evaluation of this PCC is currently underway.

Perspectives

Numerous novel genes identified by our research group have yet to undergo comprehensive and systematic investigation. Notably, HCV NS3-binding protein (NS3BP, LOC171391-SEP), HBV S promoter Ⅰ DNA-binding protein (SBP1, ITPR3-SEP), and HBx-binding protein 1 (XBP1) (Fan et al., 2013) have emerged as particularly intriguing candidates implicated in the pathogenesis of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. In-depth exploration is imperative to elucidate the precise functions and roles of these newly discovered genes in liver diseases. Nonetheless, the accumulation of research efforts over the past three decades dedicated to investigating novel genes attests to the efficacy and dependability of this approach within the realm of hepatology research.

Acknowledgement

The author wanted to express sincerely thanks to the following colleagues working together from the year of 1997 to 2003. Without your efforts, nothing is possible.

Bai GQ, Bai RL, Cai J, Chai YY, Chen GF, Chen J, Chen L, Chen LD, Chen TY, Chen XB, Chen XH, Chen YT, Cheng DY, Cheng YQ, Dang XY, Deng XJ, Dong F, Dong J, Dong JL, Dong XQ, Duan FF, Duan HJ, Duan XF, Fan WH, Feng SH, Gao LL, Gao P, Gao XS, Gong M, Gong XL, Guo FJ, Guo J, Han K, Han L, Han M, Hong Y, Hu CC, Huang YP, Huangfu JK, Ji D, Jiang Q, Jiao FP, Ju LZ, Ju W, Ju WH, Lan XY, Li C, Li HJ, Li J, Li K, Li K, Li LX, Li M, Li MH, Li Q, Li W, Li XG, Li XQ, Li Y, Li Y, Li YR, Li YR, Li ZQ, Li ZS, Liang JQ, Liang P, Liang YD, Liang YL, Lin SM, Ling W, Liu C, Liu M, Liu SA, Liu T, Liu W, Liu XC, Liu XJ, Liu Y, Liu YW, Lu HP, Lu Y, Lu YY, Lun YZ, Ma YH, Meng YX, Mou JS, Ni Q, Pan ZP, Qiu RX, Qu JH, Quan M, Rui LL, Shao DZ, Shao FJ, Shao Q, Shen J, Shi CH, Shi L, Shi L, Song H, Song R, Song SS, Sun CF, Sun M, Tao ML, Tian JK, Tian XJ, Tuerxun K, Wang CH, Wang DL, Wang DQ, Wang J, Wang JJ, Wang JJ, Wang L, Wang L,

Wang Q, Wang QX, Wang XC, Wang XJ, Wang Y, Wang YY, Wang ZL, Wei P, Wen SF, Wen ZL, Wu HJ, Wu L, Wu SH, Wu SL, Wu Y, Xiang TX, Xiao L, Xu H, Xu YF, Xu ZQ, Xue RX, Yan J, Yan J, Yang B, Yang Q, Yang S, Yang XL, Yang Y, Yang YJ, Yang YY, Yin L, Yin Y, Yu KK, Yuan J, Yuan XX, Zhang B, Zhang CY, Zhang HD, Zhang J, Zhang J, Zhang JJ, Zhang JK, Zhang L, Zhang LJ, Zhang LY, Zhang MR, Zhang Y, Zhang Y, Zhang YF, Zhang YR, Zhang YX, Zhang ZD, Zhao CS, Zhao J, Zhao LH, Zhao RX, Zheng JM, Zheng TL, Zhong LH, Zhong YW, Zhou L, Zhu LY

References

[1] Ahn SH, Chan HL, Chen PJ, Cheng J, Goenka MK, Hou J, Lim SG, Omata M, Piratvisuth T, Xie Q, Yim HJ, Yuen MF; APPROACH Working Group. Chronic hepatitis B: whom to treat and for how long? Propositions, challenges, and future directions. Hepatol Int. 2010;4(1):386-95.

[2] Bai GQ, Liu Y, Cheng J, Zhang SL, Yue YF, Huang YP, Zhang LY. Transactivating effect of complete S protein of hepatitis B virus and cloning of genes transactivated by complete S protein using suppression subtractive hybridization technique. World J Gastroenterol. 2005;11(25): 3893-8.

[3] Bai GQ, Cheng J, Zhang SL, Huang YP, Wang L, Liu Y, Lin SM. Screening of hepatocyte proteins binding with complete S protein of hepatitis B virus by yeast-two hybrid system. World J Gastroenterol. 2005;11(25):3899-904.

[4] Cai J, Wang SZ, Zhong YW, Cheng J, Ji ZJ, Sheng SL and Zhang C. Screening and characterization of human single-chain Fv antibody against β amyloid peptide 40. Neuroreport. 2003;14(2):265-8.

[5] Chainuvati S, Cheng J, Hou JL, Hsu CW, Jia JJ, Komolmit P, Kwon SY, Lee CH, Li H, Li Y, Liu CJ, Neo BL, Peng CH, Tanwandee T, Wongcharatrawee S, Wu JC, Yu ML, Zhang XX. Patterns of managing chronic hepatitis B treatment-related drug resistance: a survey of physicians in Mainland China, South Korea, Taiwan, and Thailand. Hepatol Int. 2009;3:453-60.

[6] Cai X, Liu X, Xie W, Ma A, Tan Y, Shang J, Zhang J, Chen C, Yu Y, Qu Y, Zhang L, Luo Y, Yin P, Cheng J, Lu L. Hydronidone for the Treatment of Liver Fibrosis Related to Chronic Hepatitis B: A Phase 2 Randomized Controlled Trial. Clin Gastroenterol Hepatol. 2023;21(7):1893-901.

[7] Cai X, Liu X, Xie W, Ma A, Tan Y, Shang J, Zhang J, Chen C, Yu Y, Qu Y, Zhang L, Luo Y, Yin P, Tang Y, Fan R, Xie Q, Zhang J, Liang X, Wang H, Tan D, Cheng J, Chen S, Ning Q, Bai X, Xu M, Chen X, Niu J, Shi J, Ren H, Gao Z, Wang M, Dou X, Hou J, Sun J. Impact of nonalcoholic fatty liver disease status change on antiviral efficacy of nucleos(t)ide analogues in HBeAg-positive chronic hepatitis B. J Med Virol. 2023; 95(2):e28501.

[8] Chen GF, Wei L, Chen J, Duan ZP, Dou XG, Xie Q, Zhang WH, Lu LG, Fan JG, Cheng J, Wang GQ, Ren H, Wang JP, Yang XX, Jia ZS, Fu QC, Wang XJ, Shang J, Zhang YX, Han Y, Du N, Shao Q, Ji D, Li F, Li B, Liu JL, Niu XX, Wang C, Wu V, Wong A, Wang YD, Hou JL, Jia JD, Zhuang H, Lau G. Will Sofosbuvir/Ledipasvir (Harvoni) Be Cost-Effective and Affordable for Chinese Patients Infected with Hepatitis C Virus? An Economic Analysis Using Real-World Data. PLoS One. 2016;11(6):e0155934.

[9] Chen XF, Fan YN, Si CW, Yu YY, Shang J, Yu ZJ, Mao Q, Xie Q, Zhao W, Li J, Gao ZL, Wu SM, Tang H, Cheng J, Chen XY, Zhang WH, Wang H, Xu ZN, Wang L, Dai J, Xu JH. Tenofovir disoproxil fumarate in Chinese chronic hepatitis B patients: Results of a multicenter, double-blind, double-dummy, clinical trial at 96 weeks. World J Clin Cases. 2021;9(18):4690-9.

[10] Cheng J, Chen J. Plasma superoxide dismutase measurement in children with viral hepatitis. Free Rad Res Commun. 1991;12-13:669-73.

[11] Cheng J. Progress in gene therapy for liver diseases. J Gastroenterol Hepatol. 1999;14(Suppl):A261-2.

[12] Cheng J, Zhong YW, Liu Y, Dong J, Yang JZ, Chen JM. Cloning and sequence analysis of human genomic DNA of augmenter of liver regeneration. World J Gastroenterol. 2000;6(2):275-7.

[13] Cheng J, Zhong YW, Liu Y, Dong J, Yang JZ, Zhang LX. Screening and identification of a humanized single chain variable region antibody for hepatitis C virus non-structural 3 protein. Chin J Immunol. 2000;16(4):246-9.

[14] Cheng J, Ke HN, Liu Y, Si CW, Wang QH, Hong WG and Zhong YW. cDNA cloning and expression in murine fibroblast cell line SVT2 of murine interleukin-18. US Chinese J Microbiol Immunol. 2000;2(3):36-9.

[15] Cheng J. Molecular pathogenesis of viral hepatitis. J Gastroenterol Hepatol. 2001;16(Suppl):A185.

[16] Cheng J, Wang L, Li K, Lu YY, Liu Y, Duan HJ, Hong Y, Wang G, Li L, Zhang LX. Cloning and expression of the gene of human augmenter of liver regeneration in yeast cells. Hepatobiliary and Pancreatic Dis Int. 2002;1(1):87-91.

[17] Cheng J, Li K, Wang L, Li L, Liu Y. Study on the steatosis of patients with chronic hepatitis C virus. Korean J Hepatol. 2002;8(Suppl 3):S11-S14.

[18] Cheng J. Molecular mechanisms of hepatitis virus-hepatocyte interactions. J Gastroenterol Hepatol. 2002;17(Suppl 3):S342-S343.

[19] Cheng J, Wang L, Li K, Lu Y, Wang G, Liu Y, Zhong Y, Duan H, Hong Y, Li L, Xia X, Chen J. Screening of augmenter of liver regeneration-binding proteins by yeast-two hybrid technique. Hepatobiliary & pancreatic diseases international: HBPD INT. 2003;2(1):81-4.

[20] Deng H, Dong J, Cheng J, Huangfu JK, Shi SS, Hong Y, Ren XM, Li L. Quasispecies groups in the core promoter region of hepatitis B virus. Hepatobiliary Pancreatic Dis Int. 2002;1(3):392-6.

[21] Deng YQ, Zhao H, Ma AL, Zhou JY, Xie SB, Zhang XQ, Zhang DZ, Xie Q, Zhang G, Shang J, Cheng J, Zhao WF, Zou ZQ, Zhang MX, Wang GQ; China HepB Related Fibrosis Assessment Research Group. Selected cytokines serve as potential biomarkers for predicting liver inflammation and fibrosis in chronic hepatitis B patients with normal to mildly elevated aminotransferases. Medicine (Baltimore). 2015;94(45):e2003.

[22] Dong J, Cheng J, Wang QH, Liu Y, Wang G, Shi SS, Xia XB, Shao Q and Si CW. The preliminary study on hepatitis B virus (HBV) quasispecies in patients with chronic HBV infection. Chin J Infect Dis. 2001;19(4):199-203.

[23] Dong J, Cheng J, Wang QH, Shi SS, Wang G, and Si CW. Cloning and analysis of the genomic DNA sequence of augmenter of liver regeneration from rat. Chin Med Sci J. 2002;17(2):63-7.

[24] Duan FF, Liu C, Liu YW, Chang CY, Zhai H, Xing HC, Cheng J, Yang S. Nomogram to predict the survival of Chinese patients with alcohol-related liver disease. Can J Gastroenterol Hepatol. 2021; 2021: 4073503.

[25] Duan X, Zhang J, Liu S, Zhang M, Wang Q, Cheng J. Methylation

of nucleolar and coiled-body phosphoprotein 1 is associated with the mechanism of tumorigenesis in hepatocellular carcinoma. Oncol Rep. 2013;30(5):2220-8.

[26] Fan R, Sun J, Yuan Q, Xie Q, Bai X, Ning Q, Cheng J, Yu Y, Niu J, Shi G, Wang H, Tan D, Wan M, Chen S, Xu M, Chen X, Tang H, Sheng J, Lu F, Jia J, Zhuang H, Xia N, Hou J; Chronic Hepatitis B Study Consortium. Association of central obesity with hepatocellular carcinoma in patients with chronic hepatitis B receiving antiviral therapy. Aliment Pharmacol Ther. 2021;54(3):329-38.

[27] Fan W, Cheng J, Zhang S, Liu X. Cloning and functions of the HBxAg-binding protein XBP1. Mol Med Rep. 2013;7(2):618-22.

[28] Fan X, Huang W, Pan C, Gao Z, Zhang J, Zhou B, Chen G, Wan M, Tang H, Wang G, Yang Y, Xu D, Dong P, Wang Q, Wang J, Bognar FA, Xu D, Cheng J. HBsAg and HBeAg in the prediction of a clinical response to peginterferon α-2b therapy in Chinese HBeAg-positive patients. Virol J. 2016;13(1):180.

[29] Farooq A, Hoque R, Ouyang X, Farooq A, Ghani A, Ahsan K, Guerra M, Mehal WZ. Activation of N-methyl-d-aspartate receptor downregulates inflammasome activity and liver inflammation via a β-arrestin-2 pathway. Am J Physiol Gastrointest Liver Physiol. 2014;307(7):G732-40.

[30] Feng S, Li M, Zhang J, Liu S, Wang Q, Quan M, Zhang M, Cheng J. Regulation of HepG2 cell apoptosis by hepatitis C virus (HCV) core protein via the sirt1-p53-bax pathway. Virus Genes. 2015;51(3):338-46.

[31] Feng S, Han M, Zhou L, Wang Q, Li Z, Li Y, Lu H, Liu T, Ma Y, Liu S, Cheng J. NS5ABP37 inhibits liver cancer by impeding lipogenesis and cholesterogenesis. Cancer Sci. 2017;108(1):12-22.

[32] Gao LL, Li M, Wang Q, Liu SA, Zhang JQ, Cheng J. HCBP6 modulates triglyceride homeostasis in hepatocytes via the SREBP1c/FASN pathway. J Cell Biochem. 2015;116(10):2375-84.

[33] Gao LL, Meng FP, Cheng J, Li HW, Han J, Zhang WH. Prediction of oesophageal varices in patients with primary biliary cirrhosis by non-invasive markers. Arch Med Sci. 2017;13(2): 370-6.

[34] Gao X, Wang Q, Li W, Yang B, Song H, Ju W, Liu S, Cheng J. Identification of nucleolar and coiled-body phosphoprotein 1 (NOLC1) minimal promoter regulated by NF-κB and CREB. BMB Rep. 2011;44(1):70-5.

[35] Han M, Lu H, Han K, Yuan X, Liu S, Wang Y, Zhao J, Liang P, Cheng J. XTP8 promotes hepatocellular carcinoma growth by forming a positive feedback loop with FOXM1 oncogene. Biochem Biophys Res Commun. 2019;515(3):455-61.

[36] Hladik F, Burgener A, Ballweber L, Gottardo R, Vojtech L, Fourati S, Dai JY, Cameron MJ, Strobl J, Hughes SM, Hoesley C, Andrew P, Johnson S, Piper J, Friend DR, Ball TB, Cranston RD, Mayer KH, McElrath MJ, McGowan I. Mucosal effects of tenofovir 1% gel. Elife. 2015;4:e04525.

[37] Hou JL, Gao ZL, Xie Q, Zhang JM, Sheng JF, Cheng J, Chen CW, Mao Q, Zhao W, Ren H, Tan DM, Niu JQ, Chen SJ, Pan C, Tang H, Wang H, Mao YM, Jia JD, Ning Q, Xu M, Wu SM, Li J. Study Consortium. Baseline quantitative hepatitis B core antibody titre alone strongly predicts HBeAg seroconversion across chronic hepatitis B patients treated with peginterferon or nucleos(t)ide analogues. Gut. 2016;65(2):313-20.

[38] Hou J, Wang G, Wang F, Cheng J, Ren H, Zhuang H, Sun J, Li L, Li J, Meng Q, Zhao J, Duan Z, Jia J, Tang H, Sheng J, Peng J, Lu F, Xie Q, Wei L; Chinese Society of Hepatology, Chinese Medical Association; Chinese Society of Infectious Diseases, Chinese Medical Association. Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update). J Clin Transl Hepatol. 2017;5(4):297-318.

[39] Kim IH, Kim SK, Kim EH, Kim SW, Sohn SH, Lee SC, Choi S, Pyo S, Rhee DK. Korean red ginseng up-regulates C21-steroid hormone metabolism via Cyp11a1 gene in senescent rat testes. J Ginseng Res. 2011;35(3):272-82.

[40] Huang YP, Cheng J, Zhang SL, Wang L, Guo J, Liu Y, Yang Y, Zhang LY, Bai GQ, Gao XS, Ji D, Lin SM, Shao Q. Screening of hepatocyte proteins binding with F protein of hepatitis C virus by yeast two-hybrid System. World J Gastroenterol. 2005;11(30):4709-14.

[41] Huang YP, Cheng J, Zhang SL, Wang L, Guo J, Liu Y, Yang Y, Zhang LY, Bai GQ, Gao XS, Ji D, Lin SM, Shao Q. Screening of hepatocyte proteins binding to F protein of hepatitis C virus by yeast two-hybrid system. World J Gastroenterol. 2005;11(36): 5659-65.

[42] Ji D, Cheng J, Chen GF, Liu Y, Wang L, Guo J. Study of transactivating effect of pre-S2 protein of hepatitis B virus and cloning of genes transactivated by pre-S2 protein with suppression subtractive hybridization. World J Gastroenterol. 2005;11(35): 5438-53.

[43] Johnson C, Han Y, Hughart N, McCarra J, Alpini G, Meng F. Interleukin-6 and its receptor, key players in hepatobiliary inflammation and cancer. Translational Gastrointestinal Cancer. 2012;1(1), 58–70.

[44] Ju W, Yang S, Feng S, Wang Q, Liu S, Xing H, Xie W, Zhu L, Cheng J. Hepatitis C virus genotype and subtype distribution in Chinese chronic hepatitis C patients: nationwide spread of HCV genotypes 3 and 6. Virol J. 2015;12:109.

[45] Jungst C, Berg T, Cheng J, Green RM, Jia J, Mason AL, Lammert F. Intrahepatic cholestasis in common chronic liver diseases. Eur J Clin Invest. 2013;43(10):1069-83.

[46] Li K, Wang L, Cheng J, Lu YY, Zhang LX, Mu JS, Hong Y, Liu Y, Duan HJ, Wang G, Li L, Chen JM. Interaction between hepatitis C virus core protein and translin protein-a possible molecular mechanism for hepatocellular carcinoma and lymphoma caused by hepatitis C virus. World J Gastroenterol. 2003;9(2):300-3.

[47] Li K, Ma QY, Shi L, Dang CX, Hong Y, Wang Q, Li Y, Fan WH, Zhang L, Cheng J. NS5ATP9 gene regulated by NF-κB signal pathway. Arch Biochem Biophy. 2008;479(1):15-9.

[48] Li L, Zhao J, Zhou L, Chen J, Ma Y, Yu Y, Cheng J. Tenofovir alafenamide fumarate attenuates bleomycin-induced pulmonary fibrosis by upregulating the NS5ATP9 and TGF-β1/Smad3 signaling pathway. Respir Res. 2019;20(1):163.

[49] Li M, Wang Q, Liu SA, Zhang JQ, Ju W, Quan M, Feng SH, Dong JL, Gao P, Cheng J. MicroRNA-185-5p mediates regulation of SREBP2 expression by hepatitis C virus core protein. World J Gastroenterol. 2015;21(15):4517-25.

[50] Li MH, Xie Y, Zhang L, Lu Y, Shen G, Wu SL, Chang M, Mu CQ, Hu LP, Hua WH, Song SJ, Zhang SF, Cheng J, Xu DZ. Hepatitis B surface antigen clearance in inactive hepatitis B surface antigen carriers treated with peginterferon alfa-2a. World J Hepatol. 2016;8(15):637-43.

[51] Li MH, Zhang L, Zhang D, Cao WH, Qi TL, Hao HX, Wang XY, Ran CP, Qu XJ, Liu SA, Lu Y, Shen G, Wu SL, Chang M, Liu RY, Hu LP, Hua WH, Wan G, Cheng J, Xie Y. Plasmacytoid dendritic cell function and cytokine network profiles in patients with acute or chronic hepatitis B virus infection. Chin Med J (Engl).

2018;131(1):43-9.

[52] Li X, Hong Y, Wang Q, Liu S, Wei H, Cheng J. Short hairpin RNAs with a 2-or 3-base mismatch inhibit HBV expression and replication in HepG2 cells. Hepatol Int. 2013;7(1):127-33.

[53] Li Y, Pan CQ, Ji SB, Yan GQ, Cheng J, Liu SA, Xing HC. Serum IL-21 levels predict HBeAg decline during rescue therapy in patients with partial response to nucleos(t)ide analogues. Exp Ther Med. 2021;21(3): 216.

[54] Li Y, Liu S, Han M, Lu H, Wang Q, Zhang Y, Tursun K, Li Z, Feng S, Cheng J. NS5ATP13 promotes liver fibrogenesis via activation of hepatic stellate cells. J Cell Biochem.2017;118(8):2463-73.

[55] Li ZQ, Linghu E, Cheng J. Screening of hepatocyte proteins binding with the middle protein of hepatitis B virus by the yeast-two hybrid system. Mol Med Rep. 2014;10(3):1259-63.

[56] Li ZQ, Linghu E, Jun W, Cheng J. Screening of hepatocyte proteins binding with C-terminally truncated surface antigen middle protein of hepatitis B virus (MHBst167) by a yeast-two hybrid. Mol Med Rep. 2014;9(6):2342-6.

[57] Li Z, Feng S, Zhou L, Liu S, Cheng J. NS5ATP6 modulates intracellular triglyceride content through FGF21 and independently of SIRT1 and SREBP1. Biochem Biophys Res Commun. 2016;475(1):133-9.

[58] Liang RY, Xu JH, Si CW, Wang S, Shang J, Yu ZJ, Mao Q, Xie Q, Zhao W, Jun Li, Gao ZL, Wu SM, Tang H, Cheng J, Chen XY, Zhang WH, Wang H, Xu ZN, Wang L, Dai J, Yu YY. A randomized, double-blind, double-dummy, controlled, multicenter study of Qingzhong (tenofovir disoproxil fumarate) versus Viread for the treatment of chronic hepatitis B: First-stage results at week 48. Medicine (Baltimore). 2019;98(33): e16778.

[59] Liang X, Cheng J, Sun Y, Chen X, Li T, Wang H, Jiang J, Chen X, Long H, Tang H, Yu Y, Sheng J, Chen S, Niu J, Ren H, Shi J, Dou X, Wan M, Jiang J, Xie Q, Shi G, Ning Q, Chen C, Tan D, Ma H, Sun J, Jia J, Zhuang H, Hou J. A Randomized, Three-arm Study to Optimize Lamivudine Efficacy in HBeAg Positive Chronic Hepatitis B Patients. J Gastroenterol Hepatol. 2015;30(4):748-55.

[60] Liang X, Xie Q, Tan D, Ning Q, Niu J, Bai X, Chen S, Cheng J, Yu Y, Wang H, Xu M, Shi G, Wan M, Chen X, Tang H, Sheng J, Dou X, Shi J, Ren H, Wang M, Zhang H, Gao Z, Chen C, Ma H, Chen Y, Fan R, Sun J, Jia J, Hou J. Interpretation of liver stiffness measurement-based approach for the monitoring of hepatitis B patients with antiviral therapy: A 2-year prospective study. J Viral Hepat. 2018;25(3):296-305.

[61] Liang X, Gao Z, Xie Q, Zhang J, Sheng J, Cheng J, Chen C, Mao Q, Zhao W, Ren H, Tan D, Niu J, Chen S, Pan C, Tang H, Wang H, Mao Y, Jia J, Ning Q, Xu M, Wu S, Li J, Zhang X, Zhang W, Wei L, Lim SG, Xie Q, Văn KN, Piratvisuth T, Huang Y, Wu S, Xu M, Tang H, Cheng J, Le Manh H, Gao Y, Mou Z, Sobhonslidsuk A, Dou X, Thongsawat S, Nan Y, Tan CK, Ning Q, Tee HP, Mao Y, Stamm LM, Lu S, Dvory-Sobol H, Mo H, Brainard DM, Yang YF, Dao L, Wang GQ, Tanwandee T, Hu P, Tangkijvanich P, Zhang L, Gao ZL, Lin F, Le TTP, Shang J, Gong G, Li J, Su M, Duan Z, Mohamed R, Hou JL, Jia J. Sofosbuvir-velpatasvir for treatment of chronic hepatitis C virus infection in Asia: a single-arm, open-label, phase 3 trial. Lancet Gastroenterol Hepatol. 2019;4(2):127-134.

[62] Lin SM, Cheng J, Lu YY, Zhang SL, Yang Q, Chen TY, Liu M, Wang L. Screening and identification of interacting proteins with hepatitis B virus core protein in leukocytes and cloning of new gene C1. World J Gastroenterol. 2006;12(7):1043-8.

[63] Liu M, Liu Y, Cheng J, Zhang SL, Wang L, Shao Q, Zhang J, Yang Q. Transactivating effect of hepatitis C virus core protein: A suppression subtractive hybridization study. World J Gastroenterol. 2004;10(12): 1746-9.

[64] Liu M, Zhang SL, Cheng J, Liu Y, Wang L, Shao Q, Zhang J, Lin SM. Genes transactivated by hepatitis C virus core protein, a microarray assay. World J Gastroenterol. 2005;11(22): 3351-6.

[65] Liu Y, Wang C-M, Cheng J, Liang Z-L, Zhong Y-W, Ren X-Q, Xu Z-H, Zoulim F, Xu D-P. Hepatitis B virus in tenofovir-naïve Chinese patients with chronic hepatitis B contains no mutation of rtA194T conferring a reduced tenofovir susceptibility. Chin Med J. 2009;122(13):1585-6.

[66] Liu YW, Han K, Liu C, Duan FF, Cheng J, Yang S. Clinical Characteristics and Prognosis of Concomitant Primary Biliary Cholangitis and Autoimmune Diseases: A Retrospective Study. Can J Gastroenterol Hepatol. 2021; 2021: 5557814.

[67] Lu H, Han M, Yuan X, Tursun K, Zhang Y, Li Y, Li Z, Feng S, Zhou L, Pan Z, Wang Q, Han K, Liu S, Cheng J. Role of IL6-mediated expression of NS5ATP9 in autophagy of liver cancer cells. J Cell Physiol. 2018; 233(12):9312-9.

[68] Lu H, Yuan X, Zhang Y, Han M, Liu S, Han K, Liang P, Cheng J. HCBP6 deficiency exacerbates glucose and lipid metabolism disorders in non-alcoholic fatty liver mice. Biomed Pharmacother. 2020;129:110347.

[69] Lu YY, Li K, Cheng J, Wang L, Liu Y and Zhang LX. Cloning and expression of pre-S1 gene of hepatitis B virus in yeast. Hepatobiliary Pancreatic Dis Int. 2002;1(2):238-42.

[70] Lu YY, Cheng J, Yang YP, Liu Y, Wang L, Li K, Zhang LX. Cloning and characterization of a novel hepatitis B virus core binding protein C12. World J Gastroenterol. 2005;11(36):5666-71.

[71] Lun YZ, Cheng J, Zhong YW, Zhao BC. Cloning, expression and identification of the gene of human single-chain variable fragment antibody against Hepatitis B virus surface antigen. Acta Virol. 2006;50(3):195-200.

[72] Lun YZ, Cheng J, Zhong YW, Yu ZG, Wang Q, Wang F, Feng J. Cloning, expression and identification by immunohistochemistry of humanized single-chain variable fragment antibody against hepatitis C virus core protein. Pol J Microbiol. 2011;60(1):13-7.

[73] Lun YZ, Cheng J, Chi Q, Wang XL, Gao M, Sun LD. Transactivation of proto-oncogene c-Myc by hepatitis B virus transactivator MHBst167. Oncol Lett. 2014;8(2):803-8.

[74] Lun YZ, Pan ZP, Liu SA, Sun J, Han M, Liu B, Dong W, Pan LH, Cheng J. The peptide encoded by a novel putative lncRNA HBVPTPAP inducing the apoptosis of hepatocellular carcinoma cells by modulating JAK/STAT signaling pathways. Virus Res. 2020;287:198104.

[75] Qu JH, Cheng J, Zhang LX, Zhang LY, Zhong YW, Liu Y, Wang L, Dai JZ, Xu DP. Identification of genes upregulated by recombinant interferon-alpha in HepG2 cells by suppressive subtractive hybridization analysis. Hepatobiliary Pancreat Dis Int. 2007;6(3):290-3.

[76] Quan M, Liu S, Wang Q, Li G, Zhang Y, Feng S, Liang J, Cheng J. NS5ATP9 promotes Beclin 1-dependent starvation-induced autophagy of hepatoblastoma cells. J Cell Biochem. 2015; 116(8):1574-82.

[77] Shan S, You H, Niu J, Shang J, Xie W, Zhang Y, Li X, Ren H, Tang H, Ding H, Wang X, Nan Y, Dou X, Han T, Zhang L, Liu X, Deng C, Cheng J, Wang X, Xie Q, Lin S, Huang Y, Xu Y, Xiong Y, Li W, Yan X, Piao H, Huang W, Lu Q, Gong W, Li S, Hu X, Zhang X, Liu S, Li Y, Yang D, Li H, Yang C, Cheng M, Zhang L, Zheng H, Luo X,

Lin F, Wang L, Xu G, Xu X, Wei L, Hou J, Duan Z, Zhuang H, Yang X, Kong Y, Jia J; CR-HepB study group, Beijing, China. Baseline characteristics and treatment patterns of the patients recruited to the China Registry of Hepatitis B. J Clin Transl Hepatol. 2019;7(4):322-8.

[78] Shi L, Zhang SL, Li K, Hong Y, Wang Q, Li Y, Guo J, Fan WH, Zhang L, Cheng J. NS5ATP9, a gene up-regulated by HCV NS5A protein. Cancer Lett. 2008;259:192-7.

[79] Shi L, Zhou L, Han M, Zhang Y, Zhang Y, Yuan XX, Lu HP, Wang Y, Yang XL, Liu C, Wang J, Liang P, Liu SA, Liu XJ, Cheng J, Lin SM. Calcitriol attenuates liver fibrosis through hepatitis C virus nonstructural protein 3-transactivated protein 1-mediated TGF β1/Smad3 and NF-κB signaling pathways. World J Gastroenterol. 2023;29(18): 2798-817.

[80] Quan M, Liu S, Li G, Wang Q, Zhang J, Zhang M, Li M, Gao P, Feng S, Cheng J. A functional role for NS5ATP9 in the induction of HCV NS5A-mediated autophagy. J Viral Hepat. 2014;21(6):405-15.

[81] Song H, Wang Q, Wen J, Liu S, Gao X, Cheng J, Zhang D. ACVR1, a therapeutic target of fibrodysplasia ossificans progressiva, is negatively regulated by miR-148a. Int J Mol Sci. 2012;13(2):2063-77.

[82] Song H, Wang Q, Guo Y, Liu SA, Song R, Gao XS, Dai L, Li BS, Zhang DL, Cheng J. Microarray analysis of microRNA expression in peripheral blood mononuclear cells of critically ill patients with influenza A (H1N1). BMC Infectious Diseases. 2013;13: 257.

[83] Song R, Yang B, Gao X, Zhang JQ, Sun L, Wang P, Meng Y, Wang Q, Liu SA, Cheng J. Cyclic adenosine monophosphate response element binding protein transcriptionally regulates CHCHD2 associated with the molecular pathogenesis of hepatocellular carcinoma. Mol Med Rep. 2015; 11:4053-4062.

[84] Sun J, Xie Q, Tan D, Ning Q, Niu J, Bai X, Fan R, Chen S, Cheng J, Yu Y, Wang H, Xu M, Shi G, Wan M, Chen X, Tang H, Sheng J, Dou X, Shi J, Ren H, Wang M, Zhang H, Gao Z, Chen C, Ma H, Jia J, Hou J. The 104-week efficacy and safety of telbivudine-based optimization strategy in chronic hepatitis B patients: a randomized, controlled study. Hepatology. 2014;59(4):1283-92.

[85] Sun J, Cheng J, Chen X, Xie Q, Tan D, Wang H, Chen S, Yu Y, Tang H, Niu J, Sheng J, Sun Y, Ning Q, Wan M, Bai X, Shi G, Ren H, Xu M, Dou X, Shi J, Jiang J, Long H, Wang M, Zhang H, Cao Z, Chen CLH, Ma H, Jia J, Hou J. Assessment of the change in estimated glomerular filtration rates in telbivudine- or lamivudine-based therapy in patients with chronic hepatitis B. J Hepatol 2014;60(1):S206.

[86] Sun J, Ma H, Xie Q, Xie Y, Sun Y, Wang H, SHi G, Wan M, Niu J, Ning Q, Yu Y, Zhou H, Cheng J, Kang W, Xie Y, Fan R, Wei L, Zhuang H, Jia J, Hou J. Response-guided peginterferon therapy in patients with HBeAg-positive chronic hepatitis B: A randomized controlled study. J Hepatol. 2016; 65(4):674-82.

[87] Sun J, Xie Q, Tan D, Ning Q, Niu J, Bai X, Fan R, Liang X, Chen S, Cheng J, Yu Y, Wang H, Xu M, Shi G, Wan M, Chen X, Tang H, Sheng J, Dou X, Shi J, Ren H, Wang M, Zhang H, Gao Z, Chen CHL, Ma H, Chen Y, Jia J, Hou J. Five-year on-treatment systematically monitoring of dynamic changes of liver stiffness measurement with transient elastography compared with paired liver biopsies in a randomized controlled trial in chronic hepatitis B patients. J Hepatol. 2016;64(2):S133-S134.

[88] Tang JT, Wang YF, Han T, Mao Q, Cheng J, Ding HG, Shang J, Zhang Q, Niu JQ, Ji F, Chen CW, Jia JD, Jiang XJ, Lv NH, Gao YQ, Wang ZH, Wei Z, Chen YX, Zeng MD, Mao YM. Tolvaptan therapy of Chinese cirrhotic patients with ascites after insufficient diuretic routine medication responses: a phase III clinical trial. BMC Gastroenterol. 2020; 20: 391.

[89] Wang Q, Du HB, Li M, Li Y, Gao P, Liu SA, Zhang XL, Cheng J. Mapk signal transduction pathway regulates, a novel mechanism of HSC-T6 cell apoptosis induced by FUZHENGHUAYU Tablet. Evidence-Based Complementary and Alternative Medicine. 2013;2013:368103.

[90] Wang Q, Wang YS, Li Y, Gao XS, Liu SA, Cheng J. Cheng NS5ATP9 contributes to inhibition of cell proliferation by HCV NS5A via MEK/ERK pathway. Int J Mol Sci. 2013;21;14(5):10539-51.

[91] Wang Q, Li Y, Liu SA, Xie W, Cheng J. Cell culture-adaptive mutations in hepatitis C virus promote viral production by enhancing viral replication and release. World J Gastroenterol. 2018;24(12):1299-311.

[92] Wang Y, Liang X, Yang J, Wang H, Tan D, Chen S, Cheng J, Chen Y, Sun J, Fan R, Yang W, Liu H, Liu Z, Zhang Y, Liang J, Li S, Liu Z, Hou J. Improved performance of quantitative collagen parameters versus standard histology in longitudinal assessment of non-advanced liver fibrosis for chronic hepatitis B. J Viral Hepatitis. 2017;25(5):598-607.

[93] Wang Y, Han M, Liu S, Yuan X, Zhao J, Lu H, Han K, Liang P, Cheng J. S6K1 inhibits HBV replication through inhibiting AMPK-ULK1 pathway and disrupting acetylation modification of H3K27. Life Sci. 2021;265:118848.

[94] Wang Z, Hou J, Zeng G, Wen S, Tanaka Y, Cheng J, Kurbanov F, Wang L, Jiang J, Naoumov NV, Mizokami M, Qi Y. Distribution and characteristics of hepatitis B virus genotype C subgenotypes in China. J Viral Hepat. 2007;14(6):426-34.

[95] Wei L, Xie Q, Hou JL, Tang H, Ning Q, Cheng J, Nan YM, Zhang LL, Li J, Jiang JN, McNabb B,

[96] Wu X, Shi Y, Zhou J, Sun Y, Piao H, Jiang W, Ma H, Chen Y, Xu M, Xie W, Cheng J, Xie S, Shang J, Cheng J, Xie Q, Ding H, Zhang X, Bai L, Zhang M, Wang B, Chen S, Ma H, Ou X, Jia J, You H. Combination of entecavir with thymosin alpha-1 in HBV-related compensated cirrhosis: a prospective multicenter randomized open-label study. Expert Opinion on Biological Therapy 2018;18(sup1):61-9.

[97] Xie W, Shi G, Zhang H, Zhao G, Yu Z, Lang Z, Zhao H, Yan J, Cheng J. A randomized, multi-central, controlled study of patients with hepatitis B e antigen-positive chronic hepatitis B treated by adefovir dipivoxil or adefovir dipivoxil plus bicyclol. Hepatol Int. 2012;6(2): 441–8.

[98] Xie W, Cao Y, Xu M, Wang J, Zhou C, Yang X, Geng X, Zhang W, Li N, Cheng J. Prognostic Significance of Elevated Cholestatic Enzymes for Fibrosis and Hepatocellular Carcinoma in Hospital Discharged Chronic Viral Hepatitis Patients. Sci Rep. 2017;7(1):10289.

[99] Yan J, Xie W, Wang Q, Li Y, Feng X, Cheng J. The optimal threshold: baseline serum hepatitis B virus DNA and alanine transaminase levels can predict the 2-year on-treatment virological response to lamivudine. Hepat Mon. 2011;11(5):358-63.

[100] Yan J, Xie W, Ou WN, Zhao H, Wang SY, Wang JH, Wang Q, Yang YY, Feng X, Cheng J. Epidemiological survey and risk factors analysis of fatty liver disease of adult residents, Beijing, China. J Gastroenterol Hepatol. 2013;28(10):1654-9.

[101] Yang JD, Nakamura I, Roberts LR. The tumor microenvironment in hepatocellular carcinoma: Current status and therapeutic targets. Semin Cancer Biol. 2011; 21(1), 35–43.

[102] Yang Q, Cheng J, Liu Y, Hong Y, Wang JJ, Zhang SL. Cloning and identification of NS5ATP2 gene and its spliced variant transactivated by hepatitis C virus non-structural protein 5A. World J Gastroenterol. 2004;10(12): 1735-9.

[103] Yang Q, Cheng J, Dong J, Zhang J, Zhan S-L. Molecular epidemiological study on pre-X region of hepatitis B virus and identification of hepatocyte proteins interacting with whole-X protein by yeast-two hybrid. World J Gastroenterol. 2005;11(22):3473-8.

[104] Yang S, Xing H, Wang Y, Hou J, Luo D, Xie Q, Ning Q, Ren H, Ding H, Sheng J, Wei L, Chen S, Cheng J, Wang Y, Hou J, Luo D, Xie Q, Ning Q, Ren H, Ding H, Sheng J, Wei L, Chen S, Fan X, Huang W, Pan C, Gao Z, Zhang J, Zhou B, Chen G, Wan M, Tang H, Wang G, Yang Y, Mohamed R, Guan R, Lee TH, Chang WH, Zhenfei H, Ye Z, Xu D. Peginterferon alfa-2b in the treatment of Chinese patients with HBeAg-positive chronic hepatitis B: A randomized trial. J Clin Virol. 2014;61(4):509-16.

[105] Yang S, Xing H, Wang Q, Wang X, Liu S, Cheng J. De novo entecavir+adefovir dipivoxil+lamivudine triple-resistance mutations resulting from sequential therapy with adefovir dipivoxil, and lamivudine. Ann Clin Microbiol Antimicrob. 2016;15:24.

[106] Yang S, Xing H, Feng S, Ju W, Liu S, Wang X, Ou W, Cheng J, Pan CQ. Prevalence of NS5B resistance-associated variants in treatment-naïve Asian patients with chronic hepatitis C. Arch Virol. 2018;163(2):467-73.

[107] Yang X, Han M, Liu S, Yuan X, Liu X, Feng S, Zhou L, Li Y, Lu H, Cheng J, Lin S. HCBP6 upregulates human SREBP1c expression by binding to C/EBPβ-binding site in the SREBP1c promoter. BMB Rep. 2018;51(1):33-38.

[108] Yu Y, Guo J, Li Y, Hong Y, Li K, Cheng J, Li Y. Effect of bicyclol on gene expression profiles in mice with liver injury induced by concanavalin A. Acta pharmaceutica Sinica. 2008;43(6):596-600.

[109] Yuan X, Lu H, Han M, Han K, Zhang Y, Liang P, Liu S, Cheng J. HCBP6-induced activation of brown adipose tissue and upregulated of BAT cytokines genes. J Therm Biol. 2022;109:103306.

[110] Zeng G, Wang Z, Wen S, Jiang J, Wang L, Cheng J, Tan D, Xiao F, Ma S, Li W, Luo K, Naoumov NV, Hou J. Geographic distribution, virologic and clinical characteristics of hepatitis B virus genotypes in China. J Viral Hepat. 2005;12(6):609-17.

[111] Zhang C, Wang J, Zhang H, Liu S, Lee HJ, Jin W, Cheng J. Hepatitis C virus core protein induces hepatic steatosis via Sirt1-dependent pathway. Liver Int. 2018;38(5):803-12.

[112] Zhang FQ, Camus G, Mo HM, Osinusi A, Brainard DM, Gong GZ, Mou ZB, Wu SM, Wang GQ, Hu P, Gao YH, Jia JD, Duan ZP. Ledipasvir/sofosbuvir for treatment-naive and treatment-experienced Chinese patients with genotype 1 HCV: an open-label, phase 3b study. Hepatol Int. 2018;12(2): 126–32.

[113] Zhang J, Guo J, Cheng J, Wang D, Lun Y, Zhao L, Lan X, Hong Y, Mao Y. Cloning of spliced variant HBeBP4A of hepatitis B virus e antigen binding protein 4. World Chinese Journal of Digestology. 2006;14(15):1462-5.

[114] Zhao H, Cheng J and Si CW. Effects of IL-12 on the immune response in mice inoculated with nucleic acid vaccine expressing S protein of hepatitis B virus. Chin Med J. 2001;114(1):47-52.

[115] Zhao J, Zhai F, Cheng J, He Q, Luo J, Yang X, Shao J, Xing H. Evaluating the significance of viscoelasticity in diagnosing early-stage liver fibrosis with transient elastography. PLoS One. 2017;12(1):e0170073.

[116] Zhang JK, Zhao LF, Cheng J, Guo J, Lun YZ, Hong Y. Screening of genes for proteins interacting with the PS1TP5 protein of hepatitis B virus: probing a human leukocyte cDNA library using the yeast two-hybrid system. Chin Med J. 2006;119(22):1884-91.

[117] Zhang JK, Zhao LF, Cheng J, Guo J, Wang DQ, Hong Y, Mao Y. Screening and cloning for proteins transactivated by the PS1TP5 protein of hepatitis B virus: A suppression subtractive hybridization study. World J Gastroenterol.2007;13 (10):1602-7.

[118] Zhang MR, Zhang JQ, Liu SA, Wang Q, Lin GX, Qiu RX, Quan M, Cheng J. NS5ATP9 suppresses activation of human hepatic stellate cells, possibly via inhibition of Smad3/ phosphorylated-Smad3 expression. Inflammation. 2015;38(1):278-89.

[119] Zhang XX, Ji Y, Dong J, Li J. Tenofovir disoproxil fumarate vs adefovir dipivoxil in Chinese patients with chronic hepatitis B after 48 weeks: a randomized controlled trial. J Viral Hepat. 2015;22(2):85-93.

[120] Zhao J, Han M, Zhou L, Liang P, Wang Y, Feng S, Lu H, Yuan X, Han K, Chen X, Liu S, Cheng J. TAF and TDF attenuate liver fibrosis through NS5ATP9, TGFβ1/Smad3, and NF-κB/NLRP3 inflammasome signaling pathways. Hepatol Int. 2020;14(1):145-60.

[121] Zhao J, Wang Y, Han M, Lu H, Chen X, Liu S, Yuan X, Han K, Liang P, Cheng J. P7TP3 inhibits tumor development, migration, invasion and adhesion of liver cancer through the Wnt/β-catenin signaling pathway. Cancer Sci. 2020;111(3):994-1007.

[122] Zhong Y, Cheng J, Shi S, Zhao J, Wang G, Xia X, Tian X, Li L, Zhang L. Screening and application of human-derived HBsAg bacteriophage single chain antibody in clinical diagnosis. J Exp Clin Toxicol. 2002;16:223-5.

[123] Zhong YW, Cheng J, Wang G, Shi SS, Zhang LX, Li L, Chen JM. The preparation of human single chain Fv antibody against hepatitis C virus E2 protein and its application in immunohistochemistry. World J Gastroenterol. 2002;8(5):863-7.

[124] Zhong YW, Cai J, Zhang CF, Xing XY, Qin EQ, He J, Mao PY, Cheng J, Liu K, Xu DP, Song HB. Mimotopes selected with neutralizing antibodies against multiple subtypes of influenza A. Virology Journal. 2011;8:542-52.

[125] Zhou L, Liu S, Han M, Feng S, Liang J, Li Z, Li Y, Lu H, Liu T, Ma Y, Cheng J. MicroRNA-185 induces potent autophagy via AKT signaling in hepatocellular carcinoma. Tumour Biol. 2017;39(2):1010428317694313.

[126] Zhou L, Liu S, Han M, Ma Y, Feng S, Zhao J, Lu H, Yuan X, Cheng J. miR-185 Inhibits Fibrogenic Activation of Hepatic Stellate Cells and Prevents Liver Fibrosis. Mol Ther Nucleic Acid. 2018;10:91-102.

[127] Zhou L, Zhao J, Han M, Ma A, Yang S, Zeng Y, Cheng J. Aspartate reduces liver inflammation and fibrosis by suppressing the NLRP3 inflammasome pathway via upregulating NS3TP1 expression. J Personalized Med. 2023;13(3):386.