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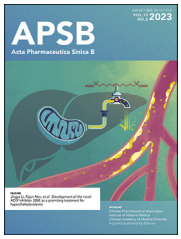

REVIEW

Expression and functions of transient receptor potential channels in liver diseases

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Abstract Liver diseases constitute a major healthcare burden globally, including acute hepatic injury resulted from acetaminophen overdose, ischemia−reperfusion or hepatotropic viral infection and chronic hepatitis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC). Attainable treatment strategies for most liver diseases remain inadequate, highlighting the importance of substantial pathogenesis. The transient receptor potential (TRP) channels represent a versatile signalling mechanism regulating fundamental physiological processes in the liver. It is not surprising that liver diseases become a newly explored field to enrich our knowledge of TRP channels. Here, we discuss recent findings revealing TRP functions across the fundamental pathological course from early hepatocellular injury caused by various insults, to inflammation, subsequent fibrosis and hepatoma. We also explore expression levels of TRPs in liver tissues of ALD, NAFLD and HCC patients from Gene Expression Omnibus (GEO) or The Cancer Genome Atlas (TCGA) database and survival analysis estimated by Kaplan−Meier Plotter. At last, we address the therapeutical potential and challenges by pharmacologically targeting TRPs to treat liver diseases. The aim is to provide a better understanding of the

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implications of TRP channels in liver diseases, contributing to the discovery of novel therapeutic targets and efficient drugs.

1. Introduction

Liver diseases have become major causes of illness and death worldwide over last decades, including acute hepatic injury due to acetaminophen overdose, ischemia−reperfusion or hepatotropic viral infection and chronic hepatitis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), as well as hepatocellular carcinoma (HCC). Especially, ALD and NAFLD exceed with climbing incidences as a result of sedentary lifestyles, chronic alcohol consumption and over-nutrition. Both can progress to more cases of end-stage cirrhosis and HCC with high mortality[1].

The transient receptor potential (TRP) channels are evolutionarily conserved membrane proteins, responding to thermal, chemical, osmotic or mechanical stimuli by regulating membrane potential and activation of calcium (Ca^{2+}), sodium (Na^{+}) and magnesium (Mg^{2+}) fluxes[2]. The TRP superfamily consists of canonical TRP (TRPC), ankyrin TRP (TRPA), melastatin TRP (TRPM), mucolipin TRP (TRPML), polycystin TRP (TRPP) and vanilloid TRP (TRPV) subfamilies, among which there are low sequence homology and marked structure divergence. A functional TRP channel contains four subunits, either homo- or hetero-tetramers[3].

TRPs are ubiquitously expressed in most cells, tissues and organs of the human body, although for individual TRP subtypes, the expression levels can differ greatly. TRPs play multiple roles in sensory perception, vessel relaxation, cell proliferation, etc. Ectopic changes in expression or function of these TRPs have been vigorously explored in skin, sensory, cardiac, ocular, skeletal and neuronal diseases[3,4]. Most TRPs are located at the cell surface, making them generally accessible drug targets. Albeit withdraw or failure of few cases, a growing number of compounds targeting TRPV1, TRPV4, TRPA1 and TRPM8 channels are still undergoing clinical trials[2].

Most TRPs are expressed in liver cells, where they are implicated in maintaining intracellular cationic homeostasis and biological functions of the liver to regulate glucose, fatty acid, amino acid and xenobiotic metabolism, bile acid secretion, protein synthesis and secretion[5]. It is not surprising that dysregulated TRPs participate in pathological processes. In recent years, liver diseases become a newly explored field to further enrich our knowledge of TRP channels. Here we delved into the currently attainable studies related to TRP channels in liver diseases across the fundamental pathological course, from initial hepatocellular injury due to acetaminophen overdose, ischemia−reperfusion, hepatotropic viral infection, high-fat diet, alcohol consumption or environmental factor, to inflammation, subsequent fibrosis and ultimate tumor formation in the liver. We also explored the expression profiles of TRPs in liver tissues of ALD, NAFLD and HCC patients from Gene Expression Omnibus (GEO) or The Cancer Genome Atlas (TCGA) database and survival analysis estimated by Kaplan−Meier Plotter. The aim is to provide a better understanding of the implication of TRP channels in liver diseases, which will enable the discovery of potential therapeutic targets and drug development.

2. TRP channels in acute and chronic liver injury

2.1. *Acetaminophen hepatotoxicity*

Acetaminophen is the most frequently used analgesic and antipyretic drug available over the counter. Acetaminophen overdose has become the most common cause of acute hepatic injury, which is difficult to reverse and if not treated timely can lead to liver failure[6,7]. Acetaminophen is initially metabolised in hepatocytes and eliminated by excretion into bile fluid. Its overdose exceeds the capacity of hepatic elimination, causing depletion of intrahepatic glutathione and aberrant accumulation of intermediate metabolites[6,8]. Glutathione depletion impairs the capacity of hepatocytes to remove reactive oxygen species (ROS), causing oxidative stress.

2.1.1. *TRPM2*

Increased ROS can activate redox-sensitive TRRM2 channels[9] by increasing their translocation to plasma membrane[10] and inducing nonselective cation current in hepatocytes, which mediates Ca^{2+} entry and a very high Na^{+} and K^{+} conductance through these channels. Increased cytoplasmic Ca^{2+} leads to mitochondrial Ca^{2+} overload, the activation of Ca^{2+}-sensitive proteases and lipases, and the stimulation of Ca^{2+}/calmodulin protein kinase II (CaMKII) and supressed autophagy[11]. On the other hand, the accumulation of Na^{+} and loss of K^{+} elicits a loss of the plasma membrane potential and activation of Na^{+}/K^{+} ATPase that contributes to the reduction of cellular ATP levels[12]. *Trpm2* knockdown using siRNA reduces membrane currents and Ca^{2+} entry induced by acetaminophen in primary rat hepatocytes. In *Trpm2* knockout mice, acetaminophen induced-liver injury is substantially ameliorated compared with that in wild-type mice[12].

2.1.2. *TRPV4*

It is not acetaminophen per se but its intermediate metabolites, *e.g.*, *N*-acetyl-*para*-benzoquinoneimine, that evokes Ca^{2+} influx responses in HEK293 cells expressing TRPV4[13,14]. Similarly in primary mouse hepatocytes, *N*-acetyl-*para*-benzoquinoneimine triggers Ca^{2+} influx by activating TRPV4 channels, therefore aggravating oxidative and nitrosative stress as well as mitochondrial membrane depolarization. Deletion and selective pharmacological inhibition of TRPV4 protect hepatocytes against acetaminophen-induced hepatotoxicity both *in vitro* and *in vivo*[14].

2.1.3. *TRPC1, TRPV1 and TRPM7*

Redox-sensitive TRPV1, TRPC1 and TRPM7 contribute to the acetaminophen-induced Ca^{2+} influx and further ROS production

in hepatoma G2 (HepG2) cells. Pre-treatment of HepG2 cells with TRP blockers prior to acetaminophen significantly improves cell viability and reduces the number of apoptotic cells. Similar results are observed using siRNA-mediated knockdown of TRPV1, TRPC1 and TRPM7. Especially, the effects resulted from the suppression of TRPV1 or TRPC1 are stronger, since their activation is triggered by oxidative cysteine modifications mediated by acetaminophen or its metabolites[13].

The overall effect of activation of the above TRPs by acetaminophen is to promote hepatocellular apoptosis. These TRPs could be potential targets for the treatment of acetaminophen induced liver toxicity.

2.2. *Ischemia–reperfusion injury*

Liver surgery including resection or transplant, has been an optimal treatment for advanced cirrhosis, HCC and other advanced liver diseases. However, it also increases the risk of ischemia–reperfusion injury (IRI)[15,16], a main cause of liver dysfunction or functional failure following liver surgery. A better understanding of the mechanisms underlying IRI will provide insights into improving the treatment strategy[15].

2.2.1. *TRPM2*

In a rat model of liver IRI, *Trpm2*, *Trpm6*, *Trpm7*, and *Trpm8* mRNA levels are significantly increased compared to sham-operated livers; verapamil (a calcium entry blocker) can ameliorate necrotic and degenerative differentiations and reduce hemorrhagic area, probably due to its inhibitory effect on the upregulation of these TRPs by IRI[17]. Similarly, adenovirus interference mediated *Trpm2* knockdown yields less hepatic injury, assessed by measurement of blood liver marker enzymes and qualitative liver histology in mice. In addition to direct inhibition on Ca^{2+} entry, *Trpm2* knockdown leads to a reduction of Rac family small GTPase 1 protein level[18], which can physically interact with TRPM2 and increase its expression at the cell membrane[19]. The overall effect is to further decrease cytoplasmic Ca^{2+} and attenuate oxidative stress. Thus, increased TRPM2 is a key molecule contributing to hepatic IRI by Ca^{2+} overload mediated oxidative stress.

2.3. *Alcohol hepatotoxicity*

Chronic alcohol consumption markedly increases the risk of ALD together with obesity, cigarettes and genetic factors[20]. ALD is a continuum from early fatty liver to alcoholic hepatitis, and subsequent cirrhosis with its complications[21]. What molecular-level alterations occur following over-burden alcohol metabolism in the liver is the key to understand the intact pathological course of ALD. Ethyl alcohol (EtOH) is acknowledged as one of extracellular stimuli to activate TRP channels, inducing Ca^{2+} influx[22] that underlies ALD pathological process.

2.3.1. *TRPV1*

Chronic alcohol consumption increases hepatic *Trpv1* mRNA expression, activates metabolic pathways of linoleic acid oxidation and upregulates plasma oxidized LA metabolites (OXLAMs), specifically 9- and 13-hydroxy-octadecadienoic acids[23,24]. As endogenous ligands for TRPV1, OXLAMs elevate intracellular Ca^{2+} levels of HepG2 cells, comparable to those elicited by capsaicin, a classic TRPV1 agonist. TRPV1–OXLAM interaction aggravates hepatic injury through induction of plasminogen activator inhibitor-1, an important alcohol-induced hepatic inflammation mediator. Consistently, genetic depletion of *Trpv1* does not blunt hepatic steatosis caused by ethanol, but prevents hepatic injury[24]. In contrast, oral capsaicin treatment causes a drastic improvement in the hepatic tissue of the alcohol-treated mice, reflected by the normalization of hepatic enzyme and protein levels along with restored histological alterations[25]. Thus, whether TRPV1 plays a protective or deleterious role in ALD development awaits further investigation.

Pertinent studies to identify the functions of other TRPs remain limited. Here, we explore the hepatic TRPs expression in ALD patients compared to normal livers from GEO database. With the exception of *TRPM4* upregulation, *TRPM2*, *-7*, *-8* and *TRPP2* show accordant decrease at RNA levels in ALD liver tissues (Fig. 1). Similarly, EtOH treatment significantly decreases RNA levels of most of these TRPs but exclusively reduces protein levels of TRPM2 and TRPM7 in brain microvascular endothelial cell. EtOH mediated TRPM7 downregulation leads to the disruption of endothelial Ca^{2+} and especially Mg^{2+} homeostasis, which further enhances endothelial cell barrier permeability[22]. Current clues suggest that alterations in hepatic TRPM2, -4, -7, -8 and TRPP2 by alcohol exposure may contribute to ALD development.

2.4. *High-fat diet hepatotoxicity*

Longstanding high-fat diet intake triggers NAFLD progression by early non-alcoholic fat liver (NAFL) with excessive fat accumulation in hepatocytes to non-alcoholic steatohepatitis (NASH) with steatosis, necro-inflammation, hepatocyte injury and different degrees of fibrosis[26]. Excessive fat accumulation in hepatocytes yields impaired intracellular Ca^{2+} homeostasis[27] and aberrant Ca^{2+} redistribution among organelles further enhances lipid accumulation by positive feedback, fuels the progression to NASH and raises insulin resistance[28,29]. As important channels for sustaining intracellular Ca^{2+} levels, TRP channels are involved in the pathological process.

2.4.1. *TRPM2*

As shown in Fig. 2, *TRPM2* RNA level displays an increase tendency in both NAFL and NASH livers compared to healthy control and healthy obese groups. In line with this is palmitic acid-treated hepatic L02 cells, which show significant increases at both *TRPM2* mRNA and protein levels. Consequently, activated TRPM2/Ca^{2+}/CaMKII pathway contributes to palmitic acid-induced cell injury and lipid accumulation. These effects are alleviated by mitigating oxidative stress with a powerful antioxidative, salidroside, which also yields TRPM2 downregulation, subsequent reduction in cytoplasmic Ca^{2+} and increased autophagic clearance in a dose-dependent manner[30].

2.4.2. *TRPM4*

TRPM4 RNA levels are significantly increased in NAFL and NASH livers, compared to both healthy control and healthy obese groups (Fig. 2). Likewise, the livers of mice fed with high-fat diets and methionine choline-deficient diets have significant elevation of *Trpm4* mRNA and protein levels. Gexia Zhuyu decoction alleviates all stages of NAFLD, by inhibiting TRPM4 expression[31], indicating that TRPM4 plays a detrimental role in NAFLD development.

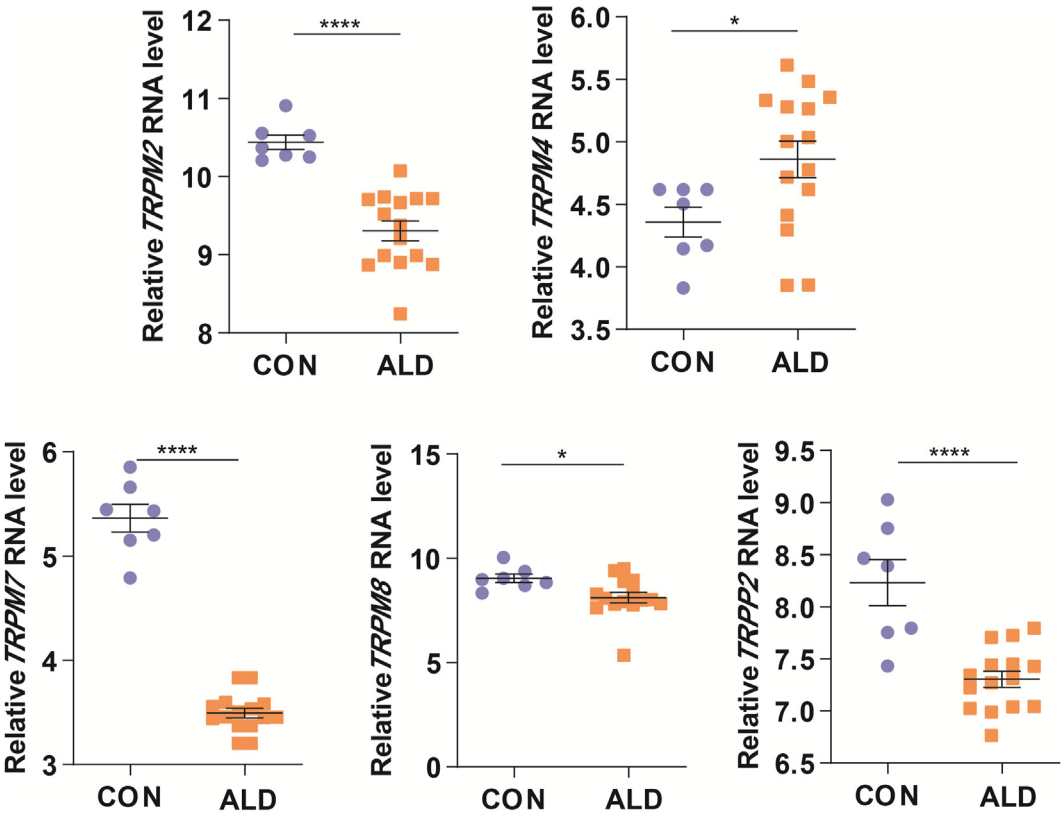


Figure 1 Hepatic expression of TRPs in ALD patients. The GEO data set GSE28619 is analysed to obtain differentially expressed genes (mean ± SEM) from the control ($n = 7$) and ALD ($n = 15$) groups. Comparisons between groups were carried out using unpaired-*t* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. ALD, alcoholic liver disease.

2.4.3. *TRPV1*

TRPV1 expression is detectable in HepG2 and mouse liver tissues[32]. Dietary capsaicin reduces lipid accumulation and triglyceride level in the livers from high-fat diet fed mice. These effects are deprived in the livers from *Trpv1*$^{-/-}$ mice[32]. TRPV1 activation upregulates hepatic uncoupling protein 2[32], phosphorylated hormone-sensitive lipase, carnitine palmitoyltransferase 1 and peroxisome proliferator-activated receptor δ[33] to promote lipid metabolism. On the other hand, capsaicin decreases the expression of key enzymes involved in the synthesis of fatty acids, such as acetyl Co-A carboxylase and fatty acid synthase[34], which could be also associated with TRPV1 activation.

2.4.4. *TRPV4*

RNA levels of *TRPV4* also show a notable increase in NAFL and NASH livers (Fig. 2). Interestingly, TRPV4 protein expression in NAFLD mouse livers show an initial increase followed by a rapid decrease with disease progression, which is regulated by CYP2E1 (a cytochrome p450 enzyme)-mediated promoter methylation[35]. *Trpv4*$^{-/-}$ mice display increased liver injury and inflammation, CYP2E1 protein levels and CYP2E1-mediated oxidative stress. TRPV4 activation leads to induction of endothelial nitric oxide synthase in Kupper cells and blockade of CYP2E1-mediated redox toxicity to hepatocytes[35]. Thus, TRPV4 may behave as an endogenous defensive mechanism to resist NAFLD.

Similar to *TRPM2*, *TRPM4* and *TRPV4*, *TRPML1* and *TRPV2* are increased in NAFL stage and maintain high expression levels in NASH stage (Fig. 2). There is still a lack of understanding the functions of TRPV2 in NAFLD. TRPML1 is predominantly localized on the membranes of late endosomes and lysosomes in all mammalian cell types to mediate Ca^{2+} efflux from these compartments into the cytosol. TRPML1 regulates autophagy[36] and *endo*-lysosomal network incorporating endocytic trafficking, lysosome reformation, lysosomal degradation, lysosomal exocytosis as well as autophagic vesicle−lysosome fusion[37]. Autophagy and *endo*-lysosomal network are both impaired during the pathogenesis of NAFLD[38,39]. Taken together, it indicates that elevated TRPML1 in NAFLD may be a compensatory mechanism to clear excessive lipid in the liver.

2.5. *PM2.5 hepatotoxicity*

PM2.5, also known as fine particles, refers to particulate matter with a dynamic diameter of ≤2.5 μm in air pollutants, carrying metals (Zn, Co, Cd) that pass through the alveolar epithelium to enter the circulatory system and other tissues[40]. Long-term PM2.5 exposure closely correlates with NAFLD and HCC[41−43].

2.5.1. *TRPV6*

Using human hepatocytes L02 exposed to PM2.5, TRPV6 was screened out to be one of the key targets associated with PM2.5 hepatotoxicity, which exerts the effect by facilitating the transportation of metals and minerals into cells. Furthermore, overexpressing TRPV6 exacerbates cell apoptosis caused by PM2.5 and positively promotes ROS production while a ROS scavenger, *N*-acetyl-L-cysteine alleviates PM2.5 and TRPV6 overexpression

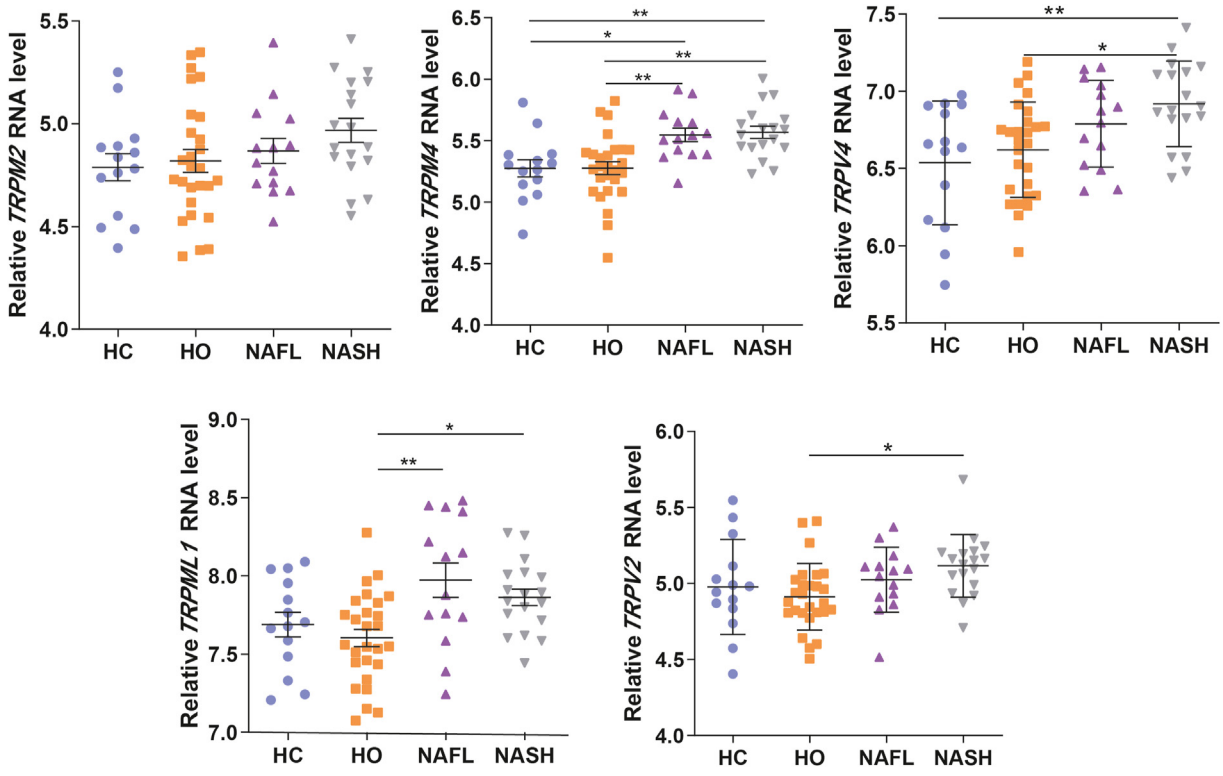


Figure 2 Hepatic expression of TRPs in NAFLD patients. The GEO data set GSE48452 is analysed to obtain differentially expressed genes (mean ± SEM) among the healthy control (HC, $n = 14$), healthy obese (HO, $n = 27$), NAFL ($n = 14$) and NASH ($n = 18$) groups. Comparisons between groups were carried out using unpaired-*t* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fat liver; NASH, non-alcoholic steatohepatitis.

induced apoptosis[40]. These findings suggest that TPRV6 promotes the toxic effects of PM2.5 by facilitating absorption of fine particles and aggravating oxidative stress in hepatocytes.

2.6. *Hepatotropic viral infection*

Global incidence and related mortality of hepatitis B virus and hepatitis C virus infection remain stably high in last decades[44]. Currently, the overwhelming severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) also causes acute liver injury or exacerbates pre-existing chronic liver disease leading to higher mortality[45,46]. Hepatitis B virus, hepatitis C virus and SARS-CoV-2 belong to enveloped viruses, which encode envelope proteins essential for binding and entry into host cells. Despite the lack of work comprehensively verifying the direct connection between TRPs and hepatotropic viral resultant liver injury in patients, convincing studies show that TRPs are indispensable for the cellular entry of enveloped viruses. TRPV2 interacts with the spike protein of SARS-CoV-2 at 39.5 °C to mediate its entry into primary bovine alveolar macrophages and THP-1[47]. TRPML2 facilitates cellular entry of enveloped RNA viruses including influenza A virus, yellow fever virus and Zika virus, by enhancement of viral vesicular trafficking and subsequent escape from endosomal compartments[48]. Increased membrane expression of TRPC1 facilitates herpes simplex virus type 1, an enveloped DNA virus, to enter host cells through an interaction between its third ectodomain with the glycoprotein D of herpes simplex virus type 1[49]. Together with ubiquitous expression of TRPs in human liver cells[5], the above findings suggest that TRPs may be important for the hepatocellular entry of enveloped hepatotropic viruses in patients.

Acetaminophen hepatotoxicity and IRI progress fast once happening, demanding timely pharmacological intervention. ALD, NAFLD, hepatotropic viral infection and PM2.5 mediated hepatotoxicity always progress slowly and have been neglected until symptoms appear or abnormal biomarkers tested by annual physical examination. With the exception of hepatitis viral infection being treated timely alongside by antiviral prescription once the diagnosis is made, therapeutic strategies for the above liver injury largely depend on the modification of personal behaviours. For instance, suggested lifestyle adjustment for NAFLD and ALD consists of healthy diet, abstinence from alcohol, exercise and weight loss[26,50]. Apparently, this heavily demands on self-discipline, which represents a huge challenge in most cases[51], turning the situation to a pursuit of superadded symptomatic treatment or even liver transplantation. Hence, efficient pharmaceutical agents to intervene early hepatocellular injury in time is urgently needed.

TRP channels may serve as novel therapeutic targets to treat acute and chronic liver injury. TRP channels respond to various etiological factors mentioned above by expression alteration or enhanced activation/inactivation, disturbing the hepatic homeostasis of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}. Mechanistically, augmented influx Ca^{2+} to the cytoplasm results in mitochondrial Ca^{2+} overload causing reduction of cellular ATP levels, the activation of Ca^{2+}-sensitive proteases and lipases yielding hepatocyte injury, and the activation of Ca^{2+}/CaMKII axis to supress autophagy[11]. Accumulation of Na^{+} and loss of K^{+} impair the plasma membrane

potential and stimulate Na^+/K^+ ATPase, further reducing cellular ATP levels[12]. Since increased intracellular Mg^{2+} promotes glycodeoxycholate-induced apoptosis in rat hepatocytes by stimulation of Mg^{2+}-dependent endonucleases[52], this indicates that permeability of TRPs to Mg^{2+} could also contribute to hepatocellular apoptosis. As a common downstream of various etiological factors, enhanced ROS generation caused by mitochondrial Ca^{2+} overload, further fuels oxidative stress, which is a crucial factor in multiple acute and chronic liver injuries. On the other hand, TRP mediated cationic-independent absorption of fine particles and possible viral cellular entry also contribute to liver injury. Nevertheless, TRPs reported to have contrary results, such as TRPV1 in ALD progression, or analyzed here by datamining the existing databases to show significantly altered expression, await further investigation to explore their function and underlying molecular mechanisms in relevant liver injury (Fig. 3 and Supporting Information Table S1).

3. TRP channels in inflammatory response

TRP channels are expressed in most inflammatory and immune cells and play critical roles in maintaining their cellular functions (such as phagocytosis, cytokines production, cell survival and polarization)[53–55]. Thus, perturbations in the expression levels or active/inactive states of TRPs in various pathological conditions could contribute to immune or inflammatory response.

In the liver, consecutive exposure to the aforementioned deleterious etiological factors results in hepatocellular death, which triggers hepatic inflammation by activation of residential Kupffer cells and subsequent chemokine-mediated recruitment of

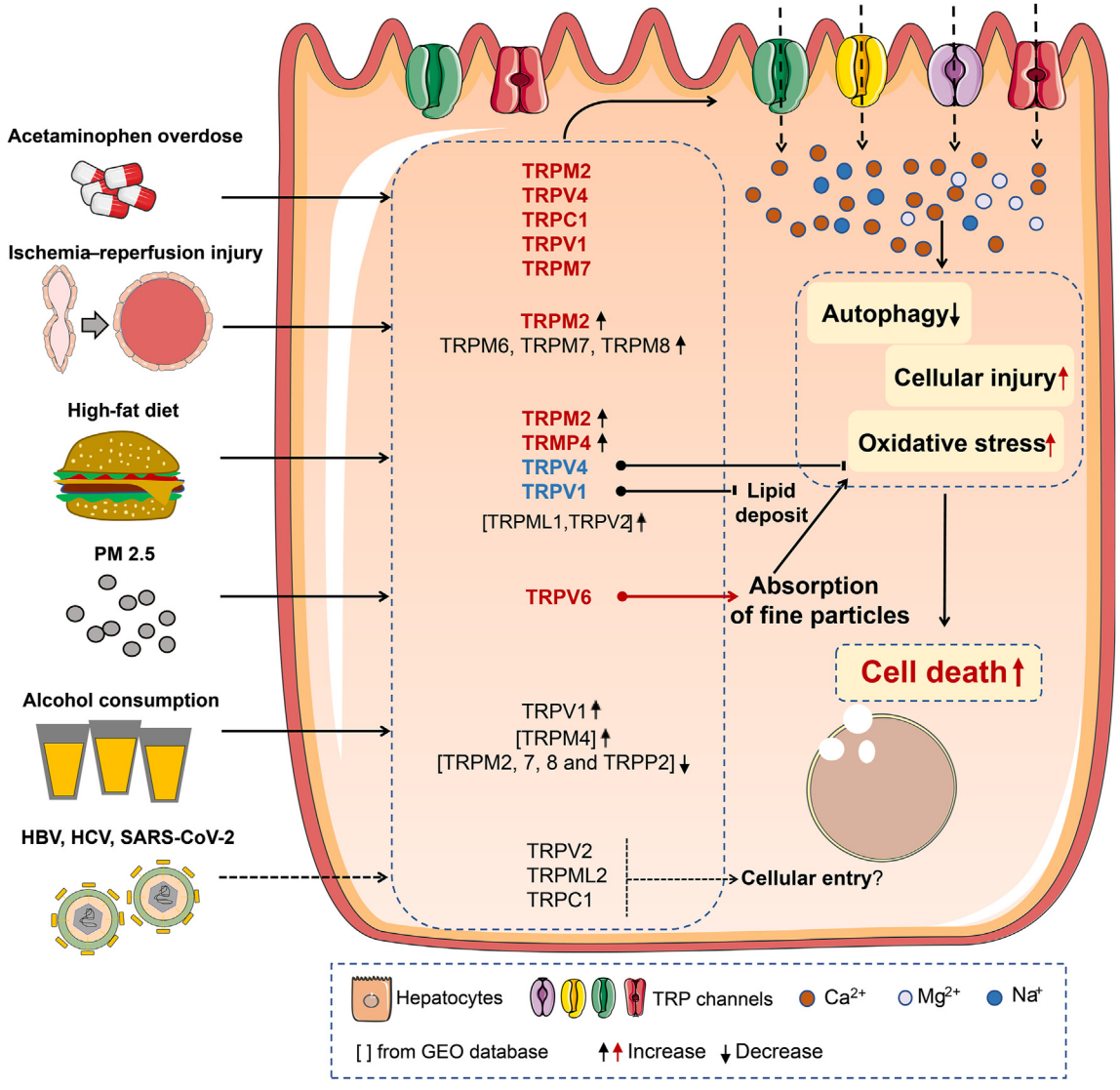


Figure 3 The scenario explaining how TRPs expression is altered and contributes to liver injury due to different etiological factors. Acetaminophen overdose, alcohol consumption, high-fat diet, ischemia−reperfusion injury, PM2.5 and hepatotropic viral infection cause upregulation and/or activation of TRPs (in red), which elevates intracellular Ca^{2+}, Mg^{2+} and Na^+ levels. Consequent oxidative stress, cellular injury and autophagy inhibition together with possible cationic-independent mechanisms (absorption of fine particles and viral cellular entry) lead to hepatocellular death. Conversely, TRPV4 and TRPV1 (in blue) play protective roles in high-fat diet mediated insult. The functions of the TRPs in black await further investigation.

blood-derived monocytes and neutrophils. The disruption of TRPV1 significantly reduces the mRNA levels of hepatic chemokines (*Ccl2* and *Cxcl2*) and proinflammatory cytokines (*Tnf-α*, *Il-1α*, *Il-1β* and *Il-6*), and attenuates neutrophil infiltration induced by chronic binge ethanol in *Trpv1* knockout mice[24]. TRPV3 inhibitor significantly reduces F4/80, a surface marker of macrophages, together with decreased mRNA levels of *Tnf-α*, *Il-1β* and *Il-6* in the livers from the carbon tetrachloride (CCl_4)-treated mice and *vice versa*[56]. Moreover, TRP channels expressed in neutrophils and monocytes/macrophages manipulate their response to chemokines[57] and their competency of subsequent migration and adhesion[58–60]. Therefore, TRPs potentiate inflammatory response following hepatocellular injury *via* controlling biological functions of Kupffer cells, monocytes and neutrophils including cytokine and chemokine production, response to chemokines, adhesion and migration from blood stream to injured hepatic sites (Fig. 4).

Despite initial exploration sprouts in liver disease, TRP channels have been widely investigated in other inflammatory diseases, such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). In RA development, TRPV1, TRPV4 and TRPA1 show increased expression and enhanced function, as downstream to TNF-α release[61,62]. Both TRPV1 and TRPV4 foster the proliferation of synovial fibroblasts, a critical cell group in the genesis and development of RA by release of pro-inflammatory cytokines[63,64]. *Trpv1*$^{-/-}$ mice display alleviated knee swelling and thermal hyperalgesia[65]. TRPV4 blocker, ruthenium red, inhibits the proliferation of synovial fibroblasts stimulated by hypotonic stimulus *in vitro*[63]. In contrast, TRPA1 selectively triggers necrosis of proinflammatory synovial fibroblasts[62]. IBD consists of ulcerative colitis and Crohn's disease, featured cumulative leukocyte infiltration and pro-inflammatory cytokine production. Growing evidence suggests crucial involvement of TPRs in the pathogenesis of IBD. TRPV1 channel is expressed in $CD4^+$ T cells and increases their proinflammatory properties in mouse models of colitis[66]. Whereas expressed TRPA1 counteracts TRPV1 activity to deter $CD4^+$ T cells activation and colitogenic responses[67]. Differently, TRPV4 is expressed in intestinal epithelial cells[68]. And its upregulation and activation facilitate IBD progression by elevating chemokine release[68] and impairing epithelial barrier[69].

These findings indicate that TRP are ubiquitously expressed in immune, parenchyma and mesenchymal cells. In various pathological conditions, they show similar or even inverse effects, orchestrating inflammatory response in the liver, arthrosis and intestine (Table 1). TRP channels may therefore have therapeutic implications for hepatitis, RA and IBD.

4. TRP channels in hepatic fibrosis

Hepatic fibrogenesis is a dynamic procedure with accumulation of extracellular matrix occurring across chronic liver injury caused by various aetiology[70]. Fibrogenesis is provoked by the activation of hepatic stellate cells (HSCs), *i.e.*, transdifferentiation from quiescent, vitamin-A-storing cells into proliferative, fibrogenic myofibroblasts, which is well acknowledged nowadays as a pivotal driver in both experimental and human fibrotic liver tissues[71].

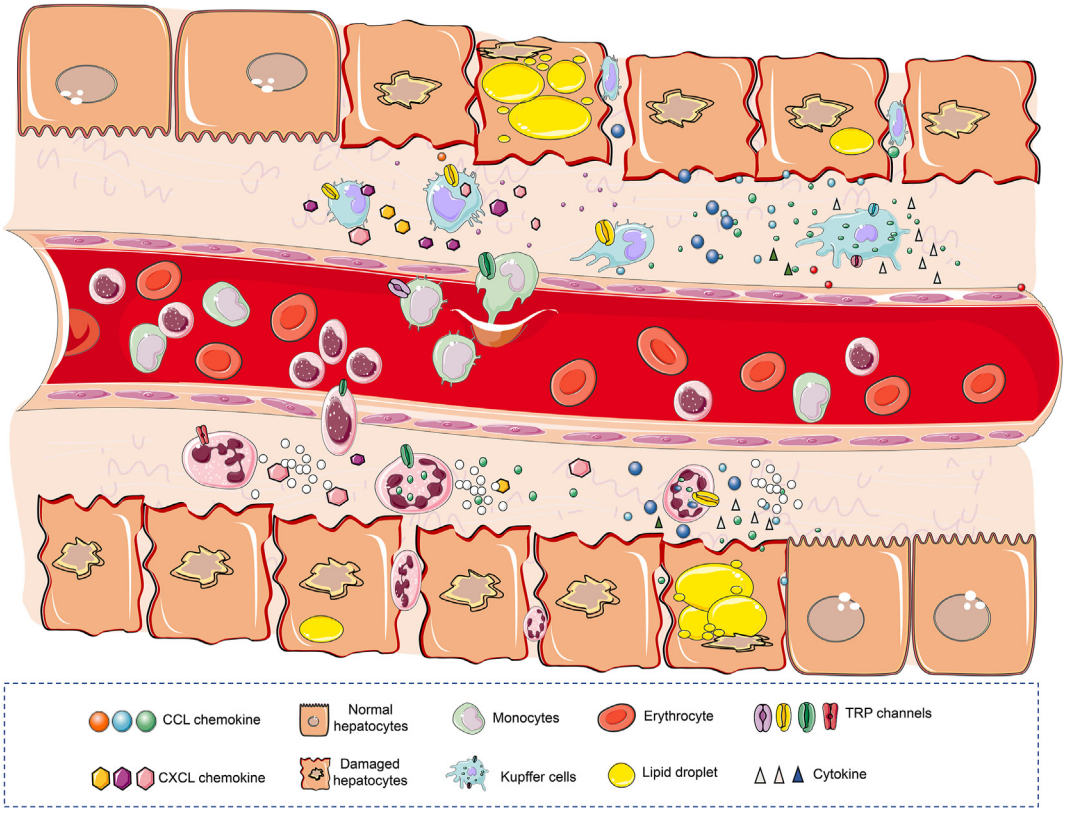


Figure 4 The scenario explaining how TRP channels mediate inflammatory response following hepatocellular injury. TRPs are important for the activation of residential Kupffer cells and the release of cytokines and chemokines. TRPs expressed in monocytes and neutrophils regulate chemokine production, response to chemokines, adhesion and migration from blood stream to injured hepatic sites.

Table 1 TRP channels in inflammatory response.

Disease	Channel	Cell type	Functions	Ref.
Hepatitis	TRPV1	Neutrophil	Promoting neutrophil infiltration; increasing the mRNA levels of hepatic chemokines (*Ccl2* and *Cxcl2*) and proinflammatory cytokines (*Tnf-α*, *Il-1α*, *Il-1β* and *Il-6*)	24
	TRPV3	Macrophage	Promoting macrophage infiltration; increasing the mRNA levels of *Tnf-α*, *Il-1β* and *Il-6*	56
RA	TRPV1	Synoviocyte;	Fostering the proliferation of synovial fibroblasts and the release of pro-inflammatory	64
	TRPV4	Synovial fibroblast	cytokines	63
	TRPA1		Triggering necrosis of proinflammatory synovial fibroblasts	62
IBD	TRPV1	$CD4^+$ T cell	Increasing proinflammatory properties	66
	TRPA1		Deterring $CD4^+$ T cells activation and colitogenic responses	67
	TRPV4	Intestinal epithelial cell	Elevating chemokine release and impairing epithelial barrier	68,69

RA, rheumatoid arthritis; IBD, inflammatory bowel disease.

4.1. *Experimental models*

Molecular mechanisms underlying hepatic fibrosis is complicated, demanding suitable experimental models. For example, *Trpc6* knockout mice fed with choline deficient, L-amino acid-defined, high-fat diet (CDAHFD) have no obvious difference in expression of TIMP metallopeptidase inhibitor 1 and collagen type I alpha 1 chain (COL1A1), two factors involved in fibrosis compared to wild-type mice[72]. What draws attention here is that these mice were fed with CDAHFD for merely 6 weeks. The time span appears too short to initiate fibrogenesis, because many established diet-induced mouse models for NASH require around 24–52 weeks[73–75]. Addition of CCl_4 exacerbates histological features of NASH, fibrosis and tumor development induced by Western diet, which almostly mimics histological, immunological and transcriptomic features of human NASH[73]. Albeit not the most optimal to simulate the natural pathological progression of human fibrotic liver, *in vivo* CCl_4-treated experimental models together with *in vitro* HSC cell lines are widely utilized to explore molecular mechanisms contributing to fibrogenesis, including TRP channels.

4.2. *HSC activation and extracellular matrix generation*

4.2.1. *TRPC6*

In addition to the CDAHFD diet-fed mouse model, the function of TRPC6 in fibrotic liver disease has been investigated in human HSC cell line lx-2 under hypoxia[76]. Briefly, the upregulated hypoxia inducible factor 1α enhances Notch intracellular domain activation, which facilitates the expression of TRPC6 in lx-2 cells. TRPC6 activation leads to increased intracellular Ca^{2+}, coupled with the activation of the calcineurin-nuclear factor of activated T-cell and TGF-β signaling pathways, which further activated the synthesis of extracellular matrix (ECM) proteins[76].

4.2.2. *TRPV3*

Cumulative work has validated the contributory role of TRPV3 activation in cardiac[77] and dermal[78] fibrosis, which as well holds true in the liver. TRPV3 expression is significantly upregulated in human hepatic cirrhosis tissues compared to normal counterparts. In CCl_4-induced hepatic fibrosis mouse model, TRPV3 inhibitor significantly ameliorates liver fibrosis, whereas its agonist exacerbates fibrosis progression. *In vitro*, *Trpv3*-siRNA impairs DNA synthesis, inhibits HSC cell proliferation and concomitantly enhances cell apoptosis[56].

4.2.3. *TRPV4*

Both mRNA and protein of TRPV4 are dramatically increased in fibrotic liver tissues of both patients and CCl_4-treated rats as well as TGF-β1 simulated HSC-T6 cells. Blockade of TRPV4 using ruthenium red or TRPV4-siRNA inhibits the proliferation of HSC-T6 cells and decreases myofibroblast markers α-smooth muscle actin (α-SMA) and COL1A1[79]. These results are in line with pharmacological inhibition of TRPV4 in a mouse model of CCl_4-induced liver fibrosis, where collagen fiber deposition and α-SMA levels are markedly diminished meanwhile the hepatic lobule disorganization is noticeably alleviated[80].

4.2.4. *TRPM7*

Akin to activated rat HSC cells, upregulated mRNA and protein levels of TRPM7 have been observed in fibrotic liver tissues of patients and CCl_4-treated rats. TRPM7 blocker 2-APB and *Trpm7*-siRNA in these studies show similar effects, *i.e.*, markedly inhibited proliferation but induced apoptosis of HSC cells and the decrease of α-SMA and COL1A1 production[81–84].

Fibrosis is a major determinant of clinical outcomes in patients with alcoholic hepatitis/NASH, increasing the risks of cirrhosis and HCC. Collectively, TRPC6, TRPV3, TRPV4 and TRPM7 are accordantly elevated to promote fibrosis development, underscoring their potential as novel diagnostic predictors (Fig. 5). Targeting these TRP channels at either expression or function level could be beneficial to facilitate fibrosis resolution and liver regeneration to protect livers from advanced cirrhosis and even HCC.

5. TRP channels in liver cancers

Hepatocarcinogenesis takes decades from hepatocellular injury, inflammation, fibrosis and further cirrhosis to ultimate malignant tumor formation. Deranged intracellular Ca^{2+} homeostasis provides a special microenvironment in the liver, to facilitate the occurrence of driver mutations[28] of vital components from Wnt/β-catenin pathway[85,86], TP53/cell-cycle pathways, telomere maintenance and chromatin regulators[87] to promote rapid growth of hepatocytes. Aberrant expression and dysfunction of TRP channels cannot be neglected in this pathological procedure[88].

TRP channels have been reported to mostly show increased expression in tumoral liver tissues or HCC cell lines[89–106], partially in line with our analysis using RNA-seq data from TCGA database, as summarized in Table 2 and Fig. 6A. Functionally, TRPC1, TRPC6, TRPM7 and TRPV4 seem pro-tumor by promoting proliferation, dedifferentiation, migration, metastasis and

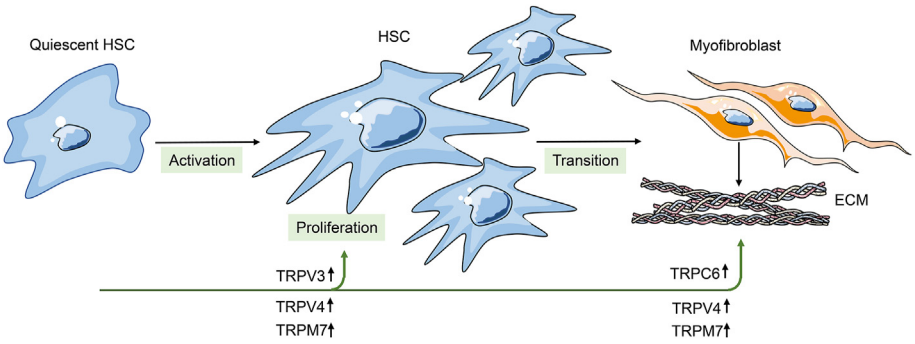
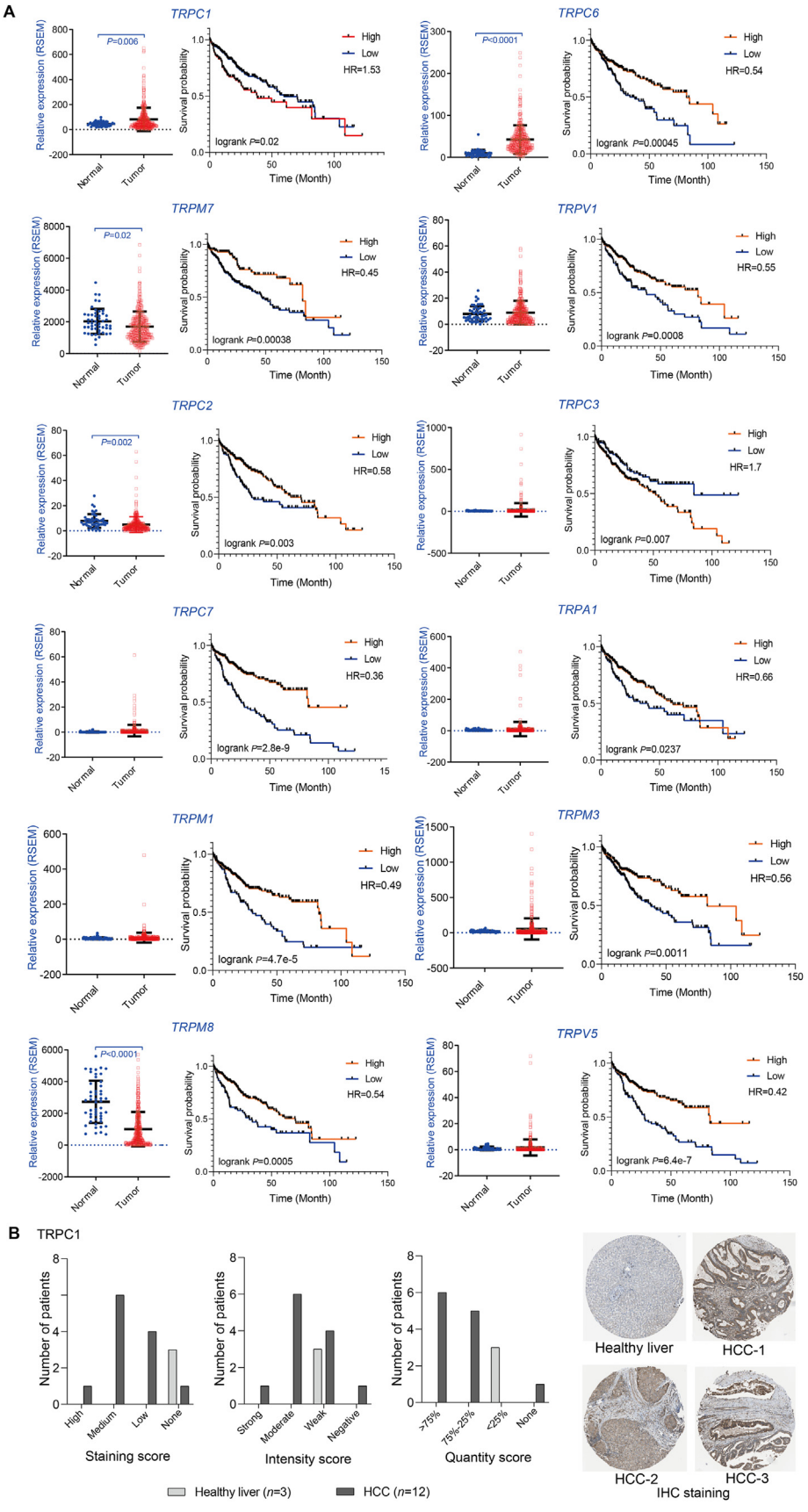


Figure 5 Functions of TRP channels on HSC to promote hepatic fibrosis. TRPC6, TRPV3, TRPV4 and TRPM7 are commonly increased in fibrotic liver tissues. TRPV3, TRPV4 and TRPM7 potentiate HSC proliferation and TRPC6, TRPV4 and TRPM7 promote the production of α-SMA and COL1A1, the main components of ECM accumulated in fibrotic foci. HSC, hepatic stellate cells; ECM, extracellular matrix; α-SMA, α-smooth muscle actin; COL1A1, collagen type I alpha 1 chain.

Table 2 Expression and functions of TRP channels and association with overall survival in liver cancers.

Channel	Expression	Experimental model	Findings regarding to liver cancers	# Association with overall survival	
				HR	*P* value
TRPC1	#mRNA↑; Not available in liver tissues or cell lines	TRPC1 shRNA mediated silence in Huh7 cell line[89,90]	Promoting Huh7 cell proliferation[89,90]	1.53	0.02
TRPC6	#mRNA ↑; mRNA↑, protein↑ in tumoral *vs.* normal liver tissues[91,92]; protein↑ in murine HCC line 1MEA[93]	TRPC6 overexpression or knockdown in Huh-7/HepG2 cells[91,92,94]; 1MEA cells treated with snail venom peptides Tv1, selectively binding to TRPC6 to impair its function[93]	Promoting HCC cell proliferation[91,93], intrahepatic metastasis[92,93] and multi-drug resistance[94]	0.54	0.00045
TRPM7	#mRNA↓; mRNA↑in HCC cell lines[95]	Pharmacological blockade of TRPM7 in Huh7 cells and related xenografts[96]; HepG2 cells treated with bradykinin[95]; adult rat hepatocytes and hepatoma WIF-B cells[97]	Promoting proliferation[96,97] and migration[95] of HCC cells; Subcellular distribution affects differentiation of HCC cells[97]	0.45	0.00038
TRPV1	#mRNA↑; mRNA↑, protein↑ in tumoral *vs.* normal liver tissues[98]	*Trpv1* knockout mice[99,100], HepG2 and Huh7[99,101−103]	Positive correlation with histopathologic differentiation[98]; *Trpv1* knockout promotes hepatocarcinogenesis and metastasis[99,100]; Agonist Capsaicin inhibits the growth of HCC cells *in vitro* and *in vivo*; Improves sorafenib sensitivity[99,101−103]	0.55	0.0008
TRPV2	#mRNA↑; mRNA↓, protein↓ in poorly differentiated liver tumors[104]; protein↓ in 5 HCC tissues compared with their nontumor counterparts[105]	*TRPV2* shRNA, agonist probenecid and antagonist tranilast treated SMMC-7721 and HepG2 cells *in vitro* and SCID mouse xenografts[105]	Positive correlation with histopathologic differentiation[104]; Converse correlation with the expression of liver cancer stem-like cellular markers[105]	1.23	0.25
TRPV4	#mRNA ↓; mRNA↑, protein↑ in tumoral *vs.* adjacent liver tissues[106]	Huh7 and HepG2 treated with antagonist HC067047[106]	Increased TRPV4 associates with poor differentiation and the number of tumors; Antagonist HC067047 inhibits HCC cell proliferation, induces apoptosis and supresses the migration both *in vitro* and *in vivo*[106]	1.24	0.23

#Data of tumoral *vs.* normal liver tissues of HCC patients from TCGA and Kaplan−Meier plotter database; ↑Increase; ↓Decrease; ━No alteration. HR value < 1 and $P < 0.05$, marked in green; HR value > 1 and $P < 0.05$, marked in grey. HCC, hepatocellular carcinoma.


A
TRPC1
P=0.006
Relative expression (RSEM)
Normal
Tumor
Survival probability
High
Low
HR=1.53
logrank P=0.02
Time (Month)
TRPC6
P<0.0001
HR=0.54
logrank P=0.00045
TRPM7
P=0.02
HR=0.45
logrank P=0.00038
TRPV1
HR=0.55
logrank P=0.0008
TRPC2
P=0.002
HR=0.58
logrank P=0.003
TRPC3
HR=1.7
logrank P=0.007
TRPC7
HR=0.36
logrank P=2.8e-9
TRPA1
HR=0.66
logrank P=0.0237
TRPM1
HR=0.49
logrank P=4.7e-5
TRPM3
HR=0.56
logrank P=0.0011
TRPM8
P<0.0001
HR=0.54
logrank P=0.0005
TRPV5
HR=0.42
logrank P=6.4e-7
B
TRPC1
Number of patients
High
Medium
Low
None
Staining score
Strong
Moderate
Weak
Negative
Intensity score
>75%
75%-25%
<25%
None
Quantity score
Healthy liver (n=3)
HCC (n=12)
Healthy liver
HCC-1
HCC-2
HCC-3
IHC staining

drug resistance of tumor cells. Whereas TRPV1 and TRPV2 are more like anti-tumor effectors by inhibiting these biological processes (Table 2 and Table S1).

Based on their prognostic value *via* the Kaplan−Meier plotter database[107](Fig. 6A and Supporting Information Figs. S1−S3), *TRPC2*, *TRPC6*, *TRPC7*, *TRPM1*, *TRPM3*, *TRPM7*, *TRPM8*, *TRPV1*, *TRPV5* and *TRPA1* display significant correlation with better survival (HR value < 1 and $P < 0.05$). Whereas *TRPC1*, *TRPC3* and *TRPM6* significantly correlate with worse survival showing HRvalue > 1 and $P < 0.05$ (Table 2 and Supporting Information Table S2). Next, the protein levels of these TRP channels in liver tissues were explored using proteomic data from the Human Protein Atlas, where most TRPs remain lacking. However, consistent with the RNA data, TRPC1 protein is clearly increased in tumoral area compared to normal counterparts (Fig. 6B).

Interestingly, *TRPC3*, *TRPC7*, *TRPM1*, *TRPM3*, *TRPM6*, *TRPV5* and *TRPA1* show no clear alteration at the mRNA level in tumoral liver tissues. It is worthy to argue that other factors, *e.g.*, subcellular distribution, probably also affect the outcome of HCC patients. As demonstrated in adult rat hepatocytes and proliferating hepatoma WIF-B cells, TRPM7 subcellular distribution, rather than expression, is altered in parallel with differentiation status. Terminally differentiated quiescent hepatocytes exhibit greatest immuno-reactive TRPM7 in the nuclear envelope whereas hepatoma cells also present nucleoplasmic labelling with intense signal in the nucleolus[97]. These observations highlight the dynamic and transient distribution of TRP channels inside hepatocytes to regulate cell differentiation.

Apparently, TRP channel superfamily remains to be systematically explored in the development of liver cancers. The adopted methodology to disclose the function of these channels in most studies so far is restricted to cell line and tumor xenografts, except the one for TRPV1, which used established *Trpv1* knockout mice to induce liver cancer. Future investigations should rely more on liver specific knock-out/knock-in mouse models, functional genetic screening[108] on human liver cancer cells or liver cancer organoids and large-scale cohorts.

6. Therapeutic potential and challenges

Dysregulation of TRP channels causes aberrant cationic turbulence in the liver, facilitating early hepatocellular injury, inflammatory response, fibrogenesis and late HCC formation. Pharmacological agents targeting TRP channels have been vigorously under development for the goal of treating various diseases for years, especially skin, sensory, cardiac, ocular, skeletal and neuronal disturbances[2−4]. Nevertheless, none of TRP agonists or antagonists as potential therapy particularly aimed at liver diseases has entered clinical trials yet. Because of the low sequence homology, disparate three-dimensional structures[54] and diverse expression profiles of TRP channels in different liver diseases, efficacious agents are highly anticipated to emerge in the following years.

Due to ubiquitous expression and multiple biological functions of TRP channels, unacceptable on-target adverse effects largely hamper the development of TRP channel drugs. For example, TRPV1 antagonists were withdrawn from clinical trials due to the burn injuries that they caused[2]. As such, it is highly demanded to pay attention to this aspect if current candidates are adopted to treat liver diseases. Reassuringly, a safe hepatic-targeted prodrug system has been recently established and validated in NAFLD and HCC *in vitro* as well as *in vivo* models. The system relies on the excellent affinity of the galactose to asialoglycoprotein receptors specifically expressed in hepatocytes[109−111]. This efficient drug delivery system supplies therapeutic potential to optimize liver disease treatment using TRP channel drugs by minimizing adverse effects resulted from other organs or tissues.

7. Conclusions

A plethora of cellular and molecular mechanisms collectively promote liver damage and liver cancer, resulting in the complex nature of live diseases and the limitations on corresponding treatment efficiency. Identification of the substantial factors underlying liver disease progression is highly demanded.

As addressed above, TRP channels respond to various etiological factors to provoke hepatocellular injury, which subsequently triggers and magnifies inflammatory response and promotes final HCC formation. For example, increased activation or expression of TRPM2 promotes hepatotoxicity caused by acetaminophen overdose, ischemia−reperfusion or high-fat diet. Likewise, increased TRPC1 at the mRNA and protein levels promotes the proliferation of hepatocellular carcinoma cells and exacerbates the survival quality of liver-cancer patients in late stage. Hence, TRPM2 and TRPC1 may offer suitable pharmacological targets for the prevention or management of associated liver disease. Based on the rich knowledge of TRP channel drug development, targeting TRPs may represent a novel avenue for liver-disease treatment.

Of course, several key aspects need to be further explored, including molecular mechanisms of activation/inactivation or up/down-regulation of TRPs by distinct etiological factors, cationic-independent functions of TPRs affecting hepatic metabolic functions in different pathological settings, small molecule compounds selective for TRP subtypes, and efficient drug delivery systems to target hepatic TRPs.

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Figure 6 TRP expression in liver cancer tissues (A) TRPs mRNA levels (mean ± SEM) in normal ($n = 50$) and tumoral liver tissues ($n = 374$) from TCGA and HCC specific survival Kaplan−Meier curves. Comparisons between groups were carried out using unpaired-*t* test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. (B) The level of TRPC1 protein expression is analyzed using proteomic data and representative images downloaded from the Human Protein Atlas, including the IHC staining, intensity, and quantity of TRPC1 in normal and tumoral liver tissues.

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Author contributions

Wenhui Wang: Conceptualization, Formal analysis, Writing-Original Draft, Writing- Review & Editing and Supervision; Pengyu Liu: Formal analysis, Visualization, Writing-Original Draft; Yalin Zhang: Formal analysis, Visualization, Resources; Li Yan: Formal analysis, Visualization; Michael X. Zhu: Writing - Review & Editing; Ye Yu and Jin Wang: Supervision, Project administration.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2022.09.005.

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