

Involvement of transient receptor potential in liver diseases

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Abstract

Transient receptor potential (TRP) family is a large superfamily of widely expressed ion channels. In recent years, several studies have been conducted on the mechanism of action of TRP. However, studies on the role of TRP in liver diseases are limited. In fact, TRP plays a vital role in the progression of many liver diseases, such as hepatic fibrosis, hepatocellular carcinoma, alcoholic fatty liver disease, and nonalcoholic fatty liver disease. This review aimed to summarize the recent reports of the involvement of TRP in different liver diseases and introduce the achievements of our team in recent years about the role of the TRPV subfamily in hepatic fibrosis and hepatocellular carcinoma.

Keywords: TRP, Liver fibrosis, Hepatocellular carcinoma, Vanilloid duplication cyst, Embryology

Introduction

Various liver diseases affect people's quality of life, even life-threatening in severe cases. The prevalence of chronic liver disease (CLD) is predicted to increase in coming years due to the global epidemic of non-alcoholic fatty liver disease (NAFLD) and its risk factors, which involve obesity, the metabolic syndrome, and type 2 diabetes mellitus [1]. To date, specific therapies for liver disease have primarily been etiology-driven by eliminating or ameliorating the causative agent of chronic liver diseases (CLD) [2].

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide with prevalence estimates ranging from 25% to 45% in most studies, increasing in parallel with that of obesity and diabetes [3]. Nonalcoholic fatty liver disease is divided into the histological categories of (1) nonalcoholic fatty liver, which includes patients with isolated hepatic steatosis and patients with steatosis and mild nonspecific inflammation, and (2) nonalcoholic steatohepatitis, which is distinguished from the former by the additional presence of features of hepatocellular injury with or without fibrosis [4]. The presence of hepatic fibrosis is the most important determinant of outcome. Clinical risk factors, such as the presence of the metabolic syndrome and its features, as well as emerging biomarkers can help select patients for liver biopsy and identify those at highest risk of nonalcoholic steatohepatitis and advanced liver disease [5]. Patients with NAFLD overall, and those with nonalcoholic steatohepatitis in particular, are at increased risk of mortality from liver disease (13%), and more commonly from cardiovascular disease (25%) and malignancy (28%) [6]. Visceral adipose tissue generates multiple signals that alter lipid and glucose metabolism, which lead to hepatic fat accumulation, and creates a proinflammatory milieu that triggers cellular injury in the liver and other tissues. The inability to quell injurious processes, such as oxidative stress, dysregulation of the unfolded protein response (leading to endoplasmic reticulum stress), lipotoxicity, and apoptotic pathways, contribute to liver damage, progressive fibrosis that can lead to cirrhosis, and the development of hepatocellular cancer in some patients [5]. Diet and exercise are the mainstay treatment for the majority of patients with NAFLD.

Alcoholic liver disease (ALD) is one of the main causes of chronic liver disease worldwide. ALD ranges from steatosis and steatohepatitis to advanced injury, such as fibrosis, cirrhosis, and

hepatocellular carcinoma. Most patients are diagnosed at advanced stages and data on the prevalence and profile of patients with early disease are limited [7]. Alcohol is also a frequent co-factor in patients with other type of liver disease such as hepatitis C virus (HCV) infection where it accelerates hepatic fibrosis [8]. Dietary fat and alcohol both play important roles in the pathogenesis of ALD. It has been estimated that 15% to 30% of heavy drinkers develop advanced ALD [9]. To date, the most effective therapy to attenuate the clinical course of ALD and even reverse liver damage is prolonged alcohol abstinence [10]. Liver transplantation is a definitive treatment option in patients with advanced alcoholic cirrhosis [7].

The development of fibrosis in chronic liver disease (CLD) presents an extensive unmet clinical challenge. Liver fibrosis is pathologically universal in the progression of various chronic liver diseases. At present, there is no effective antifibrotic treatment that reverses the progression of liver fibrosis in patients with CLD successfully [11]. Findings in both human studies and animal models nicely emphasize that liver fibrosis is a dynamic process that can be modulated either by halting progression and/or promoting resolution. Hepatic stellate cells (HSCs) are the key drivers of liver fibrosis, which are liver-specific pericytes distributed perivascularly, both around major blood vessels (surrounding the portal tracts) and throughout the liver parenchyma in a peri-sinusoidal location [12]. Individuals with liver fibrosis are at risk of developing cirrhosis including related complications such as liver failure and liver cancer, where often liver transplant presents the only remaining treatment option [2]. Fibrosis resolution occurs when activated HSC/myofibroblasts undergo deactivation, senescence, or apoptosis, and degradation of matrix exceeds deposition [13]. Thus, targeting activated HSC is an attractive antifibrotic strategy.

Primary liver cancer, mainly comprising hepatocellular carcinoma (HCC), is the fifth most common type of cancer globally and the third leading cause of cancer-related deaths worldwide [14]. Curative therapies (resection, radiofrequency ablation, and liver transplantation), palliative treatments (including trans-arterial chemo- or radio- embolization), and supportive care are the major treatment modalities for patients with HCC based on the staging evaluation of tumor extent, liver function, portal pressure, and clinical performance status [15]. However, the prognosis of HCC patients after treatments generally tends to be poor due to the high frequency of tumor recurrence [16]. Hence, more effective and safer anticancer strategies to suppress HCC progression need exploration on an urgent basis.

Transient receptor potential (TRP) channels were initially discovered in a blind strain of *Drosophila* [17]. When exposed to prolonged intense light, these spontaneously mutant fruit flies showed transient calcium influx into their photoreceptor cells; this is why the mutant gene was termed *trp*, 'transient receptor potential'. TRP channels are widely expressed across eukaryotic taxa and form ion channels with varying cation selectivity [18].

TRP channels have six transmembrane spanning domains (S1–S6) with a pore-forming loop between S5 and S6, and their C termini and N termini are intracellular [19]. They are gated by a wide variety of mechanisms and stimuli (including thermal, pain, mechanical and chemical), and function in a plethora of systems, chiefly as signal transducers [20]. TRP channels are nonselective cation channels located in the plasma membrane. Some function as Ca²⁺ entry channels. Upon activation, they generate cell depolarization that can

result in activation or inactivation of voltage-dependent ion channels and modulate the driving force of ion flux through channels and transporters, translocating important signaling ions crossing the cell membrane, altering enzymatic activity, and initiating endocytosis/exocytosis [21,22]. TRPs can also function as intracellular ion channels, mainly as Ca²⁺ release channels, in several cell organelles such as lysosomes, endosomes, the Golgi network, the endoplasmic reticulum, and synaptic vesicles [23].

The TRP superfamily is divided into nine subfamilies: TRPP (polycystin or polycystic kidney disease), TRPML (mucolipin), TRPA (ankyrin), TRPV (vanilloid), TRPVL (vanilloid-like), TRPC (canonical), TRPN (nompC, or no mechanoreceptor potential C), TRPM (melastatin) and TRPS (sorumelastatin) [18,24]. In yeast, another TRP family was recently identified and named as TRPY in which Y stands for yeast.

The canonical TRP (TRPC) subfamily was the first *trp* gene family to be cloned in mammals. Inserted in the plasma membrane, most of the TRPCs are spontaneously active. All TRPC channels contain a conserved TRP box, EWKFFAR, in their C-terminal tail and three to four N-terminal ankyrin repeats. All TRPC channels are nonselective cation channels and are permeable for Ca²⁺. On the basis of sequence homology and similarities in function, TRPCs fall into four groups: TRPC1, TRPC2, TRPC3/ TRPC6/TRPC7, and TRPC4/TRPC5 [25]. TRPC channels typically function as part of the phospholipase C signal transduction pathway [26].

TRPV (vanilloid) is named due to TRPV1's sensitivity to two vanilloid compounds, capsaicin and resiniferatoxin. The TRPV subfamily comprises six members that, based on homology, fall into four groups: TRPV1/ TRPV2, TRPV3, TRPV4, and TRPV5/ TRPV6. Members of the TRPV subfamily function as tetrameric complexes with each subunit containing six N-terminal ankyrin repeats [27]. All members have a TRP box in their C terminus. TRPV1, TRPV2, TRPV3, and TRPV4 are modestly permeable to Ca²⁺, whereas TRPV5 and TRPV6 are the only highly Ca²⁺ selective channels in the TRP family. TRPV channels serve as temperature, chemical and osmotic sensors in vertebrates [28].

TRPM was named after melastatin/MLSN1 (now known as TRPM1), a transcript correlated with melanoma aggressiveness [29]. The TRPM family comprises eight members. All have a 25-amino acid TRP box. TRPM channels lack the N-terminal ankyrin repeats. Members of the TRPM family, on the basis of sequence homology, fall into three subgroups: TRPM1/TRPM3, TRPM4/TRPM5, and TRPM6/TRPM7 with TRPM2 and TRPM8 representing structurally distinct channels. At least among mammals, most TRPMs serve as Ca²⁺-permeable sensors of various stimuli [30]. TRPM channels exhibit highly variable permeability to Ca²⁺ and Mg²⁺, ranging from Ca²⁺ impermeable (TRPM4 and TRPM5) to highly Ca²⁺ and Mg²⁺ permeable (TRPM6 and TRPM7) [31].

Recent studies have shown that TRPC, TRPM and TRPV subfamilies are related to the progression of liver diseases. This review described recent findings on the role of TRP in liver diseases and detailed the research progress of our team about the involvement of TRPV subfamilies in liver diseases.

TRP in Liver Fibrosis

Fibrosis is a kind of chronic disease which was characterized by the formation of excessive accumulation of myofibroblasts

secreted extracellular matrix (ECM) components in tissues and organs, especially alpha smooth muscle actin (α -SMA) collagen and fibronectin [32]. Hepatic stellate cells (HSCs) are the primary source of activated myofibroblasts that produce ECM in the liver. The inflammatory response is a vital mechanism for activating HSCs and promoting the development of liver fibrosis [33]. Chronic liver inflammation is usually associated with the upregulation of inflammatory factors, such as IL-6, IL-1 β , and TNF- α . These cytokines can activate HSCs directly and promote the secretion and deposition of ECM [34]. Increasing evidence shows that TRP modulated fibroblasts proliferation and differentiation to myofibroblasts by integrating mechanical and soluble signals that are derived from ECM stiffness and TGF- β 1.

TRPV in liver fibrosis

The TRPV4 cation channel, a member of the TRPV subfamily, exhibits cation permeability and participates in multiple cellular processes [35].

Recent studies have demonstrated that TRP channels-mediated Ca^{2+} signaling plays an important role in the progression of fibrosis. Zhan et al. found that the expression of TRPV4 mRNA and protein dramatically increased in HSC-T6 in response to TGF- β 1 stimulation [36]. They also indicated that TRPV4 could inhibit HSC apoptosis partially by regulating autophagy-dependent Akt signaling pathway activation. Song et al. found that the blockade of TRPV4 inhibited the proliferation of activated HSC-T6 cells and decreased the expression of α -SMA and Col1 α 1 [37]. The overexpression of miR-203 inhibited TGF- β 1-induced HSC proliferation likely by targeting on TRPV4. In recent years, many researchers focused on finding drugs that acted on TRP to achieve therapeutic purposes. Our recent study indicated that the pharmacological activation or inhibition of TRPV4 channels regulated the progression of advanced liver fibrosis through different expression levels of α -SMA [38].

Transient receptor potential vanilloid 3 (TRPV3), a member of the TRPV superfamily, is a nonselective cation channel protein with high Ca^{2+} permeability [23]. It is widely distributed in skin keratinocytes, oral and nasal epithelium, nervous system, liver, and kidney. It has a wide range of functions, including protection of the skin barrier, hair growth, wound healing, and perception of itching, pain, and temperature. Our latest research found that the inhibition of TRPV3 could alleviate liver fibrosis and account for the underlying mechanisms of liver fibrosis development [39]. We found that TRPV3 upregulation significantly promoted inflammatory response not only by promoting the mRNA levels of IL-1 β , TNF- α , and IL-6 but also by upregulating the expression of F4/80, which is a surface marker of macrophages. The inhibition of TRPV3 could attenuate liver injury through downregulating collagen I and α -SMA, which are both markers related to fibrosis. The knockdown of the TRPV3 channel could influence the cell cycle by suppressing proliferation, reducing DNA synthesis, and increasing the apoptotic rate of HSCs. Besides affecting cell proliferation and apoptosis, TRPV3 also regulates the inflammatory response at both transcription and protein levels by upregulating LOX-1 encoded by the *Olr1* gene.

TRPM in liver fibrosis

TRPM7 is a membrane protein with dual functions as an ion channel and kinase; it participates in the activation and proliferation of various cells by regulating the influx of Ca^{2+} and Mg^{2+} ions

[40]. TRPM7 channels mediate a variety of physiological and pathophysiological processes, such as cell proliferation, survival, differentiation, migration, adhesion, embryonic development and neurotransmitter release [41].

Cai et al. indicated that carvacrol, a nonselective TRPM7 inhibitor, could inhibit the expression of TRPM7 and inhibit the proliferation and activation of HSCs to alleviate liver fibrosis by modulating the MAPK signaling pathway [42]. Fang et al. found that TRPM7 up-regulation contributes to the activation of the *ERK* and *AKT* pathways in activated HSC-T6 cells [43]. Knockdown of TRPM7 strongly inhibited the proliferation of activated HSC-T6 cells, which was related to a decrease in cyclin D1, CDK4 and PCNA expressions, suggesting that TRPM7 is required for HSC activation and proliferation, and target silencing TRPM7 may restore liver fibrosis.

TRP in Liver Cancer

Tumor formation and metastasis are complex processes involving multiple genes and multiple steps, including oncogenesis, basement membrane degradation, matrix permeability, cell adhesion, and vessel formation. During these processes, many genetic alterations induce changes in TRP channels expression, and the abnormal expression of these channels may promote the growth, proliferation, and metastasis of tumor cells [44,45]. Apoptosis is considered as a major mechanism of programmed cell death. Epithelial-to-mesenchymal transition (EMT), defined as the phenotypical conversion of epithelial cells into mesenchymal cells, and its reverse mesenchymal-to-epithelial transition have been delineated in various types of cancers including HCC and regarded as a key process in cancer progression. Liver cancer stem-like cells are endowed with self-renewal ability and are involved in hepatocarcinogenesis. TRP channels exhibit cation permeability and therefore participate in many cellular processes controlling the fate of cancer cells, such as cell proliferation, apoptosis, and cell motility.

TRPV in liver cancer

Transient receptor potential vanilloid 6 (TRPV6), the major constituent of TRP channels, is an epithelial calcium channel located in the epithelium of organs including the digestive tract, testis, placenta, and kidney with highly selective affinity to divalent cations, particularly Ca^{2+} [46]. KOH et al. found that low TRPV6 expression was significantly associated with adverse histologic features, and patients with low TRPV6 expression had shorter recurrence-free and disease-free survival [47]. Our team first determined the expression of TRPV4 in HCC tumor tissues and cell lines [48]. In addition, HC-067047, a potent and selective reversible TRPV4 antagonist, and GSK1016790A, a novel and potent TRPV4 channel agonist, were introduced to discover the influence of TRPV4 inhibition on HCC progression and the underlying molecular mechanisms. We indicated that TRPV4 knockdown markedly attenuated cell proliferation by reducing DNA synthesis in HCC cells. We also demonstrated that TRPV4 inhibition induced HCC cell apoptosis by suppressing ERK1/2 activation. Further inhibition of ERK1/2 activity enhanced the apoptosis of HCC cells through the upregulation of Bax and caspase-3 cleavage and downregulation of Bcl2 and caspase-3 protein levels. Furthermore, TRPV4 channel inhibition suppressed the migration of HCC cells by attenuating the EMT process.

The transient receptor potential vanilloid 1 (TRPV1), a member of the vanilloid TRP family, is a nonselective cation channel identified as the capsaicin receptor [49]. Capsaicin treatment significantly inhibited HCC cell proliferation through binding with TRPV1, causing the downregulation of several markers associated with tumor-initiating cells, including SOX2, SALL4, CD133, OCT3/4, and NANOG [50]. We also found that that TRPV1 knockout impaired the bile secretion and biliary differentiation during hepatocarcinogenesis by downregulating the expression of markers related to biliary differentiation and bile secretion, including JAG1, NUMB, HNF4 α , and BSEP. We discovered a novel pathway via which TRPV1 loss of function activated *Zeb1* and inactivated *Ovol2*, both of which coordinated with *Sox10* to regulate downstream genes. TRPV 1 induced EMT with the regulation of *Ovol2* and *Zeb1*, which played a very important role in TRPV1-induced hepatocarcinogenesis.

TRPV2, a nonselective cation channel exhibiting Ca²⁺ permeability, can be activated by noxious heat, at a temperature higher than 52°C, as well as exogenous chemicals including novel cannabinoids [51]. Our study indicated that TRPV2 knockdown enhanced the stemness of cancer stem-like cells through increased expression levels of the cancer stem cell markers CD133, CD44, and ALDH1 [52]. In addition, the overexpression of TRPV2 attenuated the stemness of cancer stem-like cells by reducing the levels of the markers. Our data indicated that probenecid, a TRPV2 pharmacological agonist, resulted in the upregulation of TRPV2 protein expression and then inhibited the stemness of cancer stem-like cells.

TRPC in liver cancer

TRPC6 is a member of the TRPC subfamily that has been reported to promote tumor cell growth in several types of cancers, including esophagus cancer, gastric cancer, colorectal cancer, prostate cancer, breast cancer and neuroblastoma and etc. [53]. Wen et al. found that TRPC6 expression was much higher in HCC tissues than in pericancerous tissues and was markedly related to a higher TNM classification, suggesting that TRPC6 was closely associated with the malignant behaviors of HCC [54]. Their study demonstrated that TRPC6 played a vital role in the sustained aggregation of [Ca²⁺]_c under various stimuli, which in turn was directly involved in regulating EMT, hypoxia-inducible factor 1 α (HIF1 α) signaling, and DNA damage repair. Xu et al. found that TRPC6 and Na⁺/Ca²⁺ exchanger 1 (NCX1) mediated the effects of TGF- β on the migration, invasion, and intrahepatic metastasis of human HCC cells, and the expression levels of TRPC6 and NCX1 positively correlated with the stage and pathologic grade of HCC [55].

TRPC1 serves as a non-selective cation channel which mediates Ca²⁺- and Na⁺-entry in response to physiological and pathophysiological stimuli, and is principally located in intracellular organelles with some expressed at the plasma membrane [56]. TRPC1 suggested to be the essential component of store-operated Ca²⁺ entry (SOCE) channel via forming heteromultimeric combinations with other TRPCs and co-localization with the lipid raft proteins such as caveolin-1 [57,58]. Selli et al. found that TRPC1 knockdown facilitated the proliferation of Huh7 cells, both ER Ca²⁺ release and SOCE upregulated [59]. However, TRPC1 suppression did not affect the morphology and migration of Huh7 cells.

TRPM in liver cancer

TRPM7, a member of the transient receptor potential channel TRP superfamily, is a cation channel permeable to divalent cations such as Mg²⁺, Ca²⁺, and Zn²⁺ fused to a C-terminal serine/threonine protein kinase domain [60]. TRPM7 has been correlated with malignant growth and cancer progression [61]. Myocardin-related transcription factors (MRTF-A/B, MKL1/2) are coactivators of serum response factor (SRF) that mediate the expression of immediate early genes and genes involved in cell growth, migration and differentiation [62]. Voringer et al. identified TRPM7 as the first druggable target at the plasma membrane to block MRTF/SRF function and HCC xenograft growth [63]. They reported that TRPM7 blockade inhibits MRTF/SRF target gene expression, resulting in growth arrest of HCC cells and HCC xenografts due to oncogene-induced senescence.

TRP in Other Liver Diseases

TRP in nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) and its more advanced form nonalcoholic steatohepatitis are the most common chronic liver diseases in developed countries. Seth et al. found that TRPV4 loss of function increased liver injury and inflammation in progressive NAFLD. Their results indicated TRPV4 to be functional in downregulating CYP2E1 expression and function in NAFLD progression [64]. In addition, TRPV4 knockdown inhibiting CYP2E1 in NAFLD decreased oxidative stress and attenuated the NAFLD pathology. Smedlund et al. found that the downregulation of TRPC3 in liver sinusoid endothelial cells reduced their susceptibility to endoplasmic reticulum stress-induced apoptosis, suggesting that a proapoptotic effect of TRPC3 might add to other fibrogenic factors *in vivo*. These novel findings showed a positive association between augmented expression of an endothelial TRPC channel, development of early steatohepatitis, and atherosclerotic burden in a hyperlipidemic mouse model of NAFLD fed a conventional Western-type diet [65].

TRP in alcoholic liver disease

Recent publications have shown that experimental and clinical alcohol-induced liver steatosis and injury were associated with elevated oxidized linoleic acid metabolites (OXLAMs), specifically 9- and 13-hydroxy-octadecadienoic acids (9- and 13-HODEs) [66]. It has been reported that 9- and 13-HODEs are natural endogenous ligands for the transient receptor potential vanilloid 1 (TRPV1) [67]. Liu et al. found that ethanol/OXLAM/TRPV1-mediated increase in intracellular Ca²⁺ might be a possible mechanism contributing to the development of ALD. TRPV1 deficiency significantly attenuated chronic alcohol-induced liver injury by suppressing an alcohol-mediated increase in hepatic cleaved caspase-3 activity, which was a marker of apoptosis [68]. TRPV1 depletion could inhibit hepatic inflammation induced by chronic alcohol by downregulating the expression of proinflammatory cytokines and chemokines. TRPV1 depletion also prevented alcohol-induced activation of hepatic NF- κ B and ERK 1/2 MAPK signaling pathways.

TRP in polycystic liver diseases

The polycystic liver diseases (PLD) comprise a group of genetic disorders characterized by the progressive growth of cholangiocyte-derived fluid-filled cysts that gradually replace liver tissue. PLD occurs

in combination with two forms of Polycystic Kidney Disease (PKD) Autosomal Dominant PKD (ADPKD) and Autosomal Recessive PKD (ARPKD) as well as alone [69,70]. Gradilone et al. found that activation of TRPV4 increases calcium levels subsequently suppressing cell proliferation and cyst growth in culture by activating AKT and inhibiting B-RAF-ERK signaling pathway. Importantly, TRPV4 activator, GSK1016790A, also reduced cystogenesis *in vivo* [71]. This means that intracellular calcium regulated by TRPV4 might influence cyst progression in PLD.

Conclusion and Perspective

Nowadays, accumulating studies are conducted on TRP in liver diseases and have made some progress. Our team made some achievements on the mechanism of TRPV subfamily in liver diseases. Our results found that the activation of TRPV4 channels aggravated liver fibrosis and the inhibition of the TRPV4 channel alleviated liver fibrosis *in vivo*. TRPV3 inhibition exhibited antifibrosis effects on both cell proliferation and inflammatory responses. As for TRPV in liver cancer, our study illustrated the essential role of the TRPV4 channel in HCC development and that its pharmacological inhibition inhibited proliferation and induced apoptosis in HCC cells, which was, at least in part, ascribed to the inactivation of the ERK pathway. TRPV4 channel blockade could also attenuate the migration capacity of HCC cells by suppressing the EMT process. TRPV1 induced hepatocarcinogenesis by promoting EMT and influencing the bile secretion and biliary differentiation. TRPV2 showed targeting potential on the treatment of human liver cancer via forming stemness. Yet, little is known about the exact role of other TRP subfamilies in initiation and/or progression for many liver diseases, though the involvement of calcium signaling via some of TRP channels has already been demonstrated. The mechanism of action of TRP in liver disease will be gradually clarified with the deepening of research and provide us a new way to treat liver diseases.

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