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Ion channels in liver diseases and hepatocellular carcinoma: potential tools for diagnosis, prognosis, and therapy

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Abstract

Cancer is a major cause of death worldwide. Hepatocellular carcinoma (HCC) is one of the malignancies with the highest mortality-to-incidence ratio (> 0.9), and in some countries this value is up to 1. Unfortunately, many patients are diagnosed at advanced stages of the disease. Therefore, HCC early markers, as well as novel therapeutic approaches, are urgently needed. HCC is the main type of liver cancer and it is associated with different factors including alcohol use, viral infections, and fatty liver disease. A significant percentage of HCC patients previously had liver cirrhosis. Several ion channels have been proposed as novel potential markers and therapeutic targets for diverse cancers including HCC. Here, we review most of the findings associating ion channel expression with HCC and its etiological factors, as well as some possible pro-tumorigenic mechanisms of action for ion channels in HCC. Novel therapies for HCC treatment and prevention are also discussed. Ion channel targeting offers a plethora of opportunities for HCC prevention, early diagnosis, and therapy that may help to reduce the extremely high mortality-to-incidence ratio of this malignancy.

Keywords: Ion channels, hepatocellular carcinoma, hepatitis virus, cirrhosis, liver disease, alcohol

INTRODUCTION

Cancer is a leading cause of death worldwide, despite the existence of hundreds of clinical trials testing novel therapies[1,2]. There are several types of liver cancer including hepatoblastoma, cholangiocarcinoma, and angiosarcoma, but hepatocellular carcinoma (HCC) accounts for up to 90% of primary liver cancers[3-7]. Liver cancer is one of the malignancies with the worst prognosis, representing the second leading cause of cancer-related deaths in the world[3-5].

The liver plays a central role in regulating whole-body carbohydrate, lipid, and protein homeostasis, as well as playing additional very important physiological roles including the synthesis and transport of bile acids and the detoxification of endogenous and exogenous metabolites[6,8]. This very important organ is exposed to several factors including infections by hepatitis viruses B and C, alcohol use, aflatoxin B1, and fatty diet. Several of these factors lead to liver cirrhosis, which is the major HCC-associated risk factor[6,9-11]. In fact, a significant percentage (> 80%) of HCC patients previously had liver cirrhosis. Unfortunately, HCC is rarely detected at early stages, and is usually fatal within a few months of diagnosis. The percentage of mortality-to incidence ratio of liver cancer is very high; it is more than 90% globally, reaching up to 100% in some countries[1]. Therefore, novel early HCC markers and therapeutic strategies are urgently needed. In this regard, ion channels have gained great interest in oncology, as novel tools for both diagnosis and treatment[5]. Here, we summarize most of the research associating ion channels with HCC. We also discuss the potential tumorigenic mechanisms of action of ion channels in HCC, as well as ion channel-based therapies for HCC prevention and treatment. The growing research field of ion channels in cancer may lead to reduce the incidence and mortality of liver cancer.

ION CHANNELS AND CANCER

Ionic channels are pore-forming membrane proteins allowing ion flux across membranes, including the plasma membrane and those from of intracellular organelles. In most cases, these proteins selectively transport specific ions and the vast majority need special stimulus to be activated[12]. These gating stimuli include changes in membrane potential (voltage-gated ion channels), different ligands such as hormones or neurotransmitters, temperature, mechanical forces, *etc.* Thus, the role of ion channels in human physiology comprises very important phenomena such as neural transmission, cardiac function, hormone release, sensory physiology, *etc.* Accordingly, many channelopathies exist including epilepsy, cardiac arrhythmias, renal diseases, blindness, skeletal muscle disorders, *etc.*[12]. Cancer is a multi-factorial disease characterized by an increased cell proliferation rate; ion channels are essential for regulation of proliferation and are also involved in many relevant processes occurring during carcinogenesis, which convert these proteins into potential cancer diagnostic tools and therapeutic targets.

Ion channels associated with cancer

The roles that ion channels have during carcinogenesis depend on the step of tumor development and the tissue type[13,14].

Calcium channels participate in pivotal functions in the body such as regulation of blood pressure, muscle contraction, secretion, metabolism, excitability, and cell proliferation[15]. These channels are important in the cell cycle, especially to enter and accomplish the S and M phase[14-18]; thus, their participation in cancer cell proliferation is very relevant[14,16,17]. In addition, because these ions are very important for cell migration, they also play a very important role in cancer cell migration and metastasis[16-19].

Potassium channels play crucial roles in every cell type and in all species. Based on their structure and function, they are categorized into three major classes: the voltage-gated (Kv), inwardly rectifying (Kir), and tandem pore domain (K2P) channels. Furthermore, various messengers can stimulate the ligand-

gated (Kligand) channels[20]. Membrane hyperpolarization due to potassium channel activity is needed for cell cycle progression from G1 to S phase. Potassium flux is also very important for apoptosis, cell volume regulation, and cytokine release. Therefore, even though the precise molecular mechanism of K^+ channel participation in cancer remains elusive, these channels have a significant role in the cell proliferation, migration, and angiogenesis of a variety of carcinoma cells[14,21,22].

Different subtypes of voltage-gated sodium channels (VGSC) are differentially expressed throughout the body, and they have essential roles in the generation and propagation of action potentials in electrically excitable cells such as neurons and cardiac and skeletal muscle[23]. Several carcinoma cells express VGSC[14,24,25]. Interestingly, these channels are active in metastatic cells[25]. Accordingly, sodium currents through VGSC enhance migration, invasion, and metastasis *in vivo*[26].

Chloride channels are involved in many biological functions such as epithelial fluid secretion, cell-volume regulation, modulation of excitability, smooth-muscle relaxation, and pH regulation. Cystic fibrosis is a disease where the relevance of alterations in chloride flux has been shown[27]. Cl^- channels are involved in apoptosis, and in cancer cells these proteins promote proliferation, migration, and invasion[14,21,28-30]. These channels are over-expressed in many cancer tissues including liver compared to noncancerous tissues, and are significantly associated with tumor size, metastasis, and poor prognosis[31].

Before going into the details of ion channels in liver diseases leading to HCC, we first review some of the channels for which expression has been reported in the normal liver.

ION CHANNELS IN HEALTHY LIVER

The importance of ion channels in different functions of the normal healthy liver has been reported by several studies. Water crosses the plasma membrane either directly through the lipid bilayer or via protein water channels [aquaporins (AQPs)][32]. The liver expresses at least six AQPs (AQP1, -3, -7, -8, -9, and -11). Immunohistochemical studies showed the expression of AQPs in different hepatic cell types including cholangiocytes (AQP1 and -7), endothelial cells (AQP1), Kupffer cells (AQP3), and hepatocytes, (AQP7, -8, and -9)[33,34]. AQP8 and -9 are relevant for bile synthesis regulation, secretion, and modification[33]. Additionally, AQP9 functions as a glycerol channel in the liver[35].

The ATP-sensitive potassium channel (K_{ATP}) is composed of two types of subunits, namely an inwardly rectifying K^+ channel (Kir6.x) and a sulfonylurea receptor. Kir6.x subunits form the pore, while sulfonylurea receptor subunits have regulatory activity. Depending on its localization at the plasma membrane or in organelles, these channels are classified as sarcolemmal ("sarcK_{ATP}"), mitochondrial ("mitoK_{ATP}"), or nuclear ("nucK_{ATP}") channels[36,37]. Interestingly, K_{ATP} channel opening has been shown to alleviate liver injury by preventing inflammation and increasing the liver tolerance to ischemia/reperfusion injury[38,39]. Besides, DNA synthesis demonstrated that these channels play significant roles in liver growth control[37].

Nucleotides act as extracellular signaling molecules via purinergic receptors. These receptors are separated into seven P2X ionotropic receptors and eight P2Y G protein-coupled receptors[40,41]. For instance, the P2X4 receptor is the dominant P2X isoform expressed in cholangiocytes in the liver[42,43]. ATP is released by hepatocytes, and it regulates hepatocyte glycogen metabolism, cell volume, bile formation, and other cell functions. When activated by ATP, P2X receptors function as cation-permeable channels that allow the influx of sodium and calcium ions[42,43]. Interestingly the expression of P2X7 receptors is decreased in HCC Huh-7 cells[43].

Acid sensing ion channels (ASICs) are H^+ channels that mediate tumor cell migration and invasion[44], and store-operated calcium entry (SOCE) controls HCC cell proliferation and migration[45]. T-type Ca^{2+}

channels participate in modulating the proliferation of some HCC cells[46]. Because the expression levels of these channels (ASICs, SOCE, and T-type)[44,45] are increased in HCC in comparison with the normal liver, they may be used as markers of the disease.

In the following, we describe several findings associating ion channels with HCC, beginning with some liver diseases representing important HCC etiological factors.

ION CHANNELS IN LIVER DISEASES

Several liver diseases have been identified as HCC etiological factors, and many ion channels have been found to have a role in liver diseases. Table 1 summarizes the ion channel expression changes for the most common liver diseases.

Viral hepatitis and ion channels

It is estimated that 350 million people are chronic hepatitis B virus (HBV) carriers in the world, and that up to 30% of them develop progressive chronic liver disease appearing as hepatitis, fibrosis, cirrhosis, and HCC[47]. HBV infection produces chronic necro-inflammation with subsequent fibrosis and hepatocyte proliferation. One of the viral factors potentially involved in HBV-related hepatocarcinogenesis is the HBx protein, which promotes cell cycle progression and inactivates negative growth regulators. This protein also binds to and inhibits the expression of *p53*, as well as other tumor suppressor genes and senescence-related factors[3,48-50]. The HBx protein regulates calcium signaling through the activation of store-operated calcium channels (SOCs), which stimulate HBV replication[51,52]. In addition, HBx can activate SOCs by binding C-terminal of Orail protein channels[53]. Interestingly, co-immunoprecipitation experiments and pull-down assays demonstrated the interaction between HBx and the Orai1 protein; the C-terminus of the Orai1 protein was involved in such interaction. The authors concluded that the HBx protein binds to the STIM1-Orai1 complexes regulating the activity of SOCs[53]. In this same direction, the HBV PreS2-mutant large surface antigen activates store-operated calcium entry and promotes chromosome instability[54].

On the other hand, miR-125b inhibits HBV expression *in vitro* by targeting the sodium channel *SCNN1A* gene[55]. It has also been observed that P2X7 function is necessary for the infection of human hepatocytes by HBV. Because P2X7 activation is a major component of inflammatory responses, HBV may contribute to liver inflammation[56].

In the case of hepatitis C virus (HCV) infections, it is estimated that 130 million people have chronic HCV infection and most of them develop chronic liver disease[47]. Continuous inflammation and hepatocyte regeneration in the setting of chronic hepatitis and subsequent progression to cirrhosis are thought to lead to chromosomal damage, and possibly to initiate hepatic carcinogenesis. HCV also induces steatosis; oxidative stress causes steatohepatitis and these pathways lead to liver injury or HCC in chronic HCV infection[3,57,58]. Interestingly, the HCV p7 protein forms a cation channel *in vitro*[59-61], and p7 deletions and point mutations markedly reduce the production of infectious virions in cell culture[61-63]. p7 is a proton channel required for the production of infectious virions[64]. There are some small molecules that block the p7 channel function and virion production in culture, rendering it an attractive antiviral target[59,65-71].

P2X4 receptors expression form part of the purinergic signaling complex in HCV-induced liver pathogenesis[72,73]. Additionally, the modulation of the gamma-aminobutyric acid type A (GABA-A) receptor activity was observed in several chronic hepatitis failures, including hepatitis C. Increased expression of GABA-A α1 receptor subunit, and decreased expression of GABA-A β3 subunit have been found in chronic hepatitis C patients. Thus, the expression of GABA-A receptor subunits may be associated with either current or previous HCV infection[74].

Table 1. Ion channel expression in major liver diseases

Liver disease	Channel/ Transporter	Gene symbol	Developed name	Trans-ported ion(s)	Genomic mapping (chromosome in homo sapiens)	Expression change (compared to normal tissue)	Ref.
Viral hepatitis	p7	--	Hepatitis C virus p7 protein	Ca^{2+}	--	Overexpression	[59-61]
	SCNN1A	*SCNN1A*	Sodium channel non-voltage-gated 1 alpha	Na^{+}	12	Overexpression	[55]
	P2X7	*P2RX7*	Purinergic receptor P2X, ligand-gated ion channel 7	Na^{+}, Ca^{2+}	12	Overexpression	[56]
	P2X4	*P2RX4*	Purinergic receptor P2X, ligand-gated ion channel 4	Na^{+}, Ca^{2+}	12	Overexpression	[72,73]
	SOCs	*ORAI1*	Calcium release- activated calcium modulator	Ca^{2+}	12	Overexpression	[51-54]
	GABA Aα1	*GABRA1*	Gamma-aminobutyric acid type A receptor alpha 1 subunit	Cl^{-}	5	Overexpression	[74]
NAFLD	$K_{Ca3.1}$	*KCNN4*	Calcium-activated potassium channel subfamily N member 4	K^{+}	19	Overexpression	[79,80]
	P2X7	*P2RX7*	Purinergic receptor P2X, ligand-gated ion channel 7	Na^{+}, Ca^{2+}	12	Overexpression	[81-83]
	SOCs	--	Store-operated calcium channels	Ca^{2+}	NS	Overexpression	[8,84]
	TPC2	*TPCN2*	Two-pore segment channel 2	Ca^{2+}	11	Overexpression	[21,85]
	TRPV1	*TRPV1*	Transient receptor potential cation channel subfamily V member 1	Non selective cation	17	Overexpression	[86,87]
Fibrosis	TRPV4	*TRPV4*	Transient receptor potential cation channel subfamily V member 4	Non-selective cation	12	Overexpression	[88]
	TRPC6	*TRPC6*	Transient receptor potential cation channel subfamily C member 6	Ca^{2+}	11	Overexpression	[89]
	TRPM7	*TRPM7*	Transient receptor potential cation channel subfamily M member 7	Ca^{2+}, Mg^{2+}	15	Overexpression	[90]
	ASIC1a	*ASIC1*	Acid sensing ion channel subunit 1	Na^{+}	12	Overexpression	[91]
Cirrhosis	TRPV2	*TRPV2*	Transient receptor potential cation channel subfamily V member 2	Non-selective cation	17	Overexpression	[95]
	TRPC6	*TRPC6*	Transient receptor potential cation channel subfamily C member 6	Ca^{2+}	11	Overexpression	[96]
	$Nav_{1.2}$	*SCN2A*	Voltage-gated sodium channel alpha subunit 2	Na^{+}	2	Overexpression	[96]
	$K_{Ca3.1}$	*KCNN4*	Calcium-activated potassium channel subfamily N member 4	K^{+}	19	Overexpression	[96]
	ABCC3	*ABCC3*	ATP binding cassette subfamily C member 3	--	17	Overexpression	[96]
	ITPRs	*ITPR*	Inositol 1,4,5-trisphosphate receptor	Ca^{2+}	NS	Overexpression	[97]
	AQP1	*AQP1*	Aquaporin 1	water channel	7	Overexpression	[98-100]
	$K_{Ca1.1}$ (BK)	*KCNMA1*	Calcium-activated potassium channel subfamily M alpha 1	K^{+}	10	Overexpression	[21,101]
	NCC	*SLC12A3*	Solute carrier family 12 member 3	Na^{+}, Cl^{-}	16	Overexpression	[102]
HCC	$K_{Ca3.1}$	*KCNN4*	Calcium-activated potassium channel subfamily N member 4	K^{+}	19	Overexpression	[103,104]
	KCNQ1	*KCNQ1*	Voltage-gated potassium channel subfamily Q member 1	K^{+}	11	Downregulation	[105]
	KCNJ11	*KCNJ11*	Inwardly rectifying potassium channel subfamily J member 1	K^{+}	11	Overexpression	[106]
	K_{ATP} channels	--	ATP-sensitive potassium channels	K^{+}	NS	Overexpression	[37]
	Eag1	*KCNH1*	Voltage-gated potassium channel subfamily H member 1	K^{+}	1	Overexpression	[115]

T-type Ca^{2+} channels	*CACNA1G* *CACNA1H* *CACNA1I*	Voltage-gated calcium channels	Ca^{2+}	17 16 22	Overexpression	[117]
P2X3	*P2RX3*	Purinergic receptor P2X, ligand gated ion channel 3	Na^+, Ca^{2+}	11	Overexpression	[119]
SOCs	*ORAI1*	Store-operated calcium channels	Ca^{2+}	12	Overexpression	[120,121]
CIC-3	*CLCN3*	Voltage-gated chloride channel 3	Cl^-	4	Overexpression	[122]
CLIC1	*CLIC1*	Chloride intracellular channel 1	Cl^-	6	Overexpression	[123]
VGSCβ1	*SCN1B*	Voltage-gated sodium channel beta subunit 1	Na^+	19	Downregulation	[124]
$Nav_{1.2}$	*SCN2A*	Voltage-gated sodium channel alpha subunit 2	Na^+	2	Overexpression	[96]
AQP5	*AQP5*	Aquaporin 5	water channel	12	Overexpression	[125,126]
AQP9	*AQP9*	Aquaporin 9	water channel	15	Downregulation	[127,128]
TRPC6	*TRPC6*	Transient receptor potential cation channel subfamily C member 6	Ca^{2+}	11	Overexpression	[129-131]
TRPC1	*TRPC1*	Transient receptor potential cation channel subfamily C member 1	Non-selective cation	3	Overexpression	[132,133]
TRPV1	*TRPV1*	Transient receptor potential cation channel subfamily V member 1	Non-selective cation	17	Downregulation	[134,135]
TRPV2	*TRPV2*	Transient receptor potential cation channel subfamily V member 2	Non-selective cation	17	Overexpression	[136]
TRPV4	*TRPV4*	Transient receptor potential cation channel subfamily V member 4	Non-selective cation	12	Overexpression	[137]
TRPM7	*TRPM7*	Transient receptor potential cation channel subfamily M member 7	Ca^{2+}, Mg^{2+}	15	Overexpression	[138]
ASIC1a	*ASIC1*	Acid sensing ion channel subunit 1	Na^+	12	Overexpression	[139]
ITPR3	*ITPR3*	Inositol 1,4,5-trisphosphate receptor type 3	Ca^{2+}	6	Overexpression	[141]

NS: no specific channel indicated in the original source; HCC: hepatocellular carcinoma; NAFLD: nonalcoholic fatty liver disease; NCC: NaCl cotransporter

Ion channels in nonalcoholic fatty liver disease and liver fibrosis

Nonalcoholic fatty liver disease (NAFLD) defines liver abnormalities ranging from simple steatosis (abnormal hepatic fat accumulation) or nonalcoholic fatty liver to nonalcoholic steatohepatitis (NASH) that have been identified as a cause of fibrosis, cirrhosis, and HCC. It is closely related to obesity and metabolic syndrome. The precise mechanism of HCC development from NAFLD has not yet been fully elucidated[3,75-77].

$K_{Ca3.1}$ potassium channels are expressed in non-excitable tissues such as epithelia affecting proliferation, migration, and vascular resistance, and play an important role in the modulation of Ca^{2+} signaling[78]. In liver disease, the $K_{Ca3.1}$ channel inhibitor TRAM-34 downregulates fibrosis-associated gene expression and reduces portal perfusion pressure[79]. It has also been found that the $K_{Ca3.1}$ channel inhibitor senicapoc mitigates both steatosis and fibrosis in non-alcoholic liver disease models[80]. P2X7 deficiency[81] or blockage attenuates nonalcoholic steatohepatitis[82] and liver fibrosis[83].

Intracellular Ca^{2+} homeostasis is altered in steatotic hepatocytes. Decreased Ca^{2+} concentration in the endoplasmic reticulum may lead to endoplasmic reticulum stress, which has been identified as an important mediator of the progression from liver steatosis to nonalcoholic steatohepatitis, type 2 diabetes, and HCC. SOCs are responsible for proper Ca^{2+} maintenance in the hepatocyte endoplasmic reticulum

lumen. Accordingly, SOCE is substantially inhibited in steatotic hepatocytes. This inhibition enhances lipid accumulation by positive feedback and may contribute to the development of NASH and insulin resistance[8,84]. The antidiabetic drug exendin-4 reverses the lipid-induced inhibition of SOCE and decreases liver lipid with rapid onset[8].

Two-pore channels (TPCs) are cation-selective intracellular ion channels, and their activation mediates calcium release from lysosomal stores. TPC2-deficient mice show hepatic cholesterol accumulation, hyperlipoproteinanemia, and finally NASH[21,85]. Interestingly, the activation of transient receptor potential type vanilloid 1 (TRPV1) by capsaicin prevents nonalcoholic fatty liver disease[86,87]. Additionally, the TRPV4, TRPC6, TRPM7, and acid-sensing ion channels (ASIC1a) have been suggested as liver fibrosis mediators. The blockage of these channels inhibits hepatic fibrosis, positioning them as promising therapeutic targets[88-91].

Liver cirrhosis and ion channels

Liver cirrhosis from any cause is the most important clinical risk factor for HCC with an annual incidence between 2% and 4%. The transition from chronic liver disease to cirrhosis involves inflammation and activation of hepatic stellate cells with ensuing fibrogenesis and angiogenesis. Liver cirrhosis is characterized by diffuse regenerative nodule of hepatocytes surrounded by dense fibrotic septa with subsequent parenchymal extinction and liver structure collapse. Over time, compensated cirrhosis may progress to decompensated cirrhosis that results in liver failure and death[3,92-94].

As stated above, TRPV4, TRPC6, TRPM7, and ASIC1a channels could act as liver fibrosis mediators. Fibrosis is the prelude to cirrhosis, thus these channels might somehow also modulate cirrhosis. Other studies have also observed over-expression of TRPV2[95], TRPC6, Nav1.2, and $K_{Ca3.1}$ channels as well as the Abcc3 transporter[96] in liver cirrhosis, suggesting them as potential markers of the disease. Ca^{2+} signals mediate the hepatic effects of numerous hormones and growth factors. Liver Ca^{2+} signals are elicited by the intracellular Ca^{2+} channel inositol trisphosphate receptor (ITPRs). Three isoforms of this receptor have been identified, and cirrhosis affects the isoform expression[97].

Some reports have shown an overexpression of AQP1 in liver cirrhosis[98]; this protein contributes to microvascular resistance in cirrhosis[99]. It has been also proposed that AQP1 polymorphism may be involved in the genetic susceptibility to develop water retention in patients with liver cirrhosis[100]. The large conductance $K_{Ca1.1}$ K^+ channels (BK) are activated by membrane depolarization and/or elevations in intracellular Ca^{2+} concentration. Cirrhotic livers display increased activity of BK channels; accordingly, blockage of these channels increased the baseline portal perfusion pressure in cirrhotic livers[21,101]. Liver cirrhosis is associated with enhanced renal tubular sodium retention, but the mechanism involved is unknown. Interestingly, liver cirrhosis is associated with increased renal abundance of the NaCl cotransporter[102]. Then, diverse ion channels may serve as potential markers and drug targets for several liver diseases leading to HCC; if so, these proteins could be used as targets for HCC prevention.

Ion channels in hepatocellular carcinoma

Because the above-mentioned liver diseases may lead to HCC, and because cancer is a multi-factorial disease, a significant amount of ion channels have been studied as potential markers and therapeutic targets of this very poor prognosis malignancy.

Potassium channels play an important role in a variety of carcinoma cells. $K_{Ca3.1}$ channels are over-expressed in HCC and the channel blockade with TRAM-34 inhibits HCC cell proliferation in a time- and dose-dependent manner[103,104]. A recent work showed that KCNQ1 was frequently downregulated in HCC cell lines and HCC tissues, and that HCC patients with low KCNQ1 expression had poor prognosis. Gain-of-

function studies showed that KCNQ1 exhibited remarkable inhibitory roles on tumor metastasis *in vitro* and *in vivo*; thus, this channel could represent a prognostic marker, as well as a promising therapeutic target for HCC[105]. Another study found that the KCNJ11 channel was differentially expressed in HCC, and it predicted the poor prognosis in HCC patients. KCNJ11 promotes tumor progression through interaction with lactate dehydrogenase A (LDHA). Pharmacological inhibition of LDHA or knockdown of KCNJ11 expression inhibited cell proliferation, promoted apoptosis, and reduced cell invasive capacity[106]. K_{ATP} channels regulate mitogen-induced proliferation in the human liver cell lines HepG2, which could have implications for liver growth control and serve as a potential therapeutic target[37]. The voltage-gated potassium channel ether à-go-go-1 (Eag1) has gained enormous interest in cancer research because of its oncogenic properties[107-109]. Eag1 channels have also been proposed as early tumor biomarkers and therapeutic targets for different types of cancers[110,111]. Moreover, the inhibition of Eag1 reduces tumor cell proliferation *in vitro* and *in vivo*[112-114]. We reported that HepG2 and HuH-7 HCC cells displayed Eag1 channel expression, and that the anti-histamine astemizole (a non-specific Eag1 inhibitor) decreased cell proliferation and induced apoptosis in both cell lines. In addition, an increase in Eag1 expression was found during HCC development in rats. Astemizole treatment prevented HCC development and seems to induce tumor regression in rats with HCC[115].

T-type calcium channels play an important role in cell cycle progression in different types of cancer[116]. The expression of the three T-type calcium channel subunits was observed in HCC cell lines and T-type channel blockage with mibefradil decreased cell proliferation in the SNU449 cell line[117]. P2 purinergic receptors are overexpressed in certain cancer tissues; the levels of P2Y2 receptor are enhanced in HCC compared with human normal hepatocytes. These receptors are involved in ATP-induced $(Ca^{2+})_i$ increase. Silencing P2Y2R expression inhibited ATP-induced human HCC cell proliferation and migration, and P2Y2R blockage inhibited cell growth in mice[21,118]. In addition, high P2X3 receptor expression is associated with poor recurrence-free survival in HCC, while high P2Y13 expression is associated with improved recurrence-free survival. Moreover, extracellular nucleotide treatment induce cell cycle progression and extracellular ATP-mediated activation of P2X3 receptors promotes proliferation of HCC cells[119]. SOCE is a major Ca^{2+} influx pathway controlling the intracellular Ca^{2+} concentration in normal hepatocytes and HCC cells, and Ca^{2+} influx has been demonstrated to be involved in liver oncogenesis. Accordingly, the blockade of SOCE inhibits hepatocarcinoma cell migration and invasion, by regulating focal adhesion turnover[120]. The activation of SOCE channels is implicated in cancer cell chemoresistance, although the underlying molecular mechanisms are not well understood. However, inhibition of Orai1-mediated Ca^{2+} entry enhances chemosensitivity to 5-fluorouracil of HepG2 hepatocarcinoma cells[121]. The specific roles and molecular mechanisms of calcium entry in drug response deserve further investigation.

CIC-3 chloride channels have multiple functions in tumorigenesis and tumor growth in HCC; the CIC-3 channel blocker DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid) arrests the cell at the G1 phase, inhibiting the proliferation of Hep3B HCC cells[122]. Proteomic approaches found that the chloride intracellular channel 1 (CLIC1) is upregulated in HCC tissues, and that it participates in HCC migration and invasion by targeting maspin[123].

The voltage-gated sodium channel β1 subunit was proposed as a cell adhesion molecule in some HCC cell lines. The analgesic-antitumor peptide (a scorpion toxin polypeptide with antitumor activity) inhibits the migration and invasion of HepG2 cells by an upregulated VGSC β1 subunit[124]. Additionally the over-expression of $Nav_{1.2}$ channels has been observed in an HCC *in vivo* model[96].

AQP5 is highly expressed in HCC cell lines and its downregulation inhibits HCC cell invasion and tumor metastasis. Downregulation of AQP5 suppressed the epithelial-to-mesenchymal transition process in HCC cells[125]. Another report found that microRNA-325-3p inhibits cell proliferation and induces apoptosis

in HBV-related hepatocellular carcinoma by downregulation of AQP5[126]. These findings suggest that AQP5 may be a potential therapeutic target for HCC. AQP9 is the main aquaglyceroporin in the liver and its mRNA and protein levels are downregulated in HCC tissues compared to normal hepatocytes. Moreover, AQP9 over-expression inhibits hepatocellular carcinoma by upregulating FOXO1 expression, and suppresses invasion by inhibiting epithelial-to-mesenchymal transition. These findings suggest that the restoration of AQP9 expression can inhibit development of liver cancer[127,128].

The TRP channel family has gained great relevance due to its role in several diseases. A recent study investigated the roles of the Na^+/Ca^{2+} exchanger 1 (NCX1) and the canonical transient receptor potential channel 6 (TRPC6) in regulating TGFβ in human HCC. They found that TGFβ induces the formation and activation of a TRPC6/NCX1 molecular complex, which mediates the effects of TGFβ on the migration, invasion, and intrahepatic metastasis of HCC. These findings suggest TRPC6 and NCX1 as potential targets for HCC therapy[129,130]. HCC develops multi-drug resistance in most cases; interestingly, multi-drug resistance regulation by TRPC6 and calcium-dependence has been shown in HCC cells[131]. Silencing of TRPC1 channels suppressed cell proliferation while store-operated Ca^{2+} entry was significantly increased[132,133]. On the other hand, it has been found that high expression of the vanilloid receptor-1 (TRPV1) is associated with better prognosis of HCC patients[134].

The combined effect of static magnetic field and anti-cancer drugs has gained great interest in cancer. Static magnetic field enhances the anti-cancer effect of capsaicin on HepG2 cells through the mitochondria-dependent apoptosis pathway. This synergy may be explained if static magnetic field increased the binding efficiency of capsaicin to TRPV1 channels[135]. TRPV2 contributes to the stemness of liver cancer and is a potential target in the treatment of human liver cancer patients[136]. TRPV4 is over-expressed in HCC tissues when compared with non-tumoral liver. Furthermore, pharmacological inhibition of TRPV4 suppressed cell proliferation, induced apoptosis, and decreased the cell migration capability by attenuating the epithelial-to-mesenchymal transition process in HCC via modulation of the ERK signaling pathway[137]. TRPM7 channels play a role in the migration and invasion of different types of cancer; actually, bradykinin promotes cell migration and invasion of HCC cells via TRPM7 channels[138].

ASICs are H^+-, Ca^{2+}-, and Na^+-gated cation channels activated by changes in the extracellular pH, and ASIC1α (ASIC1a) has been associated with tumor proliferation and migration. ASIC1α is overexpressed in HCC tissues and associated with advanced clinical stage. Silencing of ASIC1α expression inhibited the migration and invasion of HCC cells, suggesting a novel approach for HCC therapy[139].

The R-Tf-D-LP4 cell-penetrating peptide derived from the mitochondrial multifunctional protein VDAC1 (voltage-dependent anion channel) induced apoptosis in liver cancer cell lines and inhibited liver tumor growth *in vivo*, representing a promising therapeutic approach for HCC[140]. Inositol 1,4,5-trisphosphate receptors (ITPRs) are intracellular Ca^{2+} channels. ITPR3 is either absent or expressed at low levels in normal hepatocytes, but it is over-expressed in HCC patients; its increased expression level was associated with poor survival. Besides, cell proliferation and liver regeneration were enhanced *in vivo*, and ITPR3 deletion in human HCC cells increased apoptosis[141].

Discussion: ion channels as potential tools for chronic liver diseases and HCC prevention, diagnosis, and therapy

HCC is a leading cause of cancer-death worldwide and is one of the most chemo-resistant tumors[3,142]. The combination of new therapeutic targets with existing therapies may be very helpful. Several ion channels play very important roles in cancer-associated processes including inflammation, oxidative stress, cell proliferation, apoptosis, migration, invasion, angiogenesis, metastases, and drug response. These proteins are differentially expressed in HCC and liver diseases compared to their expression in the healthy

Table 2. Ion channel inhibitors as potential therapeutic agents studied in HCC

Inhibitor	Targeted ion channel	Ion channel gene symbol*	Ref.
TRAM-34	$K_{Ca3.1}$	*KCNN4*	[103,104]
ASTEMIZOLE	Eag1, Herg	*KCNH1, KCNH2*	[115]
MIBEFRADIL	T-type Ca^{2+} channels	--	[117]
2-APB, SKF96365	SOCs	--	[21,118,121]
DIDS	CIC-3	*CLCN3*	[122]
MicroRNA-325-3P	AQP5	*AQP5*	[126]
CAPSAICIN	TRPV1	*TRPV1*	[135]
HC-067047	TRPV4	*TRPV4*	[137]

*When the specific ion channel has been reported to be targeted. HCC: hepatocellular carcinoma

Table 3. Ion channels suggested as HCC prognostic markers

Channel	Gene symbol	Expression in HCC	Association to prognosis	Ref.
KCNQ1	*KCNQ1*	Downregulated	Poor prognosis	[105]
KCNJ11	*KCNJ11*	Differentially expressed	Poor prognosis	[106]
P2X3	*P2RX3*	Overexpression	Poor recurrence-free survival	[119]
TRPV1	*TRPV1*	Overexpression	Better prognosis	[134]
ASIC1a	*ASIC1*	Overexpression	Advanced clinical stage	[139]
ITPR3	*ITPR3*	Overexpression	Poor survival	[141]

HCC: hepatocellular carcinoma

liver. Thus, patients at risk of developing some liver diseases, e.g., people infected with hepatitis viruses, patients with liver cirrhosis, or those suffering from alcoholism, might be candidates in whom ion channel expression can be studied. Nevertheless, an important issue to solve is how to detect ion channel expression in not easily accessible tissues such as the liver. An option may be ion channel detection by imaging studies. For instance, Eag1 channel expression has been detected *in vivo* with labeled antibodies and near-infrared imaging techniques, even in non-palpable tumors, in mice[143]. Another option may be the detection of ion channels in extracellular vesicles released to the bloodstream by the liver. The investigation of ion channel expression in extracellular vesicles released by the liver in different pathological conditions is needed. These approaches should benefit patients by being diagnosed at earlier stages of the disease.

The precise molecular mechanisms involved in the association of ion channel function with cancer remain elusive. The antiproliferative effect of channel blockage on cell proliferation indicates that ion flux may play an important role. However, non-canonical functions of ion channel may also play a role, as occurs in other tissues and diseases[144]. For instance, mutant non-conducting Kv10.1 potassium channels partially preserve their oncogenic potential[145]. On the other hand, cleavage and translocation to the nucleus of a fragment of the carboxy-terminus of some calcium channels induce the transcription of genes associated with proliferation[146]. Thus, the potential role of non-canonical functions of ion channels in liver diseases warrants investigation.

In accordance with the potential role of ion channels in liver diseases, blockage of over-expressed ion channels or activation of downregulated channels results in the inhibition of hepatitis virus replication, development of NAFLD, NASH, liver cirrhosis, and/or HCC [Table 2].

However, because of the relevance of ion channels in normal physiology, targeting these proteins may have non-desirable side-effects. In this direction, drug repurposing is a very good alternative to reduce costs and time for approval, as well as unknown side effects. Actually, several drugs have been suggested for repurposing in cancer, including anti-histamines such as astemizole (which also blocks potassium channels) and loratadine, as well as calcium and potassium channel blockers such as mibefradil and glibenclamide, respectively[147,148].

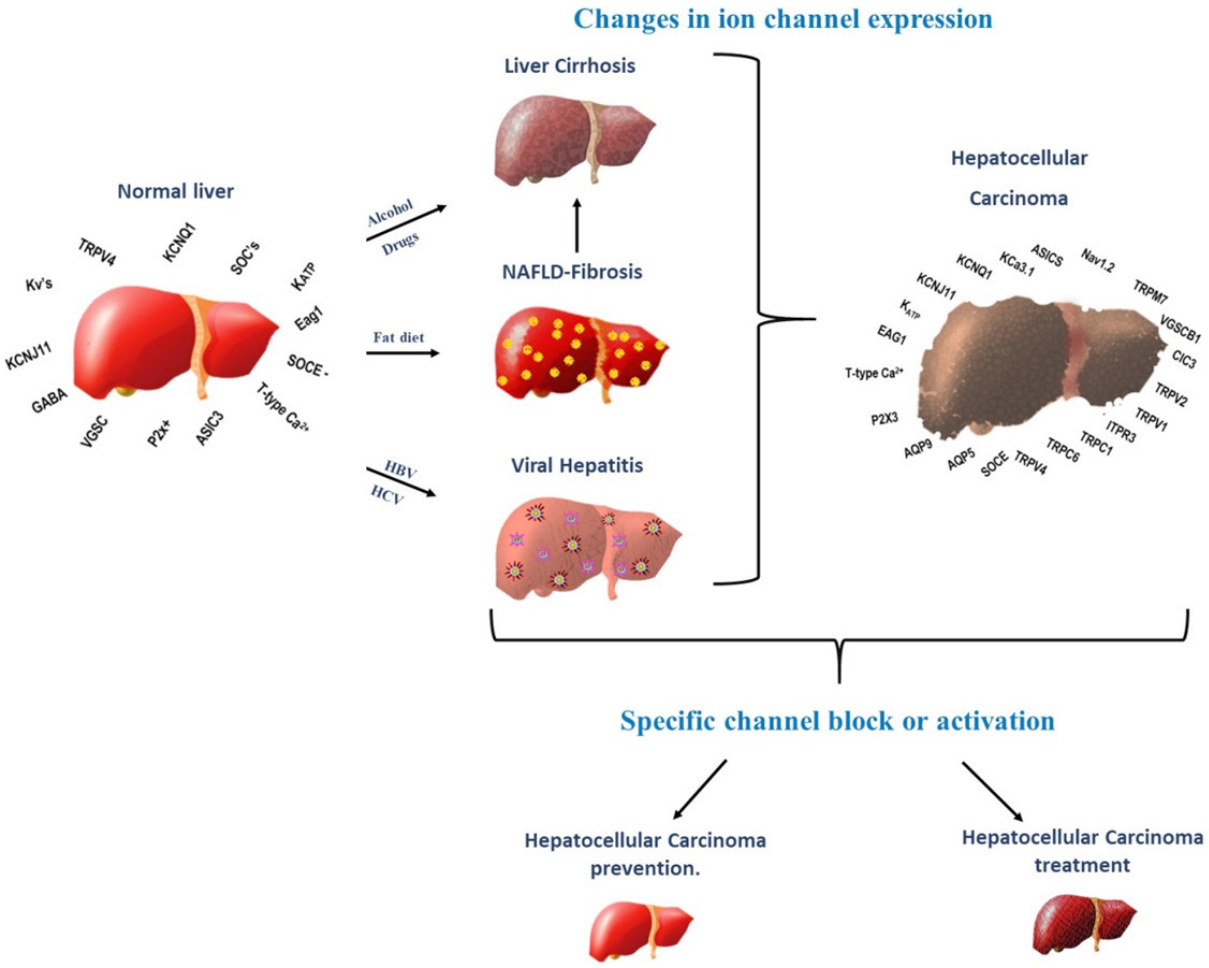

Figure 1. Ion channel-based therapy for HCC prevention and treatment. The expression of several ion channels is altered in liver disorders leading to HCC, as well as in HCC. Because of the very relevant participation of ion channels in cellular processes leading to HCC, targeting either the expression or activity of these proteins may lead to the prevention and treatment of liver diseases including HCC. HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBV: hepatitis B virus; NAFLD: nonalcoholic fatty liver disease

CONCLUSION

Ion channels offer a plethora of opportunities for the prevention, diagnosis, and treatment of liver diseases [Figure 1], as well as represent potential tools as HCC prognostic markers [Table 3]. This ion-channel-based approach may help to reduce the mortality of this very poor prognosis disease.

DECLARATION

Authors' contributions

Contributed to the conception and design of the review, wrote the paper and revised the final draft: Chávez-López MG, Cruz-Díaz A, Tlapalcoyoa-Apanco KN, Pérez-Carreón JI, Camacho J

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All authors declared that there are not conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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