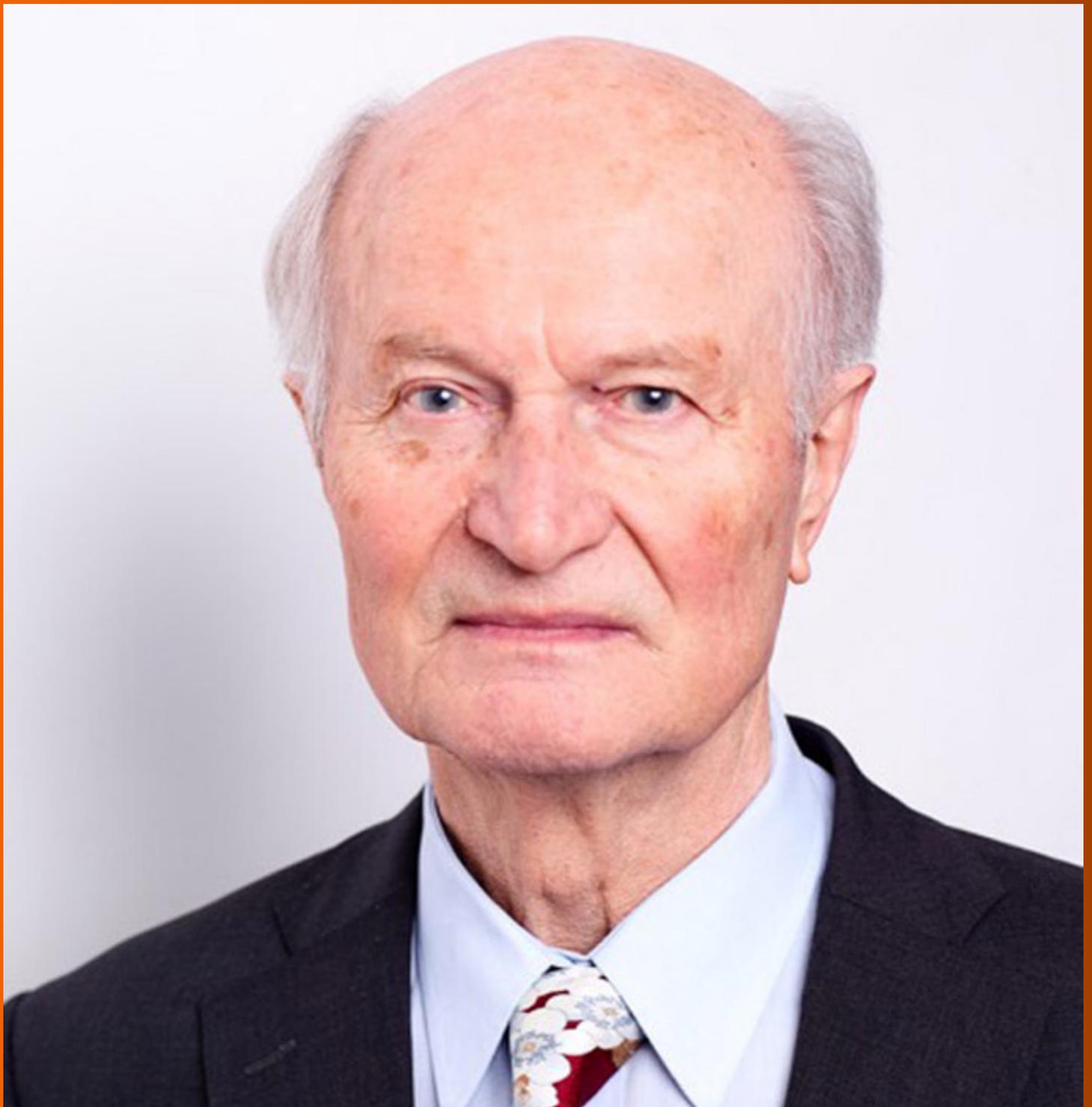


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## Inositol 1,4,5-trisphosphate receptor in the liver: Expression and function

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### Abstract

The liver is a complex organ that performs several functions to maintain homeostasis. These functions are modulated by calcium, a second messenger that regulates several intracellular events. In hepatocytes and cholangiocytes, which are the epithelial cell types in the liver, inositol 1,4,5-trisphosphate (InsP<sub>3</sub>) receptors (ITPR) are the only intracellular calcium release channels. Three isoforms of the ITPR have been described, named type 1, type 2 and type 3. These ITPR isoforms are differentially expressed in liver cells where they regulate distinct physiological functions. Changes in the expression level of these receptors correlate with several liver diseases and hepatic dysfunctions. In this review, we highlight how the expression level, modulation, and localization of ITPR isoforms in hepatocytes and cholangiocytes play a role in hepatic homeostasis and liver pathology.

**Key words:** Inositol 1,4,5-trisphosphate receptor; Liver; Calcium signaling; Hepatocytes and cholangiocytes

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**Core tip:** Calcium regulates a variety of functions in our body. In the liver, inositol 1,4,5-trisphosphate receptors (ITPR) are the only expressed intracellular calcium release channels. ITPR regulates liver functions under healthy situation, but they can also be involved in liver diseases, depending for instance, in which isoform is expressed in a specific cell type, level of expression and where inside the cell each isoform is expressed. In this review, we discuss about ITPR roles in hepatic cells in physiological

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## INTRODUCTION

The liver is an important and vital organ that regulates several functions, ranging from drug and macronutrient metabolism to immune system support<sup>[1-5]</sup>. Essentially all liver functions are at some point regulated by intracellular calcium (Ca<sup>2+</sup>). In hepatocytes and cholangiocytes, the principal epithelial cell types of the liver, inositol 1,4,5-trisphosphate (InsP<sub>3</sub>) receptors (ITPR) are the only intracellular Ca<sup>2+</sup> release channels<sup>[6,7]</sup>. There are three types of ITPR: type 1 (ITPR1), type 2 (ITPR2) and type 3 (ITPR3)<sup>[8,9]</sup>, and these receptors are expressed mainly along the endoplasmic and nucleoplasmic reticulum<sup>[10,11]</sup>.

Dysregulation in the expression of ITPR can be a cause of several liver disorders, or can be involved in the development of diseases, such as cholestasis<sup>[12]</sup> and non-alcoholic fatty liver disease (NAFLD)<sup>[13]</sup>. In this review, we will discuss the expression and the physiological functions of each isoform of ITPR present in liver hepatocytes and cholangiocytes as well as their role in disease (Table 1).

## LIVER

The liver is the largest internal organ<sup>[5]</sup> and is responsible for drug metabolism, albumin production, glycogen storage, cholesterol synthesis, bile secretion, and many other functions<sup>[14]</sup>. The liver is mostly composed of hepatocytes, which account for 80% of all cells in this organ<sup>[15]</sup>. The remaining 20% is composed mostly of cholangiocytes, Kupffer cells, stellate cells and liver sinusoidal endothelial cells<sup>[15]</sup>. Macroscopically, the liver is divided in four anatomic lobes, called the left, right, caudate and quadrate lobe<sup>[16,17]</sup>. In each lobe the cells are organized in a specific conformation, constituting a microscopic functional and structural unit, the lobule<sup>[14,18]</sup> (Figure 1). In the lobule, the hepatocytes are arranged in cords, connecting the portal triad to the central vein. In the space formed among the hepatocyte cords are the liver sinusoidal endothelial cells, the Kupffer cells, which are the resident macrophages in the liver, and the stellate cells, a cell type that stores vitamin A in its cytosol and secretes hepatocyte growth factor (HGF)<sup>[14,19,20]</sup>.

As an epithelial cell, the hepatocyte is polarized, with a basolateral membrane in contact with the sinusoids and an apical side forming the biliary canaliculus. The biliary canaliculus is a virtual space between two hepatocytes, into which hepatocytes secrete bile acids<sup>[4]</sup>. The biliary canaliculi join to form the bile duct, which is lined by cholangiocytes, specialized cells that secrete electrolytes and fluids into the bile, altering bile composition and viscosity. The bile duct conducts the bile to the gallbladder, where it is stored until its content are needed to help lipid digestion<sup>[21,22]</sup>.

Blood from the portal vein passes throughout the sinusoids and drains into the central vein<sup>[14,23]</sup>. Near the portal vein there are two other important structures: the hepatic artery and the bile duct. Together, these structures form the portal triad. The hepatocytes around this area are more highly oxygenated than those that are closer to the central vein, because the blood reaches the portal triad first<sup>[14]</sup>. This region in the lobule is called zone 1, while zone 2 is the transitional zone, and zone 3 is the region near the central vein<sup>[24,25]</sup> (Figure 2). It has been shown that based on their zonal position, hepatocytes regulate specific liver functions. For example, hepatocytes in zone 1 are more involved in producing albumin and proteins of both the complement system and coagulation pathway, while hepatocytes from zone 3 are more important for drug metabolism and bile production<sup>[26]</sup>.

Due to the key role of the liver in metabolism, hepatic tissue is continuously exposed to insults from xenobiotics, toxic metabolites and infectious agents<sup>[2]</sup>. As result of this, the liver has a remarkable capacity for regeneration. In mice, liver functions are restored within days of removing two-thirds of the organ. This capacity

**Table 1** Functions of inositol 1,4,5-trisphosphate receptor isoforms in hepatocytes and cholangiocytes

ITPR isoform	Cell type	Function	Ref.
ITPR1	Hepatocytes	Glucose secretion	[64]
		Lipid metabolism	[63,65]
		Liver regeneration	[66,67]
ITPR2	Cholangiocytes	Bicarbonate secretion	[12,61]
	Hepatocytes	Organic anion secretion	[6,62,69]
ITPR3	Cholangiocytes	Liver regeneration	[13,71]
		bicarbonate secretion	[12,61]
	Hepatocytes	Physiologically absent	[6]
ITPR3	Cholangiocytes	Proliferation and survival of hepatocellular carcinoma	[74]
		Bicarbonate secretion	[12,61]
ITPR3	Cholangiocytes	Proliferation, migration, and survival of cholangiocarcinoma	[82]

ITPR: Inositol 1,4,5-trisphosphate receptor; ITPR1: ITPR isoform 1; ITPR2: ITPR isoform 2; ITPR3: ITPR isoform 3.

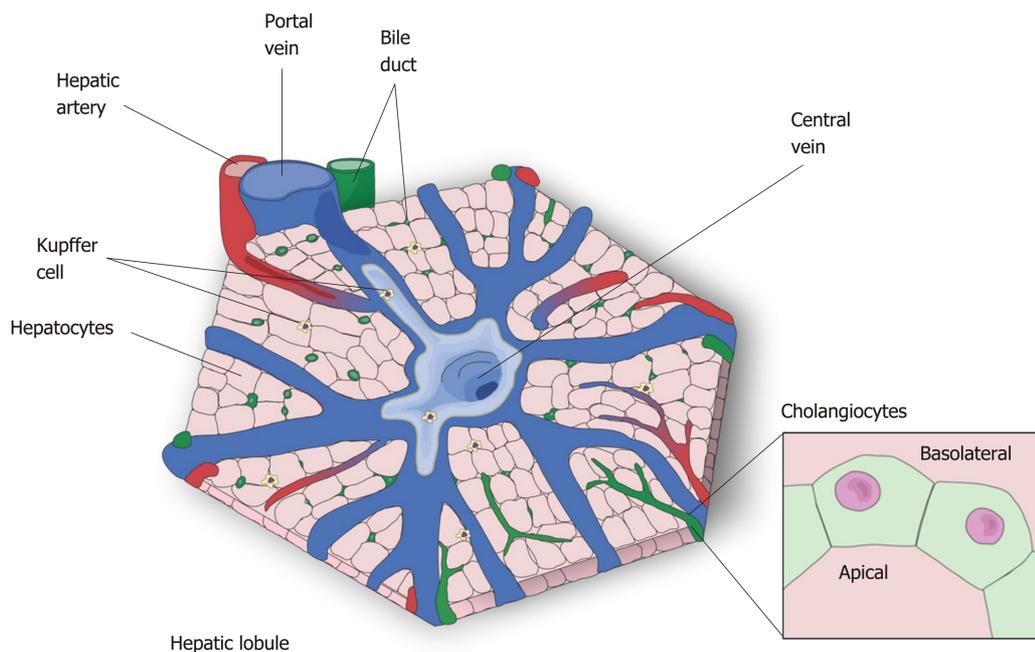
is also observed in humans for which liver function after partial hepatectomy is reestablished within a few weeks<sup>[27]</sup>. In many cases of liver disease, for which partial hepatectomy is indicated as a treatment, a small piece of healthy liver is implanted to drive hepatic tissue regeneration<sup>[27,28]</sup>. The path to regeneration depends on the extent of liver loss. When 1/3 of the liver is removed, the primary response is hepatocyte cellular hypertrophy, *i.e.*, an increase in cell size. When liver loss reaches 2/3, hepatocyte hyperplasia, an increase of the number of hepatocytes occurs to reestablish liver function. When 80-90 % of the liver is removed, the biliary epithelial cells (BEC) turn into progenitor cells, which differentiate into hepatocytes or BEC<sup>[28]</sup> that are able to regenerate the tissue. Liver regeneration is a complex process and the mechanism by which the hepatocytes stop proliferating after reestablishment of liver function is poorly understood. It is important to highlight that Ca<sup>2+</sup> signaling, and consequently the ITPR isoforms, play an essential role in liver regeneration, as discussed below.

## CALCIUM SIGNALING AND ITPRS

Many biological functions are regulated by intracellular Ca<sup>2+</sup>. These include cell proliferation, gene expression, secretion, motility and cell death, among others<sup>[29-33]</sup>. As in other tissues, Ca<sup>2+</sup> signaling in the liver starts with the binding of an agonist to a receptor, which may be a G protein-coupled receptor (GPCR) (Figure 3) or a tyrosine kinase receptor (RTK). Upon agonist-receptor binding, phospholipase C (PLC) is activated (typically isoform PLC $\beta$  when GPCR is activated or isoform PLC $\gamma$  after RTK activation), causing breakdown of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), that generates diacylglycerol (DAG) and InsP<sub>3</sub>. DAG remains at the plasma membrane while InsP<sub>3</sub> diffuses into the cytoplasm where it can bind to the InsP<sub>3</sub> receptor (ITPR) localized along the endoplasmic reticulum membrane, nuclear envelope or nucleoplasmic reticulum. InsP<sub>3</sub>-ITPR binding causes a conformational change in the ITPR, leading to the release of internal Ca<sup>2+</sup> stores<sup>[32,34]</sup>. InsP<sub>3</sub> is inactivated either after conversion to inositol 1,2-bisphosphate (InsP<sub>2</sub>) by type I inositol polyphosphate 5-phosphatase or by InsP<sub>3</sub>-kinase mediated phosphorylation, forming inositol 1,3,4,5-tetrakisphosphate (InsP<sub>4</sub>)<sup>[35,36]</sup>.

Because of the toxic effect of high concentrations of Ca<sup>2+</sup> to the cells, this ion is promptly removed from the cytosol after its release. Different mechanisms are involved in this process, including the activation of plasma membrane Ca<sup>2+</sup>-ATPase or Na<sup>+</sup>/Ca<sup>2+</sup> exchanger that exports Ca<sup>2+</sup> out of the cell, while sarco-/endoplasmic Ca<sup>2+</sup>-ATPase (SERCA) and mitochondrial Ca<sup>2+</sup> uptake 1 (MCU1) move Ca<sup>2+</sup> into the endoplasmic reticulum and mitochondria, respectively<sup>[37,38]</sup>.

The ITPRs are formed by approximately 2700 amino acids<sup>[39,40]</sup> and are organized in three domains: a N-terminal domain, which includes the InsP<sub>3</sub>-binding region, a C-terminal domain, which forms the channel pore, and a regulatory domain between the other regions (Figure 4). ITPR is an intrinsic membrane protein, with 6 transmembrane segments<sup>[41,42]</sup>. There are some sites along the ITPR structure that regulate the activity of the receptor, or determine its localization by posttranslational modifications (phosphorylation, ubiquitination, oxidation, and proteolytic frag-



**Figure 1** The hepatic lobule—the microscopic functional structure of the liver. The hepatocytes are arranged in cord, connecting the central vein to the portal triad, which is formed by a hepatic artery, a portal vein and a bile duct. The other represented cell types are: *Kupffer cell*, resident macrophages responsible for the immunologic response in the liver, and *cholangiocytes* which form the bile duct that transport bile to the gallbladder.

mentation) or by interaction with modulatory proteins, such as chromogranin A and B, neuronal  $Ca^{2+}$  sensor 1, cytochrome c, and antiapoptotic Bcl-2 family members<sup>[43-46]</sup>. There are three isoforms of ITPR: type 1 (ITPR1), type 2 (ITPR2) and type 3 (ITPR3)<sup>[8,9]</sup>. They share 70% homology<sup>[47]</sup>, however each isoform of ITPR displays a distinct affinity to  $InsP_3$ : ITPR2 has the highest affinity, ITPR1 has an intermediate affinity, and ITPR3 has the lowest affinity<sup>[48,49]</sup>. In order to open the  $Ca^{2+}$  channel, four  $InsP_3$  molecules need to bind to ITPR<sup>[50]</sup>. Moreover,  $Ca^{2+}$  ions directly modulate the open probability of the channel<sup>[51,52]</sup>. ITPR1 displays what is called a “bell shape” open probability curve, in other words, at lower concentrations of  $Ca^{2+}$  the ITPR1 releases  $Ca^{2+}$ , while higher  $Ca^{2+}$  concentrations inhibit the channel<sup>[53,54]</sup>. For ITPR3, the open probability of the channel increases with increased  $Ca^{2+}$  concentration<sup>[52,55]</sup>, and the ITPR2 dependence on  $Ca^{2+}$  concentration remains controversial. While single-channel studies show that ITPR2 displays the same configuration as observed for ITPR3, studies with whole cells exhibit similarity with ITPR1<sup>[51,56,57]</sup>, suggesting an effect of the modulatory proteins on the ITPRs channel activity.

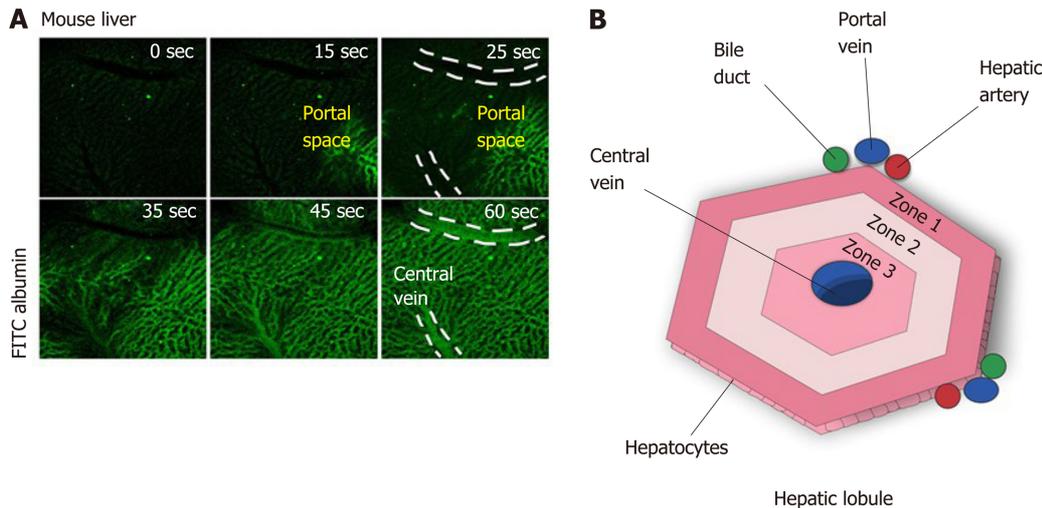
ITPRs are widely expressed, sometimes with the prevalence of a single ITPR isoform in a specific tissue. For example, in the central nervous the main ITPR isoform is ITPR1, regulating neurite formation among other functions<sup>[58]</sup>. ITPR2 is the isoform mainly expressed in cardiomyocytes, participating in heart rate and in the action potential duration<sup>[59]</sup>. In pancreatic tissue, ITPR2 and ITPR3 are involved in the exocytosis of zymogen granules<sup>[60]</sup>.

In the liver, hepatocytes express ITPR1 and ITPR2<sup>[6]</sup>, whereas all three isoforms are expressed in cholangiocytes<sup>[12]</sup>. Below, we discuss separately about the ITPR isoforms in hepatic cells, focusing on hepatocytes and cholangiocytes, while indicating their main function and expression pattern in normal condition and in liver disease.

### **ITPR1: Metabolism and electrolyte secretion**

ITPR1 is expressed in both hepatocytes and cholangiocytes, corresponding to approximately 20% of the total ITPRs present in these cells. It is localized along the endoplasmic reticulum, throughout the cytoplasm and near the nucleus<sup>[6,61-63]</sup>.

In normal liver tissue, ITPR1 regulates metabolism in hepatocytes<sup>[63-65]</sup>. After exposure to glucagon, mouse hepatocytes display an increase in ITPR1 phosphorylation by the activity of protein kinase A (PKA), raising intracellular  $Ca^{2+}$  concentration that leads to glucose secretion<sup>[64]</sup>. More evidence of the ITPR1 function in liver metabolism was shown in obese (ob/ob) and high fat diet (HFD) mouse models. Ob/ob mice and mice maintained on a high-fat diet (HFD) overexpress ITPR1, increasing the amount of these  $Ca^{2+}$  channels in close proximity to the mitochondria<sup>[65]</sup>. In accordance with the increase in ITPR expression, cytoplasmic and



**Figure 2** The microenvironment in the hepatic lobules. A: Perfusion with fluorescein isothiocyanate albumin shows that the blood flow arrives in the liver by portal vein, passes throughout the sinusoidal space, and drains into the central vein; B: Due to this blood flux, different zones of oxygenation are observed: zone 1, closer to portal vein, is the most highly oxygenated and zone 3 is the least oxygenated. zone 2 is intermediary. The direction of bile flux is opposite to that of blood flow. The bile acids excreted by the hepatocytes go to the bile duct through the biliary canaliculus.

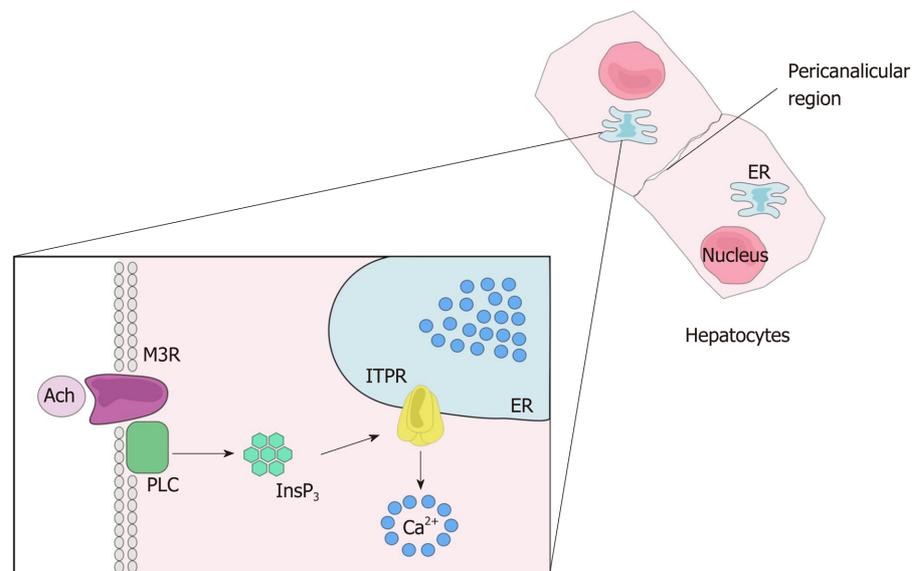
mitochondrial  $\text{Ca}^{2+}$  concentration is increased in obese mice, causing mitochondrial dysfunction and impairment of metabolic homeostasis<sup>[65]</sup>. Conversely, the reduction of ITPR1 expression in the mouse liver, by short hairpin RNA technique, improved glucose tolerance and mitochondrial metabolism<sup>[65]</sup>. These results were validated in ITPR1 liver-specific knockout mice (ITPR1 LSKO). ITPR1 LSKO mice are leaner and display less hepatic steatosis after HFD, and also have reduced levels of triglycerides and lipogenic gene expression. These metabolic alterations are in accordance with the lower mitochondrial  $\text{Ca}^{2+}$  signal observed in isolated hepatocytes from ITPR1 LSKO mice<sup>[63]</sup>. Translational studies corroborate these findings by showing that liver specimens from non-alcoholic steatohepatitis (NASH) donors display increased hepatic ITPR1 expression which is concentrated closer to mitochondria. Based on these observations, it has been suggested that ITPR1 plays a role in steatosis in human fatty liver diseases<sup>[63]</sup>.

Another function of ITPR1 in hepatocytes is related to the liver regeneration. Knocking down ITPR1 in rat with small interfering RNA (siRNA), attenuates  $\text{Ca}^{2+}$  signaling, and results in an impairment of hepatocytes proliferation after partial hepatectomy, measured by proliferating cell nuclear antigen staining positive cells. Consequently, the liver growth is diminished at the early phase (up to 48 h) of liver regeneration<sup>[66]</sup>. The involvement of ITPR1 in the beginning of the liver regeneration process is supported by the normal expression of this isoform immediately after the partial hepatectomy, followed by a downregulation of ITPR1 afterwards<sup>[67]</sup>.

In cholangiocytes, ITPR1, together with the ITPR2, are responsible for releasing bicarbonate after the activation of type 3 muscarine receptor by acetylcholine. These findings were observed by using intrahepatic bile duct units isolated from rat liver tissue, previously transfected with ITPR1 and ITPR2 siRNA, and then by measuring the luminal pH after acetylcholine exposition. It was shown that the bicarbonate secretion was reduced in ITPR1 and ITPR2 knockdown cells<sup>[61]</sup>. Moreover, in cholestasis, which is a disorder that causes bile accumulation, the expression of ITPR1 is decreased, similarly to what occur to the other ITPR isoforms<sup>[12]</sup>. These observations suggest that the downregulation of ITPRs is an early event in the pathogenesis of cholestasis. As a consequence of the decrease of ITPR1 expression in a rat model of bile duct ligation, the  $\text{Ca}^{2+}$  signal is reduced and the biliary bicarbonate secretion is impaired in isolated cholangiocytes<sup>[12]</sup>. Together, these findings show that ITPR1 isoform plays a crucial role in hepatocyte metabolism and proliferation, as well as in cholangiocyte secretory activity, which are essential functions for normal liver.

### **ITPR2: Bile acid /electrolyte secretion and liver regeneration**

ITPR2 is considered the principal intracellular  $\text{Ca}^{2+}$  release channel expressed in human and rodent hepatocytes<sup>[6,68]</sup>. This isoform is mostly concentrated in the canalicular membrane (apical region) of hepatocytes<sup>[62,69]</sup>, and due to its localization and high affinity for  $\text{InsP}_3$ <sup>[48,49]</sup>, ITPR2 plays an essential role in bile formation<sup>[6,69]</sup>. ITPR2 modulates the multidrug resistance associated protein 2 (Mrp2), which is



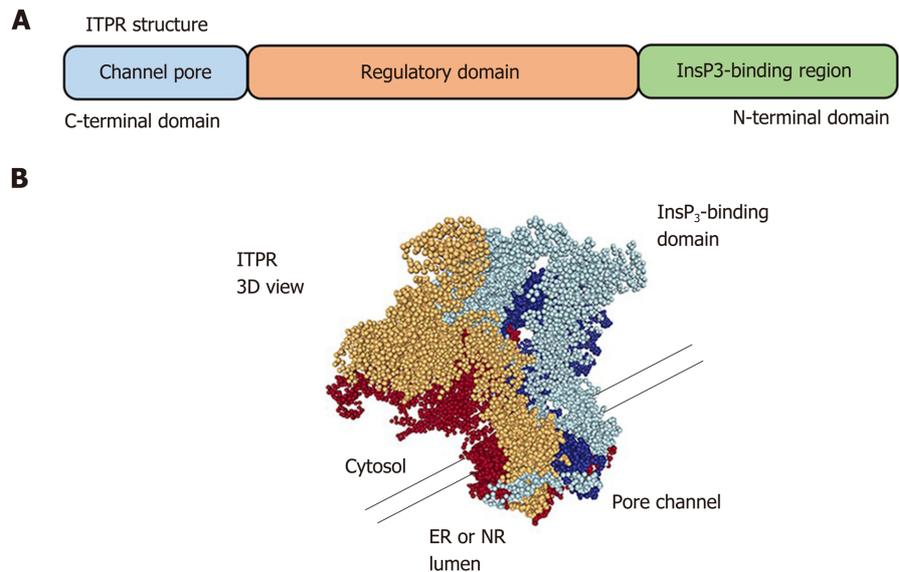
**Figure 3 Calcium signaling.** After the ligation of an agonist to its receptor, here represent by the ligation of acetylcholine to muscarinic acetylcholine receptor, phospholipase C is activated and produces 1,4,5 inositol triphosphate. The inositol 1,4,5-trisphosphate binds its receptor, inositol 1,4,5-trisphosphate receptor, that is expressed mainly along the endoplasmic reticulum, leading to  $\text{Ca}^{2+}$  release into the cytosol. Ach: Acetylcholine; M3R: Muscarinic acetylcholine receptor; PLC: Phospholipase C;  $\text{InsP}_3$ : Inositol 1,4,5-trisphosphate; ITPR: Inositol 1,4,5-trisphosphate receptor; ER: Endoplasmic reticulum.

responsible for organic anion secretion into bile, such as bilirubin, glutathione S-conjugates, and oxidized glutathione. Impairment of intracellular  $\text{Ca}^{2+}$  signal inhibits the insertion of the Mrp2 into the apical plasma membrane in hepatocytes and reduces organic ion secretion. Similarly, hepatocytes isolated from ITPR2 knockout mice also presented decreased intracellular  $\text{Ca}^{2+}$  signaling, as well as impaired organic ion secretion<sup>[69]</sup>.

Another bile salt transporter regulated by ITPR2 activity is the bile salt export pump (Bsep), an important protein normally positioned along the canalicular membrane of the hepatocyte. In a three-dimensional culture system of rat hepatocytes, siRNA against ITPR2 significantly reduced bile salt secretion, correlating with the downregulation and mislocalization of Bsep. Reduced bile secretion was also observed when the pericanalicular localization of ITPR2 was disrupted by methyl- $\beta$ -cyclodextrin to disturb lipid rafts<sup>[62]</sup>. Confirming the importance of ITPR2 to the correct bile salt transporter localization and secretory activity, immunohistochemistry analysis of hepatocytes from lipopolysaccharides (LPS) and estrogen cholestasis rat models showed a reduction of ITPR2 expression level and its diffuse distribution, different from its normal localization to the apical membrane<sup>[62]</sup>. Conversely, fasting causes a physiological upregulation of ITPR2 expression level<sup>[70]</sup>. It was shown that overnight fasting raises the mRNA and protein levels of ITPR2 in rat hepatocytes. It happens by the activation of cAMP signaling caused by a fast-dependent increase of serum glucagon levels<sup>[70]</sup>.

The correct expression level of ITPR2 is also important for hepatocyte proliferation. Downregulation of ITPR2 was observed in obese mice<sup>[71]</sup>, a condition that compromises liver regeneration<sup>[72]</sup>. ITPR2 downregulation was also observed in human liver specimens of patients diagnosed with steatosis and NAFLD, common liver diseases associated with obesity<sup>[13]</sup>. Recently, the connection between lower expression of ITPR2 and impairment of liver regeneration in some liver diseases was clarified<sup>[13]</sup>. In both human biopsies of steatosis and NAFLD, as well as in a high fructose diet induced rat model of NAFLD, the transcriptional factor c-Jun activates a pro-inflammatory environment that negatively regulates ITPR2 expression in hepatocytes<sup>[13]</sup>. As consequence of downregulation of ITPR2, a delay of liver regeneration was observed. Similarly, ITPR2 knockout mice subjected to partial hepatectomy showed more liver damage and decreased proliferation of hepatocytes<sup>[13]</sup>. This was a consequence of decreased nuclear  $\text{Ca}^{2+}$  signaling, a fundamental event for cell proliferation<sup>[10,66]</sup>. ITPR2-knockout cells markedly reduced nucleoplasmic  $\text{Ca}^{2+}$  and proliferation rates compared to WT cells<sup>[13]</sup>.

In cholangiocytes, ITPR2 represents about 10% of total ITPR, and is distributed diffusely throughout the endoplasmic reticulum membrane in the cytosol<sup>[12]</sup>.



**Figure 4 Inositol 1,4,5-trisphosphate receptor structure.** A: Linear structure of inositol 1,4,5-trisphosphate receptor (ITPR). The inositol 1,4,5-trisphosphate (InsP<sub>3</sub>)-binding domain is located on the N-terminal region and the pore channel is on the C-terminal region. The receptor spans organelle membrane six times; B: Tridimensional view of ITPR showing that the receptor is formed by a 4 single chain. It is necessary that four InsP<sub>3</sub> molecules bind to the receptor to lead the calcium releases by a pore of the channel. The tridimensional structure was adapted from Molecular Modeling Database (National Center for Biotechnology Information). ER: Endoplasmic reticulum; ITPR: Inositol 1,4,5-trisphosphate receptor; NR: Nucleoplasmic reticulum.

Functional studies showed that ITPR2 participates in the bicarbonate secretion by cholangiocytes. As discussed above, ITPR1 and ITPR2 knockdown cholangiocytes show a decrease in Ca<sup>2+</sup> signal, and a reduction in Ca<sup>2+</sup>-dependent bicarbonate secretion when stimulated by acetylcholine<sup>[61]</sup>. Similar observations have been made in some cholestatic human diseases. The expression of ITPR2 is dramatically reduced in cholangiocytes from samples of patients with bile duct obstruction and primary biliary cirrhosis<sup>[12]</sup>. In summary, the ITPR2 displays an essential function in the liver, regulating bile formation and bicarbonate secretion, as well as regenerating hepatocytes.

### ***ITPR3: Cell proliferation and electrolyte secretion***

In normal conditions, hepatocytes express ITPR1 and ITPR2 isoforms, but not the ITPR3<sup>[6]</sup>. However, ITPR3 is present in several hepatocellular carcinoma (HCC) cell lines<sup>[73,74]</sup>, as well as in NASH-related HCC<sup>[75]</sup>. The mechanism of the “de novo” ITPR3 expression in hepatocytes in the context of HCC has been partially described and involves epigenetic modification<sup>[74]</sup>, which represents changes in the genome structure that do not alter the nucleotide sequence. Examples include DNA methylation and histone modification<sup>[76]</sup>. Recently, bioinformatics analysis showed that the ITPR3 promoter region has a large number of CpG islands<sup>[74]</sup> that can be methylated by DNA methyltransferases, resulting in suppression of the gene<sup>[76,77]</sup>. Due to high level of DNA methylation at the ITPR3 promoter region, ITPR3 expression is repressed in hepatocytes under normal conditions. However, the referred methylation level is decreased in patients with HCC, allowing the expression of ITPR3 to be increased under hepatocellular disease conditions<sup>[74]</sup>. The expression of ITPR3 drives cell proliferation besides preventing the apoptotic cascade activation<sup>[74]</sup>, events closely related to tumor development. Together, these findings put the ITPR3 Ca<sup>2+</sup> channel as an essential factor that contributes to the pathogenesis of HCC.

Contrary to the normal hepatocytes, cholangiocytes constitutively express all three isoforms of ITPR<sup>[7]</sup>, with the ITPR3 being the most widely expressed, constituting approximately 80% of ITPRs in this cell type. ITPR3 mainly localizes to the apical region of the cholangiocytes in rodents and humans<sup>[7]</sup>. This apical localization of ITPR3 in cholangiocytes is important for its physiological function of secreting bicarbonate<sup>[78]</sup>. It was shown that downregulation of ITPR3 selectively disturbs the cAMP-induced bicarbonate secretion<sup>[61]</sup>. Different from ITPR1 and ITPR2, in which the bicarbonate secretion is dependent on activation of muscarinic acetylcholine receptors, ITPR3 leads to bicarbonate secretion by a cAMP-dependent cascade, wherein activation of secretin receptor indirectly stimulates InsP<sub>3</sub> production and Ca<sup>2+</sup>

release via ITPR3<sup>[61]</sup>.

As described above to the other ITPR isoforms, the ITPR3 expression is progressively decreased in bile duct ligation cholestasis rat model. Downregulation of ITPR3 was also observed after acute cholestasis, such as the endotoxin mouse model, as well as in chronic cholestatic disease in human, *e.g.*, bile duct obstruction, biliary atresia, primary biliary cirrhosis, sclerosing cholangitis, and autoimmune cholestatic<sup>[12]</sup>.

Several intracellular mechanisms have already been elucidated as being responsible for the loss of ITPR3 in cholangiocytes under pathological conditions. It was demonstrated for instance that LPS inoculation activates Toll like receptor 4 in cholangiocytes and, consequently, the transcription factor NF- $\kappa$ B. NF- $\kappa$ B then associates to the ITPR3 promoter region, inhibiting its expression in cholangiocytes. This mechanism is responsible for the loss of ITPR3 in patients affected by cholestasis due to sepsis or severe alcoholic hepatitis<sup>[79]</sup>. In cholangiopathies under oxidative stress conditions, including sclerosing cholangitis, primary biliary cholangitis, primary biliary obstruction and biliary atresia, the nuclear erythroid 2-like transcription factor 2 (Nrf2) is activated, acting negatively on ITPR3 expression<sup>[80]</sup>. Finally, the ITPR3 expression is also negatively regulated by the microRNA miR-506 in patients with primary biliary cholangitis<sup>[81]</sup>.

Conversely to the downregulation of ITPR3 in cholangiopathies and cholestasis, this Ca<sup>2+</sup> channel becomes over-expressed in cholangiocarcinoma<sup>[82]</sup>. ITPR3 accumulates in ER-mitochondrial junctions in cholangiocarcinoma cell lines, increasing mitochondrial Ca<sup>2+</sup> signaling. Moreover, ITPR3 increases nuclear Ca<sup>2+</sup> signaling in cholangiocarcinoma, which contributes to cell proliferation, migration, and survival<sup>[82]</sup>.

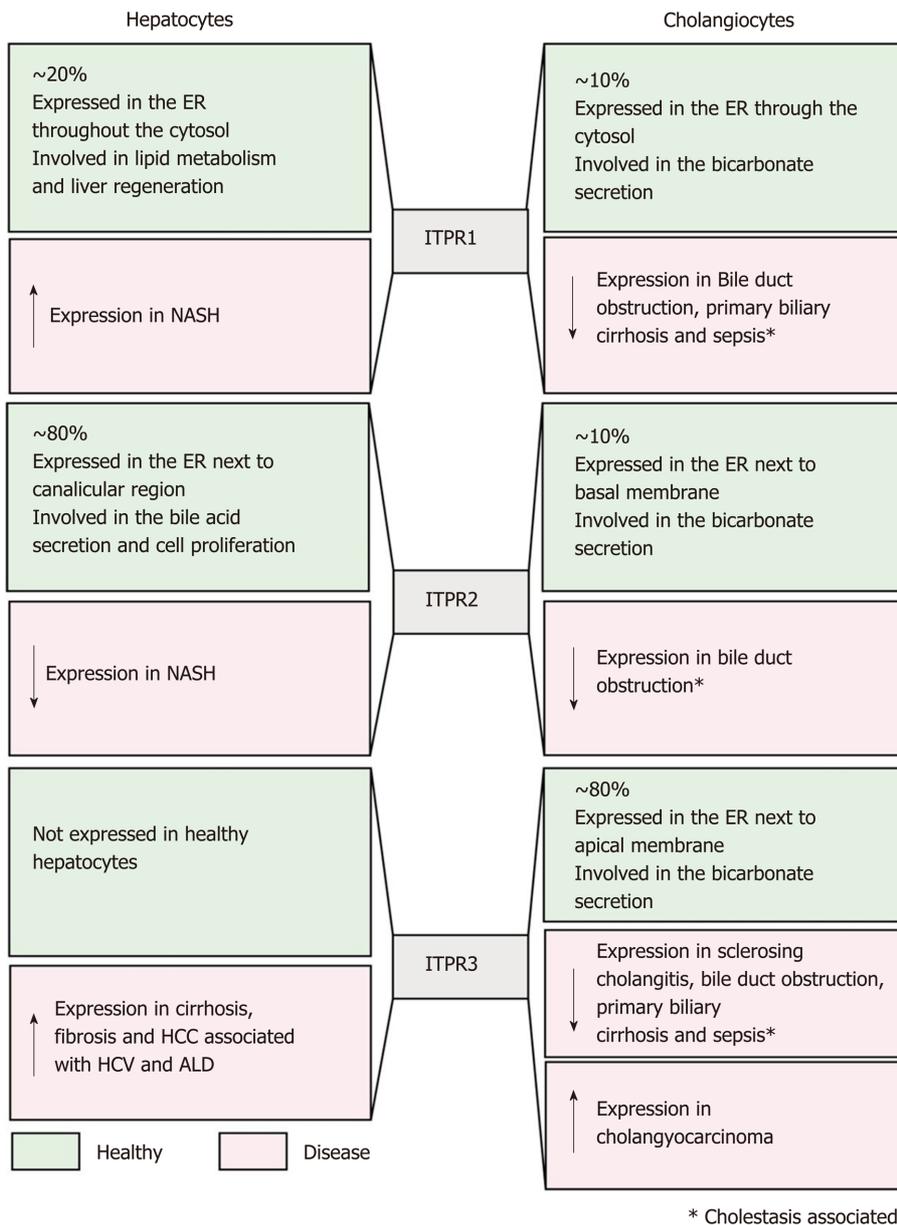
Together, these findings show that ITPR3 is absent in healthy hepatocytes but is expressed in HCC and indicates that it may be a target to understand liver cancer and its clinical implications. On the other hand, in cholangiocytes, ITPR3 is crucial to bile formation and the decrease in its expression causes cholestasis, observed in many liver diseases, while it is over-expressed in cholangiocarcinoma, contributing to malignant features, such as cell proliferation, migration and survival.

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## CONCLUSION

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In this review, we described several evidences of the role of the Ca<sup>2+</sup> signaling, and consequently the activity of ITPRs, in normal liver functions. Mislocalization and/or change in expression level of these Ca<sup>2+</sup> channels have been directly related to some liver disease (summarized in **Figure 5**). The alterations in ITPR expression and localization point these Ca<sup>2+</sup> channels as a valuable biomarker for prediction and prognosis of hepatic disease. In addition to diagnosis for liver diseases, ITPR would be a rational target for these pathological conditions. Epigenetic modification, pro-inflammatory transcription factors and miRNA have already been associated to the modulation of ITPR expression in pathological conditions. However, this field remains to be better explored to elucidate the upstream cascade that drives ITPR expression alterations. Better understanding of this pathway could open the perspective of developing pharmacological strategies for liver diseases, specifically targeting each ITPR isoforms.



**Figure 5 Inositol 1,4,5-trisphosphate receptors in the liver: Expression and functions.** This figure summarizes, in green, Inositol 1,4,5-trisphosphate receptor (ITPR) isoform expression in hepatocytes and cholangiocytes under physiological condition, and in red the expression level and function of each ITPR isoform in liver diseases. ITPR1: ITPR isoform 1; ITPR2: ITPR isoform 2; ITPR3: ITPR isoform 3; ER: Endoplasmic reticulum; NAFLD: Non-alcoholic fatty liver disease; HCC: Hepatocellular carcinoma.

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