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Type 3 inositol 1,4,5-trisphosphate receptor: A calcium channel for all seasons

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Abstract

Inositol 1,4,5 trisphosphate receptors (ITPRs) are a family of endoplasmic reticulum Ca^{2+} channels essential for the control of intracellular Ca^{2+} levels in virtually every mammalian cell type. The three isoforms (ITPR1, ITPR2 and ITPR3) are highly homologous in amino acid sequence, but they differ considerably in terms of biophysical properties, subcellular localization, and tissue distribution. Such differences underscore the variety of cellular responses triggered by each isoform and suggest that the expression/activity of specific isoforms might be linked to particular pathophysiological states. Indeed, recent findings demonstrate that changes in expression of ITPR isoforms are associated with a number of human diseases ranging from fatty liver disease to cancer. ITPR3 is emerging as the isoform that is particularly important in the pathogenesis of various human diseases. Here we review the physiological and pathophysiological roles of ITPR3 in various tissues and the mechanisms by which the expression of this isoform is modulated in health and disease.

Graphical Abstract

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Keywords

inositol 1,4,5-trisphosphate receptor; apoptosis; cancer; promoter methylation; Cholestasis

I. Introduction

Ca^{2+} signaling mediates fundamental cellular processes such as transepithelial transport, differentiation, oocyte fertilization, gene transcription, muscle contraction, learning and memory, membrane trafficking, synaptic transmission, metabolism, secretion, motility, membrane excitability, cell proliferation, and apoptosis [1]. Therefore, cytosolic Ca^{2+} levels are tightly regulated by a number of ion transporters and channels located at the endoplasmic reticulum membrane, plasma membrane, inner mitochondrial membrane as well as in the nuclear envelope and lysosomes. The ER is the main intracellular Ca^{2+} storage organelle and release of Ca^{2+} is mediated by the ryanodine receptors (RyRs) and inositol 1, 4, 5-trisphosphate (InsP3) receptors (ITPRs). RyRs are a family of three closely homologous proteins (RyR1, RyR2 and RyR3) that play a prominent role in Ca^{2+} signaling in excitable cells such as neurons and myocytes. The ITPR family is also composed of three isoforms. Each consists of approximately 2700 amino acids and is encoded by a separate gene (ITPR1, ITPR2 and ITPR3) but the isoforms are 70% homologous in terms of primary sequence [2]. These isoforms then assemble into homo- or heterotetramers to form functional channels [3]. Channel opening is triggered by binding of InsP3, which is formed by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) in the cell [4] or nuclear [5, 6] membrane by phospholipase C (PLC) isoforms. The three ITPR isoforms can control different cellular processes due to their unique biophysical properties and subcellular localization. Therefore, changes in either expression or subcellular localization of an isoform can change cell behavior. Here we review the biological role of ITPR3, factors that affect its expression and subcellular localization, and the effects that its altered expression has on the pathogenesis of disease.

II. Biophysics and Physiology of InsP3Rs

Molecular cloning studies primarily based on ITPR1 suggest that all ITPR isoforms are composed of 5 main functional domains: starting at the N-terminus, the suppressor domain (SD), the InsP3 binding core (IBC), the regulatory domain, the transmembrane domain (TD) and the carboxy-terminus domain (CTD) [7, 8]. As the name implies, the IBC contains the binding pocket for InsP3 and this property has been explored experimentally to generate genetically encoded InsP3 buffers [5, 9]. The TD is formed by six transmembrane helices embedded into the ER membrane, which form the ion conducting pore and contain residues responsible for cation selectivity. Ca^{2+} is the primary ion transported because it is the only cation with a gradient across the ER membrane. The largest portion comprises the regulatory domain, which localizes at the cytoplasmic face of the receptor. It is the site of interaction with a large number of regulatory proteins and small molecules such as ATP and the site of post-translational modifications such as phosphorylation and O-GlcNAc-glycosylation [10–12]. ITPR activity also can be modulated by reactive oxygen species [13]. Structural studies by cryo-electron microscopy have established that the ITPR tetramer has a “mushroom-like”

shape [7]. The stalk is inserted in the ER membrane and contains the TM domain. The cap instead faces the cytosol and encompasses all the remaining domains of the protein.

Although ITPR isoforms have a high degree of sequence homology and overall basic function, differences exist in terms of biophysical properties and signaling outcomes among the isoforms. Indeed, studies employing concatenated ITPR heterotetramers with multiple permutations of ITPR isoforms suggest that one of the isoforms within the assembled receptor acts as the dominant subunit and determines the final Ca^{2+} signaling outcome [3]. One contentious issue regarding differential activity of the ITPR isoforms was that of regulation by Ca^{2+} . Earlier studies employing a combination of Ca^{2+} imaging of intact cells expressing primarily one of the ITPR isoforms and measurements of purified receptor conductance in artificial lipid bilayers suggested that ITPR1 and ITPR2 but not ITPR3 displayed a bell-shaped response to Ca^{2+} in which lower concentrations of Ca^{2+} stimulate channel conductance and higher concentration inhibits channel conductance [14–16]. Later studies with recombinant ITPRs expressed in Sf9 cells and patch clamping of native receptors on the nuclear membrane of *Xenopus* oocytes however, suggested that all isoforms display the biphasic “bell-shaped” response to increasing Ca^{2+} concentrations [17, 18]. These discrepancies might be accounted for not only by differences in experimental approaches but also by isoform-specific ITPR-interacting proteins [19]. Another example of biophysical differences among the isoforms is the order of InsP3 affinity, which was established as $\text{ITPR2} > \text{ITPR1} > \text{ITPR3}$ [20, 21]. Indeed, in hepatocytes where ITPR2 is concentrated at the apical domain and ITPR1 is scattered throughout the cytosol, Ca^{2+} signals propagate as an apical to basolateral wave in response to multiple InsP3-generating agonists [22]. Regulation by cAMP and its downstream effector protein kinase A (PKA) is an additional mechanism that leads to different signaling outcomes for different ITPR isoforms. Although all three isoforms can be phosphorylated by PKA [23, 24], the open probability of both ITPR1 and ITPR2 are potentiated by PKA phosphorylation, whereas ITPR3 is unaffected by this post-translational modification [25, 26]. Additionally, the activity of all ITPR isoforms can be potentiated by high concentration of cAMP in a PKA-independent fashion [27]. At least for ITPR2, such high concentrations of cAMP may be achieved by a physical interaction between ITPR2 and adenylyl cyclase 6 (AC6), one of the 10 known isoforms of cAMP producing enzymes in mammalian cells [28]. ITPRs are targets of phosphorylation by additional kinases such as cyclic-GMP (cGMP)-dependent protein kinase (PKG), protein kinase C (PKC), and Ca^{2+} -calmodulin-activated kinase II (CamKII) [29, 30]. However the isoform-specific functional effects of these phosphorylation events are poorly understood. ITPRs are also regulated by O-linked β -N-acetylglucosamine glycosylation (O-GlcNAcylation), and the different isoforms are uniquely regulated by this post-translational modification. ITPR1 channel activity is inhibited whereas ITPR3 conductance is potentiated by O-GlcNAcylation. ITPR2 however does not appear to undergo O-GlcNAcylation [12, 31]. Moreover, the activity of ITPRs is modulated by binding to a number of protein partners and these interactions might have isoform-specific functional consequences. For example, ERp44, a luminal ER-resident protein, interacts with the intraluminal loop between transmembrane domains 5 and 6 (TM5 and TM6) of ITPR1 but not ITPR2 or ITPR3. Knock down of ERp44 increased Ca^{2+} signaling whereas its overexpression inhibited Ca^{2+} release in cells expressing predominantly ITPR1 [32].

Differences in signaling by the different ITPR isoforms cannot however be entirely explained by differences in their biophysical properties. Differences in the subcellular localization of each isoform also determines the cellular Ca^{2+} signal and the resulting cellular response. For example, ITPR1 and ITPR2 are both expressed in hepatocytes however each isoform controls a different Ca^{2+} -dependent function. ITPR2 concentrates near the apical membrane where it modulates bile solute secretion by hepatocytes [33, 34]. ITPR1 instead is distributed throughout the cytosol and controls primarily mitochondrial Ca^{2+} signaling and lipid metabolism [35]. Apical localization of ITPRs is a recurrent theme in secretory epithelia where they act as triggers of apical to basolateral Ca^{2+} waves and control fluid and electrolyte secretion [22, 36]. For example, ITPR2 and ITPR3 double knock out mice have severe salivary and pancreatic secretion defects, which compromise food digestion and animal growth [37]. Indeed, ITPR2 and ITPR3 expression is reduced in salivary glands of Sjögren's Syndrome, and autoimmune exocrinopathy associated with secretory defects [38]. Ca^{2+} in the nucleoplasm also can be independently controlled, by ITPRs localized to the nuclear envelope [39, 40]. These Ca^{2+} signals are essential for transcription of certain genes [41–43] and for cellular proliferation [44]. Similar to cytosolic Ca^{2+} signaling, nuclear Ca^{2+} signals are the result of the activation of multiple ITPR isoforms [40] and thus the role played by each individual isoform is still unclear.

Mitochondrial Ca^{2+} signals also depend on ITPRs and in part are isoform-dependent. Mitochondrial Ca^{2+} signals reflect direct transmission of Ca^{2+} from the ER via ITPRs and regulate both ATP production and cell survival [45]. ITPRs that are principally responsible for these signals are in specialized domains of the ER that localize to within 20–40 nm of neighboring mitochondria in regions known as mitochondria-associated membranes (MAMs). In these specialized domains, Ca^{2+} is directly shuttled from the lumen of the ER to the mitochondrial matrix via ITPRs opening near voltage-dependent anion channels (VDAC) in the outer mitochondrial membrane, which are Ca^{2+} permeable at membrane potentials away from zero, and then through the inner mitochondrial membrane via the mitochondrial Ca^{2+} uniporter (MCU). Here again, different isoforms might differentially regulate mitochondrial Ca^{2+} levels. In CHO cells for example, knock down of each ITPR isoform revealed that ITPR3 localizes with mitochondria more often than the other two isoforms and also contributes more to mitochondrial Ca^{2+} signaling and the induction of apoptosis [46]. In hepatocytes on the other hand ITPR1, rather than the more highly expressed ITPR2, couples to mitochondria where it modulates both mitochondrial Ca^{2+} and lipid droplet formation [35]. This finding is corroborated by independent studies, which identified the molecular chaperone glucose-regulated protein 75 (grp75) as one protein that physically links VDAC and ITPR1 at the MAM of hepatocytes [47]. In contrast, DT40 cells engineered to express single ITPR isoforms demonstrated that ITPR2 is the most efficient isoform in transmitting Ca^{2+} to the mitochondrial matrix [48]. Additionally, it was demonstrated in kidney cells that mitofusin 2 controls the relative amount of ITPR1 and ITPR3 present in association with mitochondria and the downstream mitochondrial Ca^{2+} signaling [49]. The picture that emerges from these studies is that all three ITPR isoforms are capable of coupling to mitochondria, but the relative contribution of each of them is determined in a cell-dependent manner. Given the range of proteins that can localize to the MAM [50], this may in part

reflect that these proteins may have differing abilities to couple to the different ITPR isoforms and there may be cell- and tissue-specific expression of these proteins.

Alterations in Ca^{2+} signaling pathways have been linked to a number of human diseases ranging from pancreatitis to Alzheimer's disease and cancer [51–53]. Specific mutations in ITPR isoforms in particular also have been linked to human disease, and this has recently been reviewed [54]. Most of the known mutations that are clinically relevant are associated with ITPR1. For example, deletions as well as gain-of-function mutations of ITPR1 occur in patients with Spinocerebellar Ataxia [55]. Also, a single point mutation (G7492A) in the pore forming region of ITPR2 that gives rise to a poreddead mutant occurs in individuals with generalized anhidrosis and heat intolerance [56]. In this case, the mutation prevents Ca^{2+} release and the resulting water and electrolyte secretion in sweat glands. A few mutations in ITPR3 have been linked to certain diseases through genome-wide association studies, but the relevant pathophysiology is less clear [54, 57, 58]. On the other hand, there is a growing literature demonstrating that a range of acquired, non-genetic diseases can result from alterations in ITPR expression. The responsible mechanisms have been characterized in detail for ITPR3 and are reviewed below.

III. Diseases associated with decreased amounts of ITPR3

The most extensive characterization of clinical conditions in which ITPR3 expression is lost has been in bile duct epithelial cells, or cholangiocytes. These are the polarized epithelia that line the intrahepatic bile ducts. Their primary function is to secrete HCO_3^- into bile [59], and defects in cholangiocyte secretory activity result in reduced bile secretion, or cholestasis [60]. Under normal conditions, activation of secretin receptors on the basolateral membrane of cholangiocytes promotes formation of cAMP, and then activation of protein kinase A (PKA) and phosphorylation of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride (Cl^-) channel at the apical membrane. This results in Cl^- secretion, and the Cl^- is exchanged for HCO_3^- via AE2, so the net result is biliary HCO_3^- secretion [60]. However, cAMP/CFTR-mediated HCO_3^- secretion also depends on ITPR3 that is concentrated beneath the apical membrane, plus apical P2Y receptors and luminal ATP [61, 62]. These findings have led to the idea that the primary role of CFTR in biliary HCO_3^- secretion is to mediate release of ATP into bile, where it stimulates autocrine P2Y receptors, leading to subapical Ca^{2+} release via ITPR3, and then opening of the apical, Ca^{2+} -dependent Cl^- channel TMEM16A [61, 62]. Histological evaluation of bile ducts in liver biopsy specimens from patients with a variety of disorders of bile secretion has shown that ITPR3 expression is reduced or absent in all of them [63]. Coupled with the observation that knockdown of ITPR3 directly impairs ductular HCO_3^- secretion in microdissected, microperfused bile duct segments [61], this suggests that loss of ITPR3 from cholangiocytes is part of a final common pathway in the pathogenesis of cholangiopathic disorders [63]. Three distinct mechanisms have been identified that lead to decreased ITPR3 expression in cholangiocytes. First, nuclear factor, erythroid 2-like 2 (NRF2) is an oxidant stress-sensitive transcription factor that decreases transcription of ITPR3 [64]. NRF2 binds to a musculo-aponeurotic fibrosarcoma recognition element (MARE) in the promoter of *ITPR3* that reduces promoter activity via chromatin remodeling [64]. NRF2 expression is increased and ITPR3 is decreased in bile ducts of patients with several cholangiopathic disorders,

including primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), biliary atresia, and biliary obstruction [64]. Peribiliary inflammation in these disorders has been thought to result in oxidant stress, and indeed inhibition of ITPR3 expression and cholangiocyte secretion is reproduced by directly inducing oxidant stress with H₂O₂ [64]. MicroRNA 506 (miR-506) also can decrease ITPR3 expression, by binding to the 3'-UTR of ITPR3 mRNA [65]. FISH analysis of human biopsy specimens shows that miR-506 is increased in bile ducts in patients with PBC, and work in bile duct cells and isolated bile ductules further has shown that miR-506-mediated loss of ITPR3 decreases Ca²⁺ signals and secretion [65]. Sepsis and alcoholic hepatitis are two other clinical conditions that can affect cholangiocytes and cause cholestasis [66, 67]. However, these conditions do not activate NRF2 [68]. Rather, they cause endotoxemia, which activates TLR4 to increase NFκB, which is an alternative mechanism to decrease *ITPR3* promoter activity and ITPR3 expression [68]. Indeed, examination of bile ducts in autopsy specimens from patients who died of overwhelming sepsis and liver biopsy specimens from patients with severe alcoholic hepatitis both show increased TLR4 labeling plus increased nuclear staining for the p65 subunit of NFκB, along with loss of ITPR3 (Figure 1) in the cholangiocytes [68]. Therefore these examples show that multiple pathophysiological mechanisms can lead to loss of ITPR3 from cholangiocytes and cholestasis. Although the NRF2 and NFκB pathways appear to be non-overlapping, the relative roles of NRF2 and miR-506 in PBC and other cholangiopathies remain to be determined.

In addition to decreased expression, increased degradation is another mechanism that contributes to decreased amounts of ITPR3 in certain disease states. Degradation of ITPR3 occurs via ubiquitination [69]. Upon physiological stimulation, all three ITPR isoforms can undergo poly-ubiquitination and rapid degradation by the 26S proteasome [70]. However, ITPR3 plays a particularly important role relative to the other two ITPR isoforms in mediating apoptosis [46, 71]. This likely is because ITPR3 preferentially localizes to mitochondria-associated membranes (MAMs) in some cell types [46, 49], so it can be particularly effective at transmitting the Ca²⁺ signals into mitochondria that can lead to apoptosis [46]. For this reason, conditions in which degradation of ITPR3 is increased can lead to decreased apoptosis. For example, ITPR3 degradation is accelerated in prostate cancer cells deficient in phosphatase and tensin homologue (PTEN), a common loss of function mutation in multiple solid tumors [72]. Because PTEN and F-Box and Leucine Rich Repeat Protein 2 (FBXL2) compete for binding to ITPR3, loss of PTEN enhances binding of FBXL2 to ITPR3. FBXL2 binding in turn promotes ITPR3 degradation, so that tumor cells display decreased mitochondrial Ca²⁺ signals and resistance to apoptosis. A related mechanism occurs in a congenital cancer syndrome characterized by a combination of uveal melanoma and mesothelioma [73]. In this case, a partial loss of the deubiquitylating enzyme BRCA1 associated protein-1 (BAP1) is implicated as the mechanism responsible for ITPR3 downregulation. Although most BAP1 functions have been attributed to its nuclear localization, part of the total pool of this protein localizes to the cytosol where it interacts with ITPR3. This interaction stabilizes ITPR3 expression by inhibiting its degradation. Thus, loss of BAP1 leads to decreased ITPR3 expression, decreased mitochondrial Ca²⁺ and reduced sensitivity of cancer cells to apoptosis. For these reasons, conditions that lead to

chronically increased degradation of ITPR3 are thought to predispose to development of certain types of cancers.

IV. Diseases associated with increased amounts of ITPR3

Increases in ITPR3 expression occur in a variety of malignancies, including glioblastoma, breast cancer, gastric cancer, and squamous cell cancer of the head and neck [57, 74–76]. In patients with colorectal cancer, ITPR3 becomes expressed in resected tumors although it is absent in normal colonic mucosa. The *de novo* expression of ITPR3 in the invasive edge of resected tumors furthermore is associated with a poorer 5-year overall survival, implicating expression of ITPR3 as a prognostic factor in colorectal cancer. Complementary studies in a colon cancer cell line in which ITPR3 was either overexpressed or knocked down suggested that increased levels of ITPR3 confer a survival advantage to these cells by rendering them less sensitive to apoptotic cell death, rather than by enhancing cell proliferation [77].

Cholangiocarcinoma (CCA) is another example of a type of malignancy associated with increased ITPR3 expression [78]. ITPR3 accounts for more than 90% of the total pool of ITPRs in non-transformed cholangiocytes where it is concentrated in the sub-apical region of the ER and modulates secretory activity [79]. However, ITPR3 expression is elevated in both CCA specimens and CCA cell lines when compared to histologically normal tissues and normal cholangiocyte cell lines, respectively. Furthermore, the ITPR3 in CCA redistributes so that it is no longer primarily in the apical region [78]. In contrast to what is observed in colorectal cancer however, ITPR3 in CCA appears not only to inhibit cell death but to promote cell proliferation and invasiveness as well. In addition, CRISPR deletion of ITPR3 from CCA cell lines impairs mitochondrial Ca^{2+} signaling and induces necrotic cell death. This phenomenon is similar to what has been described in prostate and breast cancer cell lines [80]. In those systems, survival of cancer cells, but not of non-transformed cells, depends on an ITPR-mediated constitutive basal transfer of Ca^{2+} into mitochondria to sustain the elevated energy metabolism of the tumor cells [80]. Once that Ca^{2+} transfer is impaired by pharmacological inhibition of ITPRs, cancer cells undergo necrotic cell death due to their inability to match the higher energy demands of rapid proliferation cycles. This phenomenon has been referred to as a “ Ca^{2+} addiction” [80]. Consistent with this interpretation, work in CCA cell lines has shown that mitochondrial Ca^{2+} is increased in cells expressing ITPR3, and that a significant fraction of the ITPR3 is in MAMs. On the other hand, Ca^{2+} signals in the nucleoplasm also are increased in CCA cells expressing ITPR3 [78]. Because progression through the cell cycle depends on nuclear Ca^{2+} signals [44], this may account for the observation that cell proliferation is increased in CCA cells as well.

Overexpression of ITPR3 also contributes to the pathogenesis of hepatocellular carcinoma (HCC) [81]. Under normal conditions, ITPR2 makes up ~80% of the total pool of ITPR in hepatocytes, whereas ~20% is ITPR1, and little to none is ITPR3 [22, 82]. ITPR3 begins to become expressed in hepatocytes in conditions of chronic liver inflammation, such as chronic infection with hepatitis B or C virus or alcoholic liver disease [81]. ITPR3 expression occurs in most hepatocytes and becomes more intense when these chronic liver diseases progress to HCC. This is observed in hepatocytes in chemically-induced HCC in mice as well [81]. Furthermore, five-year survival is lower in HCC patients whose tumors

have more elevated levels of ITPR3, consistent with the idea that the new expression of ITPR3 is of pathophysiological relevance. Moreover, ITPR3 partially co-localizes with TOMM20, a protein of the outer mitochondrial membrane (Figure 2). As in colon cancer and CCA, the presence of ITPR3 in a liver cancer cell line renders the cells more resistant to apoptotic cell death. In the case of HCC, though, this has been attributed to changes in apoptotic gene expression rather than alterations in mitochondrial Ca^{2+} signaling [81]. Therefore, loss of ITPR3 confers resistance to apoptosis in certain types of cancer, such as prostate cancer and mesothelioma, whereas gain of ITPR3 confers resistance to apoptosis in other cancers, such as colon cancer, CCA, and HCC. These seemingly contradictory roles for ITPR3 in cancer may be due to differences in adaptation to changes in ITPR3 expression. Loss of ITPR3 is likely to promote resistance to apoptosis in a direct fashion, because of decreased transmission of apoptotic Ca^{2+} signals into mitochondria [46]. In contrast, gain of ITPR3 appears to promote resistance to apoptosis indirectly. In particular, transient over-expression of ITPR3 promotes cell death, both in CCA cells and normal cholangiocytes [78]. However, chronic over-expression of ITPR3 is associated with an altered cell phenotype that includes decreased expression of pro-apoptotic genes and increased expression of anti-apoptotic genes [81], which perhaps reflects an adaptive mechanism necessary for these cells to survive. This over-expression of ITPR3 may also be responsible for the metabolic adaptation to chronically elevated mitochondrial Ca^{2+} seen in certain cancer cells [80], which likely explains why cancer cells that have undergone this adaptation cannot tolerate subsequent loss of ITPR3 [78, 80].

The increase in ITPR3 expression observed in chronic liver disease and in HCC results from progressive demethylation of CpG islands, regions of DNA ranging between 500–1500 base pairs with a high percentage (50–60%) of CG dinucleotides, in the ITPR3 promoter. In normal livers from both human and mice, ITPR3 expression is minimal and promoter methylation is high. Conversely, CpG islands in the ITPR3 promoter are mostly demethylated and ITPR3 expression is highest in HCC biopsy specimens. Hepatic expression of ITPR3 can also be induced in animals treated with a pharmacological demethylating agent, and Ca^{2+} signaling is enhanced in these hepatocytes as well [81]. These observations collectively demonstrate that ITPR3 becomes expressed *de novo* in cells that lack it or becomes overexpressed in cells that normally express it in a range of neoplasms, and that its increased expression contributes to development of cancer. Demethylation of the *ITPR3* promoter can cause overexpression of this Ca^{2+} channel, and mislocalization of the excess ITPR3 to the nucleus or to the MAM can cause excessive Ca^{2+} signals in these subcellular regions that result in the pathophysiological events responsible for neoplasia.

V. Open questions and future challenges

Collective evidence has established a clear link between altered expression of ITPR3 and the pathogenesis of various diseases. Examination of this has been particularly informative in the case of the biliary tree, because in that tissue there is an understanding not only of the physiological role of ITPR3, but also the contrasting roles that under- and over-expression of ITPR3 can play in the pathogenesis of disease (Figure 3). Although exciting, this gives rise to a number of important questions and new challenges. For example, what are the additional mechanisms controlling decreased or increased expression of ITPR3? To what

extent are these mechanisms widely applicable, and to what extent are they cell-type specific? An emerging question that may be even more important has to do with identifying the factors that regulate the subcellular localization of this Ca^{2+} channel. In diseases that are due to loss of ITPR3, can they also result from mis-localization of ITPR3? For example, sub-apical ITPR2 is important for secretion in hepatocytes [33, 34], similar to how sub-apical ITPR3 is needed for secretion in cholangiocytes. Factors such as endotoxin or estrogens cause ITPR2 to move away from the apical region of hepatocytes, which in turn slows Ca^{2+} waves and impairs secretion [34, 83], but it is not yet known what factors cause ITPR3 to localize to the apical region of cholangiocytes. It will be interesting to determine whether loss of these factors lead to impaired Ca^{2+} signaling and secretion and whether these factors play a role in development of cholestatic diseases. Similarly, it is not known why ITPR3 mislocalizes to the nucleus and/or to MAMs in conditions in which it is over-expressed. One possibility is that over-expression of ITPR3 is a primary event, and that excess ITPR3 promiscuously accumulates in these regions when apical binding sites become saturated. However, an alternative possibility would be that new or increased expression of nuclear and/or peri-mitochondrial targeting proteins is the primary event. Identification of the factors that regulate subcellular targeting of ITPR3 may help bring our understanding of the normal and abnormal regulation of subcellular Ca^{2+} signals to the next level, which may be needed to guide the development of potential new therapeutic strategies.

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Highlights

- ITPR3 modulates subcellular Ca^{2+} signaling to affect secretion, proliferation, mitochondrial metabolism and cell death.
- ITPR3 expression is controlled by multiple mechanisms including RNA silencing via microRNAs, transcriptional regulation, promoter methylation and protein degradation.
- ITPR3 expression or localization is altered in a number of human diseases, and contributes to their pathogenesis.
- Selective targeting of ITPR3 regulatory mechanisms may be of therapeutic use.

Figure 1: ITPR3 expression is decreased in cholangiocytes in alcoholic hepatitis.

Representative immunohistochemistry staining of human liver biopsy specimens shows that ITPR3 (dark brown) is most concentrated in the apical region of cholangiocytes in a histologically normal (control) specimen (left panel), whereas ITPR3 labeling is reduced and distributed throughout the cytosol of cholangiocytes in a specimen from a patient with Alcoholic Hepatitis (right panel). This loss of ITPR3 from the apical region is similar to what is observed in a variety of bile duct disorders in which bile secretion is impaired. Bile ducts are indicated by asterisks. Scale bar is 20 μ m.

Figure 2: ITPR3 is partially concentrated at ER-mitochondrial contact sites in a liver cancer cell line.

Super-resolution images of a HepG2 cell expressing a GFP-tagged ITPR3 (green) and labeled with the fluorescent tag KO2 to reveal the outer mitochondrial membrane protein TOMM20 (red) shows partial co-localization of ITPR3 with mitochondria. Bottom panels show magnified view of the region indicated in the top right panel. Scale bar is 5 μm .

Figure 3: ITPR3 expression in cholangiocytes in health and disease.

Under normal conditions, ITPR3 expression is most concentrated in the region of the ER beneath the apical cell membrane, where it regulates biliary bicarbonate secretion (left). Conditions in which ITPR3 expression is decreased result in impaired secretion (top right), whereas conditions in which ITPR3 expression is increased result in some of the excess protein promiscuously localizing to ER contact points with mitochondria, where it promotes the excess transfer of Ca^{2+} to mitochondria, leading to metabolic abnormalities that contribute to the pathogenesis of cholangiocarcinoma (bottom right).