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Mitochondrial calcium signaling in cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a primary liver cancer whose diagnosis and treatment remain challenging. Although recent developments derived from molecular characterization of CCAs have led to the availability of new pharmacological agents, a better understanding of the genetic and molecular alterations in CCA is still required for the development of more effective or broader targeting treatments. One emerging signaling pathway of interest in the pathogenesis of CCA is ER to mitochondrial Ca^{2+} signaling. This pathway is of particular importance because it regulates both cell death through apoptosis and necrosis, and metabolic reprograming of cancer cells through regulation of energy metabolism in mitochondria. Here we discuss the latest findings regarding the dysregulation of mitochondrial Ca^{2+} signals and its key regulatory molecules with a special focus on the intracellular Ca^{2+} channels of the inositol 1,4,5-trisphosphate receptor (ITPR) family. We also discuss the role of ER-mitochondrial contact sites in determining mitochondrial health and how these points of contact between organelles might represent a druggable target in CCA.

Keywords: Cholangiocarcinoma, cholangiocytes, mitochondria, inositol 1,4,5-trisphosphate-receptors, calcium signaling, membrane contact sites, mitochondria-associated endoplasmic reticulum membranes, apoptosis, cell proliferation, energy metabolism

INTRODUCTION

Cholangiocarcinoma (CCA) is the second most common liver cancer, accounting for ~10%-15% of all

primary liver cancers[1,2], and its incidence and mortality rates are on the rise in the United States[3]. CCAs have traditionally been classified by anatomical location[4], although there have been recent efforts to classify these tumors by genetic criteria[5,6]. Intrahepatic CCA (iCCA) is confined to the second-order bile ducts within the liver parenchyma, whereas perihilar CCA is found in the area between left and right hepatic ducts and proximal to the insertion of the cystic duct into the common bile duct. The third subtype, distal CCA (dCCA), localizes in the common bile duct. This anatomical classification of CCA is currently used to establish the clinical diagnosis and staging, which then leads to whether the tumor is amenable to surgery, locoregional therapy, or other treatment options such as liver transplantation[7]. Although advances in diagnostic imaging have improved the detection of CCA, pharmacological treatment options remain limited, in part because of our evolving understanding of the genetic and molecular alterations in CCA. Promising preliminary results have emerged from trials of immunotherapy in combination with the dual regimen of cisplatin plus gemcitabine. These therapies are centered on blocking monoclonal antibodies that prevent the interaction between programmed cell death ligand-1 on tumor cells with programmed cell death-1 on the cell surface of anti-tumor T cells. Durvalumab, for example, a PD-L1 blocking antibody, in combination with cisplatin and gemcitabine, has shown some improved survival in biliary tract cancer[8]. Earlier efforts in targeting treatments to genetically defined CCAs have led to the FDA approval of three new targeted drugs for the treatment of CCA. Pemigatinib and Infigratinib are approved for use in patients with fusion and rearrangements of the fibroblast growth factor receptor 2 (*FGFR2*) gene[9,10]. The third drug, Ivosidenib, is a small molecule inhibitor of a mutated form of isocitrate dehydrogenase-1 (IDH1)[6], a mitochondrial matrix enzyme that generates 2-hydroxyglutarate. Elevated concentrations of this metabolite are thought to modulate both cellular metabolism and epigenetic regulation that promote cancer progression. The potential benefits of these three therapeutic agents suggest the importance of altered Ca^{2+} signaling in the pathogenesis of CCA. For example, regarding FGFR2, growth factor/receptor tyrosine kinase signaling is a target in several types of malignancies, but recent evidence suggests that the proliferative effects of receptor tyrosine kinases (RTKs) are mediated by activation of Ca^{2+} signaling pathways in the cell nucleus[11-13]. Similarly, regarding IDH1 as a target, there is an evolving appreciation that mitochondrial Ca^{2+} signaling becomes altered in certain malignancies, including in the liver[14]. Finally, evidence suggests that there are common alterations in Ca^{2+} signaling that occur in CCA, regardless of the anatomical location of the tumor[15]. Therefore, this review will discuss normal and abnormal Ca^{2+} signaling mechanisms in cholangiocytes, with a particular focus on mitochondrial Ca^{2+} signaling machinery and mechanisms.

CA^{2+} SIGNALING MACHINERY IN CHOLANGIOCYTES

Ca^{2+} signaling in cholangiocytes is largely determined by a family of ER Ca^{2+} channels, the inositol 1,4,5-trisphosphate (IP3) receptors (ITPRs). This family of IP3-gated Ca^{2+} channels is comprised of three isoforms, ITPR1, ITPR2, and ITPR3. Although the three isoforms have over 80% homology in amino acid sequence and all are activated by binding to IP3, each isoform has a unique profile in terms of biophysical properties[16]. These include different affinities for IP3, modulation by Ca^{2+}, and regulation by post-translational modifications[17-19]. Another significant differentiator among the ITPR isoforms is their tissue distribution and subcellular localization. For example, in hepatocytes, ITPR2 constitutes 80% of the total ITPR pool and it is mostly concentrated on the portion of the ER membrane near the apical membrane[20]. ITPR1 accounts for the remaining 20% of the total, and initial immunofluorescence studies suggested this isoform was diffusely distributed throughout the cytosol[21], while more recent work has shown that it specifically localizes to ER-mitochondrial contact points[22]. In contrast to hepatocytes, cholangiocytes express all three ITPR isoforms, but ITPR3 accounts for 90% of the total, with ITPR1 and ITPR2 both contributing to the remaining 10%[23]. The subcellular distribution of ITPR isoforms is important because ITPR-containing microdomains regulate specific cell functions. The most well-established microdomains in

polarized epithelia are those beneath the apical membrane, which regulate secretion[24-26], and those at ER-mitochondrial junctions, which regulate mitochondrial metabolism and cell death[27,28]. However, ITPRs within the nucleus may constitute another type of microdomain, which regulates events that depend on nuclear Ca^{2+} signals, such as gene transcription[29-31], progression through the cell cycle[13], and cell proliferation[15,32,33]. A general feature of polarized epithelia is that ITPRs are most concentrated beneath the apical membrane, although the specific isoform(s) localized there and the nature of the molecular machinery that links to these ITPRs to result in stimulus-secretion coupling vary among cell types. In cholangiocytes, ITPR3 is the isoform that is most concentrated in the region of the ER beneath the apical plasma membrane[23]. In this region, ITPR3-mediated Ca^{2+} signals activate the Ca^{2+}-dependent chloride (Cl^-) channel TMEM16a, which allows Cl^- to move across the apical membrane. The extruded Cl^- is then exchanged with bicarbonate via anion exchanger, lower case, the product of the *SLC4A2* gene[34]. The expression and apical localization of ITPR3 are essential for normal secretory activity in cholangiocytes[25]. In fact, a variety of cholangiopathic disorders result in the loss of ITPR3, and this likely contributes to the pathogenesis of the cholestasis that occurs in these disorders. Such disorders include primary sclerosing cholangitis, primary biliary cholangitis, biliary atresia, biliary obstruction, cholestasis of sepsis, and alcoholic hepatitis[26,35,36]. However, the mechanism responsible for the loss of ITPR3 varies depending on the disorder, and can include inhibition of transcription by Nrf2, NF-kB, or c-Jun, or epigenetic regulation by miR-506[32,35,37,38]. Other conditions have been associated with gain rather than loss of ITPR3. Increased expression of ITPR3 tends to be associated with malignancies or other premalignant conditions[14,39]. Most relevant studies examined cancers other than cholangiocarcinoma and many of the studies focused on ITPR3 in the ER-mitochondrial junction, discussed below. However, ITPR3 may also play a role in Ca^{2+} signals in the cell nucleus in both hepatocytes[40] and cholangiocytes[15]. It has been established in hepatocytes that growth factors that contribute to hepatocellular carcinoma (HCC), including hepatocyte growth factor (HGF), epidermal growth factor (EGF), and insulin, selectively increase Ca^{2+} in the nucleus by inducing their cognate RTKs to translocate to the nucleus to locally generate IP3[11,12,33]. Furthermore, loss of ITPR3 results in impaired nuclear Ca^{2+} signaling in both primary cells and cell lines[15,41]. In hepatocytes, ITPR3 becomes expressed because of demethylation of its promoter[41], although transcriptional regulation by NFAT may contribute in the acute setting[42]. ITPR3 expression is increased in CCA as well[15], although the mechanism for this has not been established. ITPR3 function and its role in regulating fluid secretion in cholangiocytes have recently been reviewed[43,44], so the remainder of this review will discuss mitochondrial Ca^{2+} and how it may relate to CCA.

MITOCHONDRIAL CA^{2+} SIGNALING IN CHOLANGIOCYTES

ER-mitochondrial junctions, or mitochondria-associated membranes (MAMs), are specialized regions in which the ER comes to within 20-40 nm of mitochondria [Figure 1A and B]and their role in liver diseases has been recently reviewed[45]. ITPRs that are localized to these regions are responsible for conducting Ca^{2+} from the ER lumen into the mitochondria[46]. Each of the three ITPR isoforms may be found in the MAM, and emerging evidence suggests that mitochondrial Ca^{2+} and downstream effects may differ depending on which isoform is present[22,41,47]. ITPR3 expression is increased in both CCA liver specimens and CCA cell lines, and the overexpressed ITPR3 spills over from the apical region to accumulate in the MAM, where it enhances the transmission of Ca^{2+} from the ER to the mitochondrial matrix[15]. The overexpression of ITPR3 is associated with increased cellular proliferation as well as elevated cell spreading, both of which contribute to CCA progression[15]. A separate analysis of 59 patients with histological diagnosis of CCA, stratified according to anatomical localization, also showed that ITPR3 expression was higher in CCA than in normal bile duct cells. ITPR3 levels were particularly increased in dCCA[48].

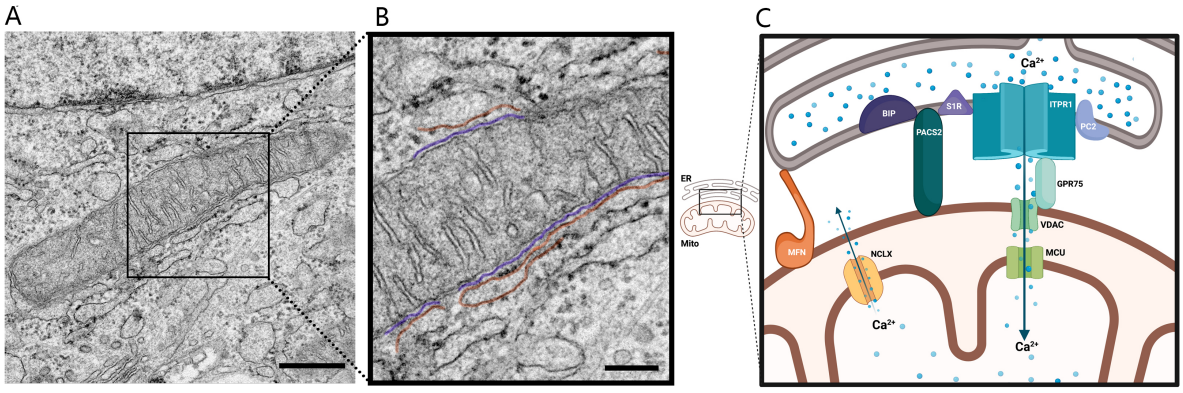

Figure 1. Mitochondria-associated membranes (MAM) as a Ca^{2+} signaling hub. (A and B) Transmission electron micrograph of a mouse hepatocyte shows the MAM as a segment of endoplasmic reticulum (ER, orange) in proximity (~20 nm) to the outer mitochondrial membrane (OMM, purple). Scale bar = 100 nm; (C) Schematic of tethering and regulatory proteins relevant for mitochondrial Ca^{2+} signaling that is present in the MAM. Glucose-regulated protein 75 (GRP75) links inositol 1,4,5-trisphosphate receptor type 1 (ITPR1) on the ER membrane and voltage-dependent anion channel 1 (VDAC1) on the OMM; polycystin 2 (PC2) is an integral ER membrane protein which can downregulate Ca^{2+} signaling to the mitochondria; BH3 interacting-domain death agonist (BID) is an pro-apoptotic factor that, once cleaved, promotes cytochrome C release from mitochondria; Phosphofurin acidic cluster sorting protein 2 (PACS2) forms a complex with Bid to promote apoptosis; Mitofusin (MFN) is part of the ER-mitochondria tethering system; voltage-dependent anion channel 1 VDAC1 allows Ca^{2+} passage to the mitochondrial intermembrane space; MCU, mitochondrial calcium uniporter is a core component of the complex that allows Ca^{2+} uptake into the mitochondrial matrix; Sigma 1 receptor (S1R) is an ER integral protein that binds ITPR1 when Ca^{2+} is released; Na^{+}/Ca^{2+} exchanger (NCLX) promotes Ca^{2+} extrusion from the mitochondrial matrix to the cytosol under physiological condition.

Mitochondrial matrix Ca^{2+} concentrations at rest are similar to those in the cytosol (~100-200 nm) and these organelles lack any mechanism for active uptake of Ca^{2+} from their surroundings. Instead, Ca^{2+} released from the ER through ITPRs is transmitted to adjacent mitochondria, where the voltage-dependent anion channel 1 (VDAC1) allows Ca^{2+} to pass through the outer mitochondrial membrane (OMM) and reach the intermembrane space. In fact, VDAC1 is physically linked to ITPR1 via Gucose-regulated protein 75 (GRP75), likely serving to maximize Ca^{2+} signal transmission[49]. Ca^{2+} is then transported to the matrix via the mitochondrial Ca^{2+} uniporter (MCU) complex. This macromolecular assembly localizes to the inner mitochondrial membrane (IMM) and it is composed of the pore-forming protein and its regulators MCUb, EMRE, MICU1, MICU2, and MICU3. Under physiological conditions, extrusion of Ca^{2+} from mitochondria predominantly occurs via the Na^{+}/Ca^{2+} exchanger (NCLX) protein[46]. Transient Ca^{2+} increases in the mitochondrial matrix, which occur physiologically, stimulate ATP production via positive regulation of three critical energy metabolism enzymes: pyruvate dehydrogenase[50], α-ketoglutarate dehydrogenase, and isocitrate dehydrogenase (IDH)[51]. In addition, glucagon-mediated ITPR1-dependent mitochondrial Ca^{2+} signaling is an essential regulator of lipolysis in the liver[47]. However, if Ca^{2+} remains elevated in the matrix for a prolonged period, the permeability transition pore (PTP) is formed in the IMM. This large protein complex forms a non-selective high-conductance pore that allows leakage of mitochondrial matrix components into the intermembrane space and the cytosol. Cytochrome *c* (Cyt *c*) is a key protein leaked from mitochondria to the cytosol via the PTP. Once in the cytosol, Cyt *c* triggers apoptosis by binding to apoptosis-protease activating factor 1, which is required for the maturation of caspase-9 and caspase-3. In fact, translocation of fluorescently tagged Cyt *c* from mitochondria to cytosol has been used to monitor PTP formation and development of apoptosis in live cells[52]. This technique was used in part to establish that the anti-apoptotic actions of MCL-1 are partly due to its buffering action on mitochondrial Ca^{2+}. In line with this mechanism, studies in CHO cells showed that ITPR3 is the most effective ITPR isoform for transmitting Ca^{2+} from the ER to mitochondria in a way that induces apoptotic cell death[53]. Moreover, buffering of mitochondrial Ca^{2+} in hepatocytes *in vivo* efficiently prevents apoptosis of parenchymal cells,

thereby accelerating liver regeneration after partial hepatectomy[54]. Therefore, increased transmission of Ca^{2+} signals into mitochondria, especially those that lead to sustained elevations in mitochondrial Ca^{2+}, are generally pro-apoptotic. More recent evidence, however, points to a more complex role of mitochondrial Ca^{2+} in cell survival. Work performed in breast and prostate cancer cell lines and melanoma *in vivo* showed that, contrary to normal cells, cancer cells rely on constitutive Ca^{2+} transfer from ER to mitochondria for survival. In normal cells, reduced mitochondrial Ca^{2+} triggers an autophagic response that is sufficient to guarantee cell survival, whereas in tumor cells, reduction in mitochondrial Ca^{2+} results in mitotic catastrophe and cell death by necrosis[55]. These findings suggest that prolonged increases in ER to mitochondria Ca^{2+} signaling can trigger an adaptation to promote cell survival responses rather than apoptosis. A similar mechanism might be at work in CCA, as the MzCha1 and HuCCA1 CCA cell lines each displayed reduced proliferation and motility together with increased death by necrosis if ITPR3 was knocked out[15]. Increased mitochondrial Ca^{2+} transients might also act synergistically with IDH mutations in a subset of CCA cases to promote cell survival. In a multiplatform study that included transcriptomic, DNA copy number and methylation profiles of 38 iCCA specimens, Farshidfar *et al.* identified a sub-cluster of CCA with gain-of-function IDH 1/2 mutations[5]. This subgroup was characterized by increased mitochondrial copy numbers and decreased global DNA methylation. These alterations positively correlated with mitochondrial biogenesis and oxidative phosphorylation gene expression programs. As mitochondrial Ca^{2+} can potentiate IDH function, ITPR3 in the MAM could further accelerate the growth of this specific subset of iCCA. Conversely, IDH mutations and their associated reduction of overall DNA methylation might positively regulate ITPR3 expression because ITPR3 promoter demethylation is one of the mechanisms by which ITPR3 expression can be upregulated in the liver[41]. Thus, targeting ITPR3-mediated mitochondrial Ca^{2+} signals might potentiate the effects of IDH inhibitors such as Ivosidenib, which is currently approved for this group of iCCA. ITPRs also interact with anti-apoptotic proteins belonging to the B-cell lymphoma 2 (BCL-2) family. Two of these proteins, BCL-2 and B-cell lymphoma-extra large (BCL-xL), have a dual role in preventing apoptosis. First, they form a scaffold in the mitochondria that binds to and therefore prevents the activation of two pro-apoptotic members of the same family, BCL-2-associated X protein (BAX) and BCL-2-associated agonist of cell death (BAD). When released from the BCL-2 scaffold, Bax and BAD form a pore at the OMM that promotes transient leakage of mitochondrial contents that act as apoptotic triggers in the cytosol[56]. Second, BCL-2 and BCL-xL bind to ITPR on the ER membrane to reduce its activation. Therefore, these two anti-apoptotic proteins prevent transmission of excessive Ca^{2+} loads into mitochondria. A similar function has been attributed to a third anti-apoptotic member of the BCL-2 family, myeloid cell leukemia-1 (MCL-1), in MzCha1 cells[52]. Notably, a recent molecular study of 72 CCA specimens showed a strong overexpression of both BCL-xL and MCL-1 in iCCA. Moreover, treatment with a combination of BCL-xL and MCL-1 inhibitors was efficient in triggering cell death in four different CCA cell lines *in vitro*[57].

THE ER-MITOCHONDRIAL INTERFACE

Dynamic contact points between organelles, also known as membrane contact sites (MCS), are ubiquitous tethering points between two opposing membranes without fusion, where inter-organellar communication takes place. One example is the contacts between lipid droplets and ER that feed building blocks for the growth of lipid droplets. Another example is the transient associations between the peripheral ER and plasma membrane that serves to refill ER Ca^{2+} stores. In the case of mitochondria and ER contact sites, referred to as MAMs, 5%-10% of the total mitochondrial surface is covered by ER tubules devoid of ribosomes. The distance of these juxtaposed organelles can range from 10 to 25 nm, whereas in the presence of ribosomes, this distance increases to 50 to 80 nm. The association between mitochondria and ER is also metabolically regulated both under physiological conditions (feeding and fasting cycles) and pathological states such as in steatohepatitis[58,59]. MAMs are critical for Ca^{2+} entry into mitochondria as they ensure the

proximity of ITPRs to mitochondria. Conversely, overexpression or mis-localization of ITPR isoforms in the MAM may disrupt this flux and result in excessive Ca^{2+} transport into the mitochondrial matrix that triggers apoptotic and other pathological types of signaling. The establishment of MCS depends on the interaction among integral tethering proteins on the surface of each organelle and the presence of auxiliary peripheral proteins. The composition of the MAMs has been among the most studied of the MCS in terms of their protein composition. In rodent and human hepatocytes, GRP75 establishes a direct link between ITPR1 on the ER membrane and VDAC1 on the OMM[49]. The extent of linkage between ITPR1 and VDAC1, as well as transmission of Ca^{2+} into mitochondria, is dynamically regulated by phosphorylation via pyruvate dehydrogenase kinase 4 (PDK4)[60]. The linkage of ITPR3 to mitochondria is likely more relevant than ITPR1 in cholangiocytes, but interactions between GRP75/PDK4 and ITPR3 are not yet known[61]. The ITPR1-GRP75-VDAC1 complex is also present in neurons and cardiac and skeletal myocytes. The integrity of this complex has been shown to regulate insulin sensitivity in both skeletal muscle and liver[62,63]. Moreover, the activity of this complex can be modulated by several accessory proteins. In CHO cells, Sigma 1 receptor (S1R), an ER integral protein, binds ITPR3 when Ca^{2+} is released from the ER. This binding prevents ITPR3 degradation and potentiates mitochondrial Ca^{2+} signals[64]. Although this mechanism has not been investigated directly in CCA, S1R is reportedly frequently activated in hilar CCA and is associated with poorer prognosis[65]. This observation is in line with the fact that ITPR3 expression is inversely related to survival in patients with HCC[41]. Another relevant protein is polycystin 2 (PC2), a large integral membrane protein expressed on the ER, where it functions as a Ca^{2+} release channel[66]. In addition to its channel activity, PC2 appears to limit mitochondrial Ca^{2+} signaling by reducing the association between ER and mitochondria in the MAMs[67]. PC2 is expressed in cholangiocytes and mutations in this gene give rise to Polycystic Liver Disease, which is characterized by large fluid-filled cysts along the intrahepatic bile ducts and reduced proliferation of cholangiocytes[68]. PC2 also physically interacts with and potentiates Ca^{2+} release by ITPR1[69]. Thus, PC2 might have opposite effects on mitochondrial Ca^{2+}. On the one hand, it potentiates ITPR-dependent Ca^{2+} release via physical interaction with this receptor; on the other hand, it might reduce mitochondrial Ca^{2+} by pulling apart ER and mitochondria. One caveat, though, is that PC2 may have different interactions with and effects on ITPR1 versus ITPR3. This is because the knockdown of PC2 decreases the expression of ITPR3 but not ITPR1 in renal cell lines[67]. Although a role for PC2 in CCA progression cannot be excluded based on the molecular evidence, the fact that individuals with PKD2 mutations, i.e., loss of function of PC2, do not have a clearly increased CCA incidence argues against PC2 being a major contributor in this malignancy. Another important protein present in the MAM is mitofusin 2 (MFN2). This small GTPase is part of the machinery of mitochondrial fusion but also functions as a bona fide ER-mitochondria tether. In mouse embryonic fibroblasts, genetic ablation of MFN2 increases the distance between ER and mitochondria and reduces IP3-dependent Ca^{2+} uptake into the mitochondria[70]. To date, no direct link has been established between MFN2 and CCA development. However, this warrants attention given the role of PC2 in regulating mitochondrial Ca^{2+} signaling and lung cancer data showing that increased MFN2 might prevent uncontrolled cell proliferation[71]. Phosphofurin acidic cluster sorting protein 2 (PACS-2) is an additional ER-mitochondria linker protein that might play a role in CCA pathogenesis. The expression of this multifunction protein is increased in hepatocytes in fatty liver disease, which brings ER closer to mitochondria, leading to an increase in mitochondrial Ca^{2+} signaling and dysregulation of mitochondrial function[72]. Moreover, PACS-2 may further control cell survival through direct interaction with the BH3 interacting-domain death agonist (Bid). In the breast cancer cell line MCF-7, PACS-2 interacts with the full-length and inactive form of Bid. Upon induction of apoptosis, the PACS-2/BID complex redistributes from ER to mitochondria, where BID is cleaved into its pro-apoptotic form, truncated BID, to induce Cyt *c* release and apoptotic cell death[73]. Lastly, the 78 KDa Glucose-regulated protein (GRP78), also known as BIP, is an additional protein regulating ITPR activity within the MAM. This ER chaperone might have a dual function in regulating ER-mitochondria contacts. First, in breast cancer cells, GRP78 forms a complex with Wiskott-Aldrich syndrome protein family

member 3 and the mitochondrial protein ATPase family AAA domain-containing 3A that has a tethering function in the MAM[74]. Second, GRP78 might indirectly regulate ITPR activity at the MAM via binding to S1R[75]. In summary, many proteins have been described that physically link the endoplasmic reticulum to mitochondria or modulate ITPR function in the MAM, thereby controlling Ca^{2+} flux between these organelles. The challenge remains to determine the exact role of these tethers in the pathogenesis of CCA. An overview of the main tethering and regulatory proteins present in the MAM of cholangiocytes is shown in Figure 1C. A number of additional MAM proteins have also been identified, including those involved in lipid transfer, which has recently been reviewed[76,77].

CONCLUSIONS AND FUTURE DIRECTIONS

Modulating the Ca^{2+} flux between ER and mitochondria represents a potential target for CCA therapy, but many challenges remain. First, we do not have a complete picture of the molecular mechanism driving the expression of ITPR3 in CCA. DNA demethylation is a possible candidate based on the data in hepatocellular carcinoma[41]. However, data generated in studies of other types of malignancies suggests that lack of ITPR3 degradation might also play a role here. All three ITPR isoforms undergo activity-dependent downregulation via ubiquitination and proteasomal degradation[78]. It is conceivable that ITPR3 in the MAM associates with other proteins in macromolecular complexes that shield it from this type of degradation. Similarly, whether ITPR3 forms hetero-tetramers with ITPR1 and ITPR2 in the MAM is not clear. This is relevant because of the unique Ca^{2+}-release properties of tetramers formed by different combinations of ITPR isoforms that would determine the extent of Ca^{2+} released into the mitochondria[79]. The factors targeting ITPR3 to the MAM also are unknown. Proteomic analysis of ITPR3-binding proteins in isolated MAMs and protein-protein interaction screens should clarify this topic. Finally, no information is available regarding ITPR3 expression in different subsets of CCA, based either on anatomical localization or gene expression/molecular profile[5]. Such information would have the potential to help us understand whether certain subtypes of CCA would be more likely to depend on mitochondrial Ca^{2+} for progression.

Additional pharmacological strategies that modulate Ca^{2+} metabolism might also be effective therapies for CCA. A retrospective study in patients who underwent liver resection as the primary treatment for CCA found that the overall survival was nearly doubled in a subgroup of patients taking Ca^{2+} channel blockers (CCBs) for hypertension[80]. Whether CCBs act directly on cholangiocytes or on immune or mesenchymal cells of the tumor stroma is not yet clear. The expression of the Ca^{2+}-sensing receptor, a G-protein coupled receptor critical for the regulation of systemic Ca^{2+} levels, is also increased in intrahepatic CCAs and cholangiocarcinoma cell lines compared to normal bile duct cells[81]. The activation of this receptor induces mobilization of intracellular Ca^{2+}, and its overexpression in CCA promotes cell proliferation via ERK activation. Therefore, multiple targets related to Ca^{2+} homeostasis other than the Ca^{2+} transfer between ER and mitochondria are implicated in the pathogenesis of CCA. Future studies should address some of the open questions regarding the role of ITPR3 in CCA development, such as: (1) Is ITPR3 a bona fide oncogene? (2) Can inhibition of ITPR3 be specifically achieved in CCA? (3) What proteins target and/or maintain ITPR3 on the MAMs? (4) Could ITPR3 be a specific and reliable biomarker for CCA diagnostics or therapy? (5) What is the functional impact of ITPR3 expression on overall mitochondrial function in CCA? (6) Are there specific somatic mutations that drive ITPR3 expression, or does overexpression of ITPR3 in CCA arise from defects in protein degradation pathways? These are some of the speculative questions that will lead to a better understanding of how ITPRs in the MAM work to sustain the oncologic growth of bile duct cancer.

DECLARATIONS

Author's Contributions

Reviewed literature, wrote the manuscript, and performed revisions: Loyola-Machado AC, Guerra MT
Supervised literature review, manuscript writing, and editing: Nathanson MH

Availability of data and materials

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Conflict of Interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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