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Recent Insights Into the Pathogenic Mechanism of Pancreatitis: Role of Acinar Cell Organelle Disorders

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Abstract

Acute pancreatitis (AP) is a potentially lethal inflammatory disease that lacks specific therapy. Damaged pancreatic acinar cells are believed the site of AP initiation. The primary function of these cells is the synthesis, storage, and export of digestive enzymes. Beginning in the endoplasmic reticulum and ending with secretion of proteins stored in zymogen granules, distinct pancreatic organelles use ATP produced by mitochondria to move and modify nascent proteins through sequential vesicular compartments. Compartment-specific accessory proteins concentrate cargo, promote vesicular budding, targeting and fusion. The autophagy-lysosomal-endosomal pathways maintain acinar cell homeostasis by removing damaged/dysfunctional organelles and recycling cell constituents for substrate and energy. Here, we discuss studies in experimental and genetic AP models, primarily from our groups, which show that acinar cell injury is mediated by distinct mechanisms of organelle dysfunction involved in protein synthesis and trafficking, secretion, energy generation, and autophagy. These early AP events (often first manifest by abnormal cytosolic Ca^{2+} signaling) in the acinar cell trigger the inflammatory and cell death

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responses of pancreatitis. Manifestations of acinar cell organelle disorders are also prominent in human pancreatitis. Our findings suggest that targeting specific mediators of organelle dysfunction could reduce disease severity.

Keywords

endoplasmic reticulum; mitochondria; endosome; lysosome; autophagy; Ca^{2+} signaling

1. INTRODUCTION

Acute pancreatitis (AP) is a potentially fatal disease with significant morbidity and mortality.[1,2] Its pathogenesis remains obscure, and there are no specific or effective treatments. Acute pancreatitis is the 3d most common reason for hospital admissions in those with gastrointestinal disease, the 6th most common nonmalignant cause of death from digestive diseases in the U.S., and is also a major risk factor for developing chronic pancreatitis and pancreatic cancer. The economic impact of AP is substantial with total inpatient, outpatient, and long-term care costs $2.8 billion annually.[1]

Key AP pathologies include the inappropriate/intra-pancreatic zymogen activation (eg, conversion of trypsinogen to trypsin), reduced and dysregulated digestive enzyme secretion, vacuole accumulation, inflammation, and apoptotic and necrotic acinar cell death. Chronic pancreatitis is manifest by exocrine pancreas atrophy, chronic inflammation (driven by macrophages and T cells), and fibrosis.[2–5] The pancreatic acinar cell is a major disease participant, and it is generally held that disordering of its functions leads to both acute and chronic pancreatitis. Although considerable progress has been achieved during the past 15 years in elucidating the inflammatory responses, modulation of inflammation has not successfully treated pancreatitis.[3,4,6] One reason for that could be the persistence of acinar cell damage sustaining the inflammatory and cell death responses.

The central physiologic function of the pancreatic acinar cell is to synthesize, transport, store and secrete digestive enzymes.[7,8] This is accomplished through coordinated and sequential actions of the endoplasmic reticulum (ER), Golgi apparatus, the endo-lysosomal system, storage and secretory organelles, and mitochondria. The work of George Palade and co-workers from 50 years ago recognized distinct cellular structures seen by electron microscopy in the pancreatic acinar cell and predicted that they have special functional roles.[9,10] Palade and his trainee Jim Jamieson worked to understand the physiologic role of these structures.[9] They found that nascent proteins are vectorially transported through acinar cell organelles to the site of storage in mature zymogen granules (ZG). Subsequent studies showed that specific steps in the biogenesis and maturation of export proteins occurred in distinct cellular compartments. Information relating to the roles of cytoplasmic organelle networks in physiologic and pathophysiologic processes in eukaryotic cells has greatly expanded during the last decade,[11,12] prompting further investigations of the functions of cytoplasmic organelles in acinar cell physiology and pathophysiology.

Here, we first review key acinar cell organelles and their physiologic functions, then summarize findings from our laboratories on the pathogenic mechanisms of organelle

dysfunction and their role in initiating and driving pancreatitis, emphasizing those that could be therapeutic targets.

2. CYTOPLASMIC ORGANELLES AND CELLULAR HOMEOSTASIS

2.1. Endoplasmic Reticulum

The ER is a major site of protein and lipid synthesis; another important ER function is maintaining Ca^{2+} homeostasis. The ER also mediates protein posttranslational modifications (e.g., glycosylation and disulfide bond formation), trafficking of newly synthesized proteins to the Golgi complex, and lipid synthesis (Fig. 1). To ensure the fidelity of protein synthesis, an ER quality control system regulates and monitors the folding and tagging of nascent proteins.[13–17] Misfolded proteins can be toxic and can also sequester substrates that are needed for new protein synthesis. When unfolded and/or misfolded proteins accumulate at their site of synthesis, cells first respond by activating an “unfolded protein response” (UPR) which temporary reduces general translation and simultaneously upregulates the levels of ER/Golgi factors (chaperones and foldases) that mediate protein folding, trafficking and degradation. If an adaptive UPR is unsuccessful to restore ER function, ER-stress signaling programs are activated leading to inflammation and cell death.[14,16]

2.2. Ca^{2+} Homeostasis

Ca^{2+} homeostasis central to both physiologic and pathologic acinar cell function because many enzymes require calcium ion as a cofactor, and because of the key role of Ca^{2+} in signal transduction. Ca^{2+} homeostasis is maintained by various organelles and at the same time impacts their function.[18,19] The intracellular Ca^{2+} concentration in a typical eukaryotic cell is about 100 nM, which is ~10,000-fold lower than in extracellular fluid. This gradient is maintained by membrane calcium pumps and channels as well as intracellular storage compartments that are primarily the ER and mitochondria. In response to physiologic hormonal or neurotransmitter stimulation, stored Ca^{2+} is released from ER into the cytosol and used by the cell to activate various Ca^{2+}-dependent functions, such as exocytosis (see below). The ER can directly transport Ca^{2+} to mitochondria through specific modifications in both organelles’ membranes (mitochondria-associated ER membranes or MAMs) that regulate the synthesis and translocation of lipids, mitochondrial dynamics, autophagy, apoptosis, and energy metabolism. Ca^{2+} interchange through MAMs is critical to fuel mitochondrial metabolism, and also modulates ER Ca^{2+} levels.[15,20] Mitochondria can also directly uptake Ca^{2+} from cytosol; this is facilitated by the highly negative mitochondrial membrane potential and finely tuned by proteins in the mitochondrial Ca^{2+} uniporter complex.[21]

2.3. Mitochondria

Mitochondria are responsible for a range of cellular functions[22,23] (Figure 1). The major one is generation of ATP through consecutive redox reactions carried out by a series of linked protein complexes within the inner mitochondrial membrane, called the electron transport chain (ETC). Electron flow through ETC generates a pH gradient and an electrical potential used by ATP synthase to transform adenosine diphosphate into adenosine triphosphate in a phosphorylation reaction. Mitochondria also mediate cell survival; a universal trigger of cell

death is mitochondrial membrane permeabilization.[24] A major mechanism of mitochondrial permeabilization is through persistent opening of the mitochondrial permeability transition pore (MPTP), a multiprotein nonselective channel traversing both the inner and outer mitochondrial membranes.[25,26] In its 'open' conformation, MPTP allows unregulated entry of solutes <1500 Da (including water) into the matrix, resulting in mitochondrial depolarization, inhibition of ATP synthesis, swelling and, ultimately, necrosis. Various stresses, such as mitochondrial Ca^{2+} overload, over-production of reactive oxygen species (ROS) and NAD/NADH shift, can cause MPTP opening. The MPTP backbone is organized around the mitochondrial resident protein cyclophilin D (CypD); genetic, molecular or pharmacologic inactivation of CypD blocks MPTP opening.[25,26]

Individual mitochondria form a tubular-circular dynamic network, which tailors ATP production to cellular demand.[27] When energy demand is low, most mitochondria are present in a circular form characterized by short ETC length and low ATP synthesis. In response to increased energy demand, mitochondria fuse, forming a tubular configuration to increase ATP synthesis. Imbalance in mitochondrial fission-fusion is associated with various diseases and can result in inadequate ATP production, increased mitochondrial ROS, and impaired Ca^{2+} transport.[28]

2.4. Vesicular Transporters and the Endosomal System

Newly synthesized proteins are moved to specific cellular organelles by vesicular transporters; related transport vesicles also mediate import of the material from outside the cell (through endocytosis). Organelles of endosomal system control trafficking of these vesicles to distinct cellular destinations such as lysosomes, the plasma membrane, or recycling back to Golgi, as well as cargo sorting and concentration (Fig. 1). Endosomal compartments are composed of highly dynamic membrane-enclosed tubulo-vesicular structures and include early/sorting, recycling, and late endosomes.[29–31] Endosomes of each subtype differ in their functions which are largely determined by the presence on their surface of distinct Rab proteins, members of the Rab family of small GTPases.[31] For example, Rab4 and Rab5 are associated with early endosomes, which mediate endocytosis and recycling; whereas Rab7 and Rab9 are markers of late endosomes, responsible for delivery of both endocytic and autophagic cargo to lysosomes for degradation. Rab proteins function as molecular switches that alternate between the active GTP-bound form and the inactive GDP-bound form.[29–32]

2.5. Lysosomes

Lysosomes are a final destination for select endosomal trafficking and are primarily responsible for protein, lipid, and carbohydrate degradation[33] (Fig. 1). Normal lysosomal activity requires several key elements. First, a unique mechanism for the sorting and delivery of a range of lysosomal enzymes to this compartment[34,35] involves a covalent mannose 6-phosphate (M6P) addition to asparagine residues of many lysosomal hydrolases in the cis-Golgi network. The modified hydrolases then bind to one of two transmembrane M6P receptors (M6P-Rs) on vesicles that are subsequently directed toward endosomes to deliver their cargo to lysosomes.[36–38] A second characteristic feature of lysosomes is their acidic pH, which matches the pH optimum of most lysosomal enzymes and is modulated by a

vacuolar H^+-ATPase that mediates proton influx.[39] Lysosomal hydrolases, such as cathepsins, are often synthesized as pro-proteins and undergo proteolytic processing first in endosomes, and then in lysosomes where acidic pH is required for their maturation to reach full catalytic activity.[40] A third critical feature of lysosomes is the presence of essential membrane proteins, including lysosome associated membrane proteins LAMP1 and LAMP2.[41,42] LAMPs are transmembrane glycoproteins whose luminal domain stabilizes content proteins. LAMP2, in particular, is important for fusion of the lysosome with autophagosomes and lysosomal proteolytic activity.

Studies from the last decade have dramatically changed our view of lysosomes from that of a simple “garbage disposal” to a dynamic organelle that regulates basic cellular processes such as nutrient sensing, vesicular protein trafficking, endocytosis and autophagy[33,43,44] (Figure 1). Recently, members of the MiT/TEE family of transcription factors (a major one is TFEB) have been shown to play a pivotal role in organelle biogenesis and metabolic processes by globally regulating lysosomal and autophagic functions.[33,44]

2.6. Autophagy

Autophagy is a collective term for several pathways through which cytoplasmic materials, in particular organelles and long-lived proteins, are delivered to the lysosome to be degraded by lysosomal hydrolases.[3,4,45,46] The degradation serves two purposes: 1) removal of damaged or dysfunctional cellular components, such as uncoupled mitochondria or ubiquitinated protein aggregates, and 2) the delivery of recycled substrates, such as amino acids and lipids, for critical cellular processes. The major, and best-studied, pathway, macroautophagy (hereon referred to as autophagy), requires de novo formation of double-membraned structures termed autophagosomes, which sequester cargo and ultimately fuse with lysosomes to form autolysosomes, where degradation occurs (Fig. 2). The process begins with the formation of so-called isolation membrane, or phagophore, followed by its elongation and closure to form the mature autophagosome. These steps are mediated by sequentially recruited complexes of evolutionary conserved ATG (autophagy-related) proteins.[46] Autophagy initiation is controlled by ULK1/ATG1-mediated complex, followed by the formation of another multiprotein complex involving phosphatidylinositol 3-kinase catalytic subunit type 3 (Vps34) and Beclin1/ATG6, which nucleates the phagophore. Phagophore expansion and elongation are controlled by the ubiquitin-like conjugation systems involving the ATG5-ATG12-ATG16 complex, and the microtubule-associated protein 1 light chain 3a (LC3). LC3, the mammalian paralog of yeast ATG8, is necessary for phagophore closure; during this process, ATG7 and ATG3 mediate lipidation of its cytosolic form (LC3-I) to become LC3-II, which specifically translocates to the autophagosome membrane. Autophagosomes then fuse with lysosomes (or with late endosomes prior to lysosomal fusion) to form single-membraned autolysosomes where cargo breakdown occurs.

Autophagy can be both non-selective or selective in its targeting specific cellular constituents for degradation.[47] Thus, in conditions of high nutrient and energy demand, such as starvation, macroautophagy is often non-selective. Other conditions, including organelle injury, can induce highly select organelle degradation. Examples include selective degradation of mitochondria (“mitophagy”) or lipid droplets (“lipophagy”).[47,48] Dysfunction

of selective autophagy can lead to reduced protein turnover, accumulation of damaged/dysfunctional organelles, and shortages of substrates for energy metabolism or protein and lipid synthesis.

3. ROLE OF ACINAR CELL ORGANELLES IN PHYSIOLOGIC SECRETION OF DIGESTIVE ENZYMES

The acinar cell secretory apparatus relies on cytoplasmic organelles to maintain a complex matrix of membrane trafficking pathways and coordinate polarized secretion according to cell demands. Physiologic secretion pathway starts in the ER where protein synthesis and folding occur; nascent proteins then travel to Golgi complex, where they are packed in secretory vesicles and through post-Golgi trafficking delivered to the plasma membrane for exocytosis[7,8] (Fig. 3). Cytosolic Ca^{2+} is a major mediator of ZG exocytosis.[18,49] Secretagogues (hormones and neurotransmitters) cause a release of calcium from ER stores though IP3 and RyR-sensitive channels. This release causes a transient increase in free cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) which is immediately offset by removal of Ca^{2+} from the cytosol by Ca^{2+}-ATPases that pump Ca^{2+} from the cytosol to the ER lumen and into the extracellular space. Therefore, increases in cytosolic Ca^{2+} during physiologic cellular functions are transient and oscillatory. This prevents sustained increase in $[Ca^{2+}]i$, which can cause aberrant activation of various calcium-dependent proteins resulting in cell damage. Further depletion of luminal ER calcium may disrupt secretion by decreasing the activities of UPR mediators, such as calreticulin.[50] Thus, maintaining low cytosolic Ca^{2+} and constantly replenished ER Ca^{2+} pools is necessary for normal acinar cell secretion.

Our recent data (unpublished) show that the UPR factor X-box binding protein 1 (XBP1) is a key regulator required for maintaining the secretory phenotype of the acinar cell. As discussed below, XBP1 and the UPR transcription factor CHOP (CCAAT/enhancer binding protein homologous protein) both play a role in the acinar cell pathobiology during pancreatitis.

Protein synthesis, trafficking and secretion require energy; Jamieson and Palade showed that mitochondrial inhibitors block post-ER trafficking and secretion of digestive enzymes,[51] indicating that export of digestive enzymes to the plasma membrane for exocytosis requires normally functioning mitochondria. $[Ca^{2+}]i$ increase by secretagogues stimulates mitochondrial ETC activity, resulting in an increase in ATP production necessary for protein trafficking,[52] and at the same time causes transient mitochondrial depolarization[53–55] limiting ATP generation. The balance between these two processes maintains ATP homeostasis during physiologic secretory responses.

Recent studies revealed a critical role of the autophagy/lysosomal pathway in maintaining acinar cell homeostasis, particularly its secretory function.[34,45,56,57] Blockade of autophagosome formation by Atg5 genetic ablation inhibits hormone-induced secretion in acinar cells.[58,59] CCK-induced amylase secretion is also inhibited in acinar cells from mice with genetic ablation of IKKα (the inhibitor of the nuclear factor κB (IκB) kinase α), which causes impaired completion of autophagy (unrelated to nuclear factor κB activity).[60] Similarly, inhibiting autophagic flux by disrupting lysosomal function in LAMP2 null mice

decreases pancreatic digestive enzymes content and inhibits CCK-induced secretion.[61] Thus, acinar cell secretion is impaired if quality control system mediated by autophagy is not working.

Most digestive enzymes are trafficked to their cellular storage sites and concentrated many-fold throughout the secretory pathway.[62] Such concentration begins at the ER exit sites in vesicles anterogradely transported to the Golgi complex, and it continues in post-Golgi compartments. Final maturation, resulting in concentration of the digestive enzyme content, is achieved by budding of small vesicles from immature secretory granules which removes both membrane and content to form ZG.[63] The M6P-Rs also extract some, but not all, lysosomal enzymes from immature secretory granules.[63] In addition to the major, ZG-mediated secretory pathway, a minority (~5%) of digestive enzymes are directly released in constitutive and constitutive-like secretory pathways (also termed the minor secretory pathway, MSP).[64–66] Some fraction of small vesicles that bud from immature secretory granules participates in secretion of nascent export proteins through MSP (Figure 3). A unique feature of this pathway[66,67] is that secretion through MSP can be enhanced (about 2-fold) by very low concentrations of secretagogues (10-fold less than those that trigger secretion from the ZG storage compartment). There is evidence that physiologic secretion through MSP is controlled by endosomes; in particular, we find that the soluble protein D52 has a major role in the regulation of both exocytic and endocytic pathways.[7,68,69] In this context, D52 interacts with Rab5, a key regulator of early endosomal compartment.[66,70,71]. We also find[68,72] that the MSP transports a portion of LAMP1 to the apical membrane before LAMP1 is directed to its final residence in the lysosome, demonstrating another unexpected link between organelles in the secretory and lysosomal pathways.

Zymogen granule exocytosis at the apical membrane involves assembly of SNARE protein complexes required for vesicle docking and subsequent membrane fusion. VAMP2 and VAMP8 (vesicle-associated membrane proteins) are SNAREs found on zymogen granules that appear to mediate the release of distinct ZG populations.[7,8,66,73] Our studies using VAMP2 and VAMP8 genetic ablation showed that VAMP2, but not VAMP8, mediates initial acinar cell secretion during the first minutes after stimulation; whereas VAMP8 mediates the subsequent phase of secretion.[66] These data indicate the existence of distinct ZG pools. VAMP8 localizes to both exocytic and endocytic compartments (as does D52), suggesting that some proteins may regulate both exocytosis and endocytosis. Supporting this, loss of the endosomal proteins Rab5 and EEA1 during 16 h acinar culture is accompanied by a loss of VAMP8-mediated (but not VAMP2-mediated) ZG secretion and is fully prevented by maintaining Rab5 and EEA1 expression via adenoviral vectors.[66,72]

Collectively, the studies discussed above reveal critical, and interconnected, roles of ER, mitochondria, endo-lysosomes and autophagy in maintaining physiologic secretion in acinar cells.

4. DISORDERING OF ACINAR CELL ORGANELLAR NETWORK IN PANCREATITIS

4.1. Experimental Models of AP

Because of the lack of access to human tissue until very recently, most studies addressing pancreatitis disease mechanisms make use of animal models or freshly isolated acinar cells (or pancreas fragments) to study AP pathogenesis.[74–76] These models reproduce the spectrum of human disease severity and have greatly advanced our understanding of the cell biology of pancreatitis and the molecular factors involved; they also allowed testing of potential therapeutic approaches. The most widely used in vivo AP models of pancreatitis include those induced in rodents by administering supramaximal doses of cerulein [CER; an ortholog of cholecystokinin-8 (CCK)], bile salts or L-arginine (Arg), and by feeding young female mice choline deficient, ethionine supplemented (CDE) diet. Incubation of isolated acinar cells with supramaximal CCK/CER or bile salts triggers early pathologic responses of AP (such as trypsinogen activation, dysregulated secretion, vacuole accumulation) and recapitulates the earliest stages of disease. These ex-vivo models have been helpful in elucidating acinar cell organelle dysfunction in AP.[76]

4.2. ER Stress

Persistent/pathologic ER stress occurs in experimental and genetic models of pancreatitis, [77–84] indicating that pancreatitis impairs homeostatic protein folding mechanisms. Further, evidence indicates that hereditary pancreatitis is caused by mutations in human cationic trypsinogen (*PRSS1*)[85]; and that mutations in PRSS1, carboxypeptidase A1, the endogenous trypsin inhibitor *SPINK1* (serine protease inhibitor Kazal-type 1), and the trypsinogen-degrading enzyme chymotrypsinogen C that are associated with protein misfolding, resulting in ER stress, predispose to pancreatitis development.[86,87] For example, the *PRSS1* mutation p.L104P is found to markedly reduce secretion of the mutated protein because of its retention and aggregation associated with ER stress.[77]

Endoplasmic reticulum stress in pancreatitis manifests by increased phosphorylation of PERK (PKR-like ER kinase), splicing of XBP1, and expression of CHOP.[77–81] We showed that increase in spliced (s)XBP1 may protect pancreas against injury, whereas activation of CHOP is associated with acinar cell injury and pancreatitis responses.[81,88] For example, chronic ethanol exposure in mice selectively enhances sXBP1 levels;[81] this response appears to protect the pancreas against injury.[81,89] We reported redox and other changes in the pancreatic acinar cell ER proteome induced by ethanol feeding in sXBP1$^{+/-}$ mice.[90] We also reported[88] that combination of ethanol and smoking results in inhibition of the sXBP1 response, associated with an increase in CHOP and pancreatitis responses, in particular acinar cell death. These findings help explain epidemiologic studies indicating that smoking promotes alcoholic pancreatitis.[91,92]

4.3. Dysregulation of Ca^{2+} Transport

The common effect seen in the first minutes of acinar cell injury in ex-vivo AP models induced by supramaximal CCK/CER or bile salts is the loss of normal cytosolic Ca^{2+}

oscillations, which are replaced by a “peak-plateau” response resulting in sustained increase in [Ca^{2+}]i.[18,93,94] This occurs because pancreatitis-causing stimuli largely deplete ER calcium stores and the depletion promotes massive Ca^{2+} entry into the acinar cell through so-called store operated Ca^{2+} channels, which sense the reduced levels of calcium in the ER lumen. This sensing is mediated by stromal interaction molecule (STIM) proteins at the ER/plasma membrane interface, which activate the plasma membrane calcium transporter ORAI1.[95] Another type of channel mediating excessive Ca^{2+} influx and acinar cell damage is the transient receptor potential (canonical) channel TRPC3.[96]

Sustained increases in [Ca^{2+}]i promote Ca^{2+} uptake by mitochondria resulting in mitochondrial Ca^{2+} overload, which in turn causes mitochondrial depolarization leading to decreased ATP synthesis and ultimately necrosis.[54,55,97,98] Further, increase in cytosolic Ca^{2+} results in activation of the phosphatase calcineurin, which promotes acinar cell injury through several pathways.[99,100]

4.4. Mitochondrial Dysfunction

Mitochondrial dysfunction is prominent in both in vivo and ex-vivo experimental and genetic models of pancreatitis.[54,55,98,101,102] Its main manifestation is the sustained opening of MPTP resulting in loss of mitochondrial membrane potential, which in turn causes mitochondrial fragmentation.[101] Our results show that the mechanism of MTPT opening in experimental pancreatitis is model-specific. In AP models induced by supramaximal CCK/CER or bile salts, increases in [Ca^{2+}]i lead to mitochondrial Ca^{2+} overload and MPTP opening.[54,98,101] However, MPTP opening in Arg-AP is through inhibition of ATP synthase in the absence of Ca^{2+} overload.[101] In alcohol-mediated pancreatitis, MPTP opening is caused by a decrease in NAD^+/NADH ratio resulting from oxidative ethanol metabolism.[55] Notably, the MPTP opening in all models of pancreatitis is CypD-dependent, and genetic or pharmacologic inactivation of CypD prevents mitochondrial depolarization and largely restores mitochondrial dynamics in pancreatitis.[98,101]

MPTP-mediated pancreas injury has been investigated in detail,[54,55,98,101] but whether pancreatitis also causes mitochondrial damage through MPTP-independent pathways is unknown. The effects of pancreatitis on mitochondrial biogenesis, activities of ETC complexes, ROS generation and mitochondrial anti-oxidant systems remain largely unexplored.

4.5. Dysregulation of Endosomal System

Our studies indicate that endosomal system is dysregulated in AP. We showed, in particular, that secretion through the MSP, which is mediated by endosomes (as discussed above), is rapidly inhibited in AP models induced by supramaximal CCK, bile salts or cigarette smoke toxin, leading to intracellular trypsin accumulation and acinar damage.[72] When inhibition of MSP secretion was prevented by molecular or pharmacological approaches, basal secretion was normalized and intracellular trypsin accumulation abolished. Further evidence for disordered endosomal function in AP is provided by our recent data[103] that experimental pancreatitis causes marked reduction in the levels of Rabs controlling the early/recycling endosomes (such as Rab5 and Rab11) and alters their cellular localization. In addition, tissue

fractionation data have shown significant increases in the density of early endosomes in CER-AP. Functionally, we find dramatic defects in receptor-mediated endocytosis (measured by transferrin endocytosis) in CCK-hyperstimulated acinar cells.[103] The data suggest dysregulation of early/recycling endosomes in experimental AP, in particular, defective transition from early to late endosomes.

4.6. Impairment of Lysosomal and Autophagy Pathways

Experimental pancreatitis is associated with severe defects in the lysosomal pathway.[3,4,45,56,61,101,104,105] These include defective processing/maturation of cathepsins, major lysosomal proteases, manifested by reduced levels of fully processed and accumulation of intermediate forms of cathepsins.[45,101,105] Concomitantly, cathepsins' enzymatic activities decrease in lysosome-enriched pancreatic subcellular fractions from rodents with pancreatitis,[45,105] as noted long ago for cathepsin B.[106] Experimental pancreatitis alters lysosomal vacuolar H^+-ATPase localization (which maintains acidic pH in the lysosomal lumen): its soluble component is driven to assemble on acinar cell membranes.[107] Finally, the levels of LAMPs, the integral membrane proteins critical for maintaining both the structure and function of lysosomes, dramatically decrease across various experimental models of nonalcoholic and alcoholic pancreatitis.[61,108] Although defects in lysosomal function in pancreatitis were observed 30 years ago,[106] the underlying mechanisms remain poorly understood. We posit that a critical defect is the incomplete processing/maturation of cathepsins. In normally functioning lysosomes, hydrolases are thought to form large luminal complexes, ensuring their spatial separation from lysosome membrane proteins.[109,110] In pancreatitis, accumulation of the intermediate forms of cathepsins may compromise this separation resulting in LAMP proteolysis by cathepsins. Indeed, we showed that cathepsin B can cleave the intra-lysosomal part of LAMP molecule and that LAMPs' degradation in CER-AP is prevented by genetic ablation of cathepsin B.[61] Dysregulation of endosomal system could also play a role in lysosomal dysfunction in pancreatitis, as suggested by a study of the effects of Rab7 genetic ablation in pancreas.[111]

Accumulation in acinar cells of cytoplasmic vacuoles, often filled with cellular debris, has long been recognized as an early marker of AP, both in various experimental models and in human disease.[45,105,112–117] Such vacuoles are also observed in pancreas of mice with genetic ablation of key mediators of autophagy or lysosomal pathways that spontaneously develop pancreatitis (see below). Our studies[45,61,101,105] show that these are autophagic vacuoles and their accumulation in acinar cells is caused by impaired autophagy. We showed that pancreatitis increases autophagosome formation but at the same time inhibits autophagic degradation, resulting in impaired autophagic flux.[3,45,101,105] The most direct evidence for this is the finding that acinar cell vacuolization in experimental AP is associated with decreased rates of long-lived protein degradation.[105] Further, electron microscopy data show that the autophagic vacuoles accumulating in experimental pancreatitis are predominantly large autolysosomes, indicating that the fusion of autophagosomes with lysosomes is not blocked (although it could be impaired).[61,101,105] The consequences of inefficient autophagic degradation are likely exacerbated by the concomitant increase in autophagosome formation in pancreatitis.[45,101]

Recent studies using genetic models that specifically target autophagy/lysosomal pathways provide further mechanistic insights into the role of these pathways in the initiation of pancreatitis. Mice with pancreas-specific knockouts of key autophagy mediators Atg5 or Atg7[118,119] develop spontaneous pancreatitis, with trypsinogen activation, inflammation, fibrosis, and acinar-to-ductal metaplasia. Disrupting lysosomal functions by LAMP2 genetic ablation in mice causes impaired pancreatic autophagy, similar to that observed in experimental AP, and the development of spontaneous pancreatitis.[61] We also found that disruption of the *Gnptab* gene, which encodes key enzyme mediating the addition of M6P moieties onto acid hydrolases (a critical step for their delivery to the lysosome), caused complete block of the autophagic flux in pancreas. These knockout mice developed severe pancreatitis.[120] In addition, Atg5 or LAMP2 deficiency worsened CER-AP and Arg-AP, compared to wild type.[58,59,61] Interestingly, CER-AP and Arg-AP responses, such as hyperamylasemia, were worsened in transgenic GFP-LC3 mice in which the increased autophagosome formation (due to overexpression of LC3) is not balanced by increased lysosomal degradation, resulting in retarded autophagic flux.[121,122]

Collectively, the findings in experimental and genetic models[3,4,45,56–61,76,101,105,118–120,122] show the essential role of autophagy/lysosomal pathways in maintaining pancreatic acinar cell homeostasis; and strongly implicate the disordered pathways in initiation and development of pancreatitis. Of note, pancreatitis develops regardless of whether these pathways are disrupted at the level of autophagosome formation, as in Atg5 and Atg7 knockout mice, or at the completion of autophagy, as in LAMP2, IKKα or *Gnptab* knockout mice.

4.7. Interrelations Between the Dysfunctions of Individual Organelles in Exocrine Pancreas

It is increasingly clear that in eukaryotic cells cytoplasmic organelles are integrated into a highly dynamic, cooperative network which exchanges signals and material to maintain and balance cellular homeostasis, metabolism and survival;[15,123] and that each organelle functions in a milieu of coordinated exchanges with other organelles through both vesicular trafficking pathways and membrane contact sites.[124] Little is known about inter-organellar interactions in the exocrine pancreas but our data indicate that disordering of a particular type of organelle in acinar cells results in the failure of the whole network, whereas restoring the function of one type of organelle improves work of others.

The most detailed information on interrelations between organelle disorders in AP has been obtained for the autophagy/lysosomal and mitochondria-mediated pathways (Figure 4). For example, blocking autophagy by genetic ablation of Atg5, Atg7 or IKKα all caused ER stress and accumulation of dysfunctional mitochondria that over-produce ROS and generate less ATP.[60,118,119] Conversely, mitochondrial damage in Arg-AP and CER-AP caused activation of pancreatic autophagy (in particular, mitophagy) and, at the same time, its impairment.[98,101] Restoring mitochondrial function by CypD genetic ablation largely normalized pancreatic autophagy.[101] Endoplasmic reticulum stress caused by dysregulation of UPR in XBP1-deficient pancreas led to mitochondrial dysfunction manifest by reduced oxidative phosphorylation and pathologic alterations in autophagy.[81] Restoring

mitochondrial function with CypD genetic ablation or by enhancing autophagic efficiency with disaccharide trehalose both alleviated ER stress in experimental pancreatitis.[101]

Reduced acinar cell secretion is a hallmark of acute pancreatitis. Studies discussed above in relation to the role of organelles in secretion reveal that disordering of autophagy/lysosomal[60,61] or endosomal[69] pathways causes inhibition of secretion in acinar cells. CCK-induced amylase secretion is inhibited in Atg5 null acinar cells.[58,59] D52 and Rab5, proteins that regulate MSP and the endosomal compartment, are depleted from CCK-hyperstimulated acinar cells and in vivo, in mouse CER-AP and CDE-AP.[69,72] Inhibition of VAMP8-dependent secretion is associated with inhibition of autophagy.[69] Finally, the SNARE protein Syntaxin 2 has beeen shown to regulate both autophagy and exocytosis in acinar cells in pancreatitis.[125] Whether and how these effectors maintain homeostatic balance between autophagy and secretion in acinar cells remains to be investigated.

5. DISORDERING OF ACINAR CELL ORGANELLES IN HUMAN PANCREATITIS

Two approaches have been applied to detect acinar cell organelle disordering in human pancreatitis. The first is to analyze pancreatic tissue specimens from patients with pancreatitis. These have shown patterns of organelle dysfunction in human disease similar to those in rodent models, including the dramatically reduced levels of LAMP1 and LAMP2[61,108] and impaired autophagy evidenced by large autophagic vacuoles (seen in acinar cells with both electron and light microscopy),[105] the accumulation of LC3-positive autophagic vacuoles,[101] and by increased levels of both LC3-II and the autophagy substrate p62/SQSTM1 (sequestosome 1).[60,101] Mitochondrial dysfunction in human pancreatic tissue specimens is manifest by massive mitochondrial fragmentation.[101]

The second approach is to use human acinar cells (obtained as byproducts of islet isolation from cadaveric pancreata of organ donors) and subject them to ex-vivo pancreatitis insults. We and others showed that healthy human acinar cells isolated from cadaveric tissue retain their morphology and functional characteristics, which has allowed examination of early pancreatitis responses to a number of stressors, e.g., bile salts.[72,98,100,113,126,127] We characterized physiologic and pathophysiologic responses of the human acinar cell preparations and found them similar to those observed in mouse ex-vivo models.[126] In particular, disordered organelle responses: the ER stress, aberrant Ca^{2+} signal, mitochondrial depolarization, and impaired autophagy – are all prominent in ex-vivo pancreatitis on human acinar cells.[98,126] Similar results were obtained on human pancreas slices.[125,128]

6. RESTORING THE FUNCTION OF ORGANELLAR NETWORK TO TREAT PANCREATITIS

6.1. Potential Pharmacologic Approaches

Studies discussed in this review indicate that restoring organellar homeostasis is a promising strategy for pancreatitis treatment (Fig. 5).[129] Recently, the development of small molecule enhancers of autophagy has become a major approach for various neurologic diseases.[130]

One such agent, the natural disaccharide trehalose, was shown to reduce injury in animal models of neurodegenerative diseases[131] and is now in clinical trials for spinocerebellar ataxia type 3. We found that trehalose enhanced autophagic degradation and alleviated essentially all pancreatitis responses in both Arg-AP and CER-AP.[101] Though the mechanism of trehalose action, as well as the treatment regimens to obtain a therapeutic response, need to be further elucidated, it may provide a valuable therapeutic option.

Restoring mitochondrial potential in pancreatitis with CypD genetic ablation alleviated pancreatitis in multiple experimental models. In particular, experimental pancreatitis in CypD null mice showed lesser or no trypsinogen activation, reduced necrosis, inflammatory infiltration, and improved pancreas histopathology (compared to wild type).[55,98,101] Further, small molecule inhibitors of CypD restored mitochondrial function and prevented or markedly alleviated AP responses.[98,132] Several new CypD inhibitors were recently synthesized and shown to protect mitochondrial functions and reduce necrosis in ex-vivo pancreatitis,[132] and thus should be considered for pancreatitis treatment.

Finally, abnormal (global and sustained) increases in cytosolic Ca^{2+} associated with several AP models cause both mitochondrial dysfunction and defects in the endosomal system. Modifying acinar cell Ca^{2+} signaling has been used in animal models to reduce AP injury – in particular, inhibition of excessive Ca^{2+} entry by ORAI1 inhibitors[133] or TRPC3 inhibitor.[96] Some of the Ca^{2+} damaging effects are through activating the phosphatase calcineurin. Calcineurin inhibition by genetic or pharmacologic means largely alleviated pancreatitis responses in Ca^{2+}-dependent models of AP.[100] The results suggest that pharmacologic approaches aimed to reduce $[Ca^{2+}]i$ can be developed for the treatment of patients with pancreatitis.[133]

6.2. Future Directions

Despite the recent progress, detailed analyses of organelle disorders in exocrine pancreas have just begun and there is much to be learned about their role in pancreatitis and the underlying mechanisms.[3,4,134–143] One important area is the interrelations between different types of organelles in acinar cells and how these are altered by pancreatitis. A key question is whether there is a critical pathologic defect common for all types of pancreatitis and leading to failure of the whole organellar network, or whether organelle dysfunction is mediated through different mechanisms and more than one organelle disorder should be restored (for example, normalizing both autophagy and mitochondrial function at the same time) to re-establish the organellar network homeostasis.[136] Another important research direction is the mechanisms that link disordering of cellular organelles to “classic” pancreatitis responses such as inflammation and cell death. In particular, recent studies began elucidating the mechanisms whereby impaired autophagy and mitochondrial dysfunction in acinar cells cause inflammation in pancreatitis.[3,4,141–143]

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Abbreviations:

AP	acute pancreatitis
Arg-AP	L-arginine-induced acute pancreatitis
CCK	cholecystokinin-8
CDE-AP	pancreatitis induced with choline-deficient, D,L-ethionine supplemented (CDE) diet
CER-AP	cerulein-induced acute pancreatitis
CHOP	CCAAT/enhancer binding protein homologous protein
CypD	cyclophilin D
ETC	electron transport chain
ER	endoplasmic reticulum
$[Ca^{2+}]i$	free cytosolic Ca^{2+} concentration
IKK	inhibitor of the nuclear factor κB kinase
MSP	minor secretory pathway
M6P-R	mannose 6-phosphate receptor
MPTP	mitochondrial permeability transition pore
ROS	reactive oxygen species
sXBP1	spliced X-box binding protein 1
UPR	unfolded protein response
VAMP	vesicle-associated membrane protein
ZG	zymogen granule(s)

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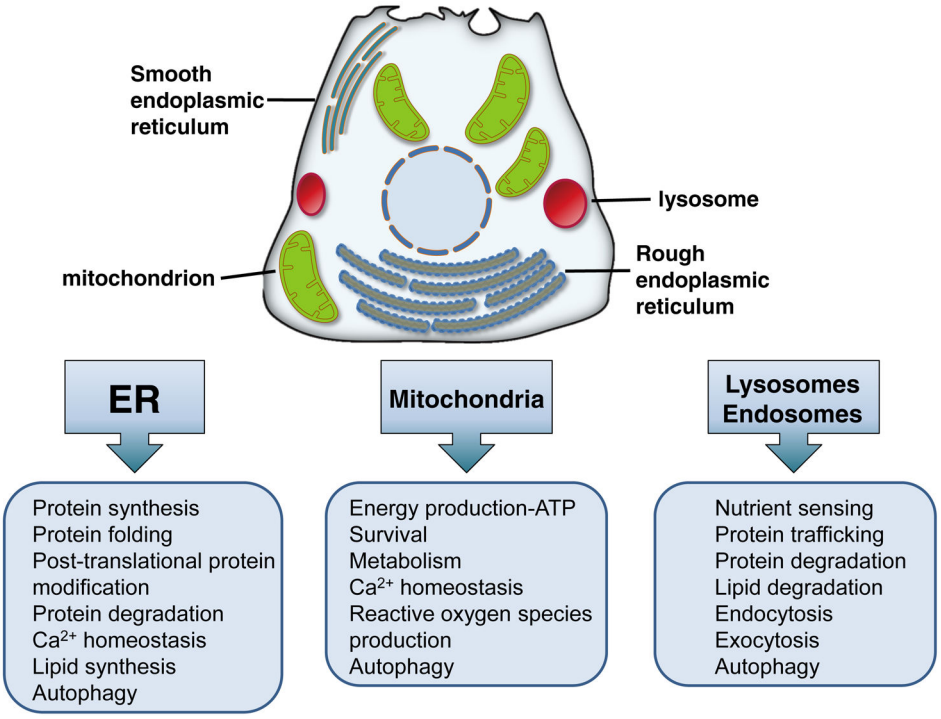

FIGURE 1.

Cellular localization and functions of cytoplasmic organelles in eukaryotic cells that are most relevant to acute pancreatitis.

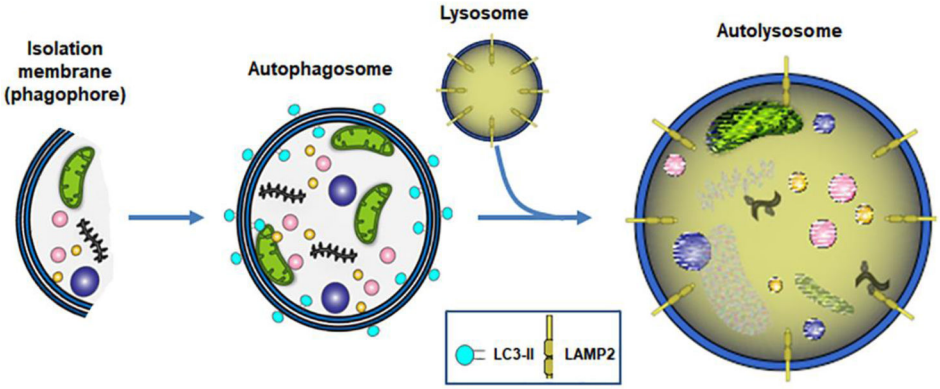

FIGURE 2.
Schematic of macroautophagy.

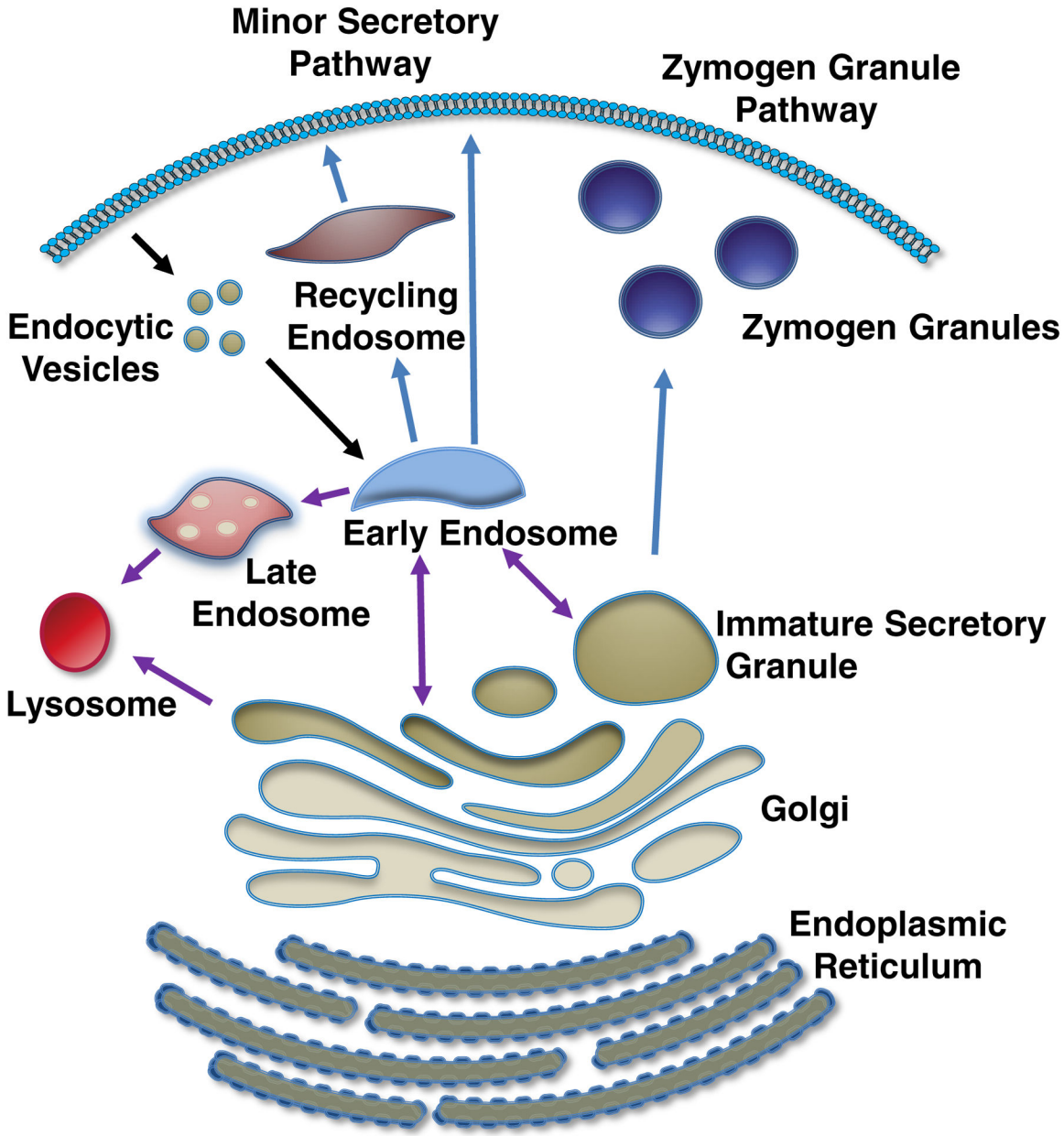

FIGURE 3.
Digestive enzymes' trafficking through secretory pathways.

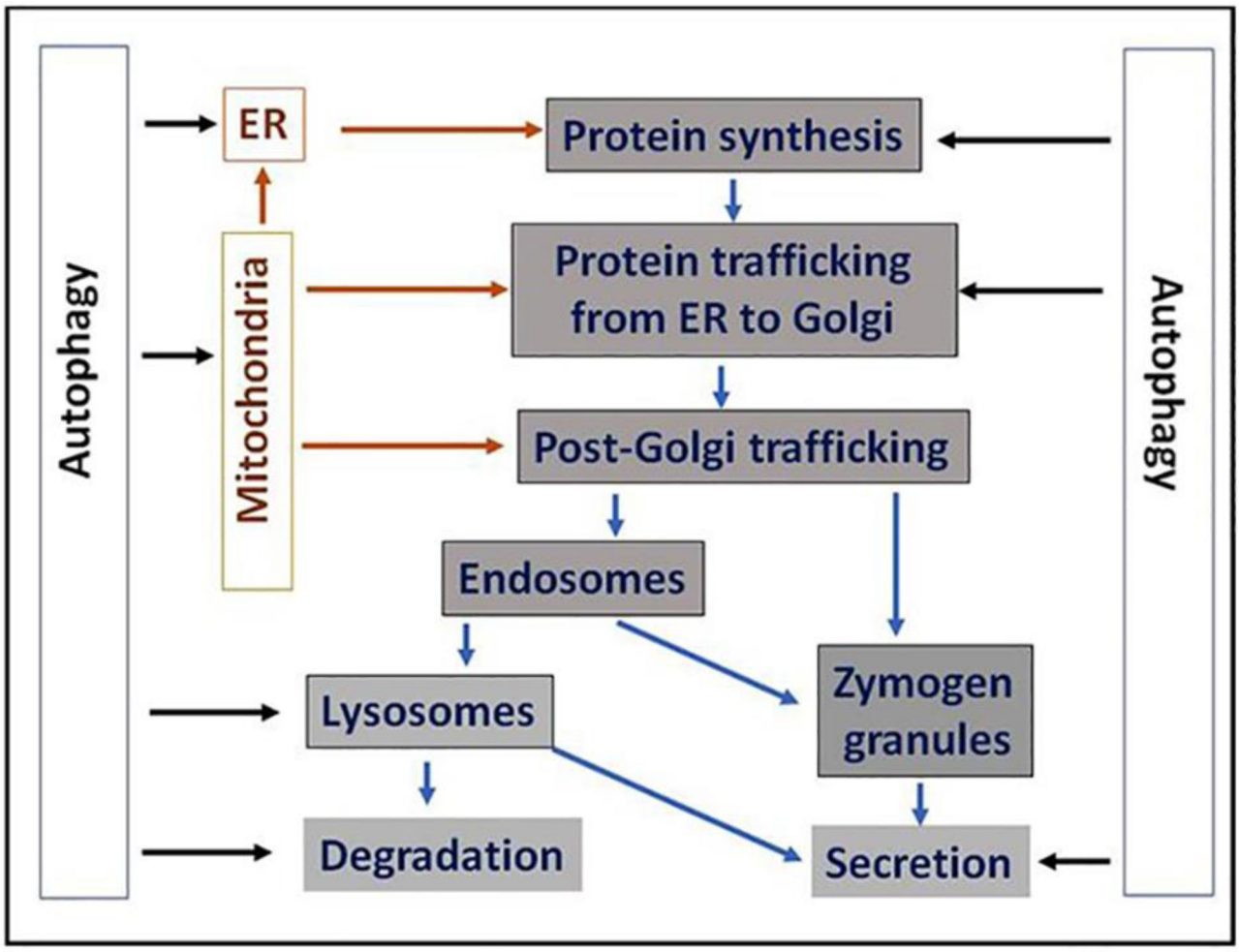

FIGURE 4.

Coordinated action of ER, mitochondrial, endo-lysosomal, autophagic and secretory pathways is required to maintain acinar cells homeostasis and physiologic functions.

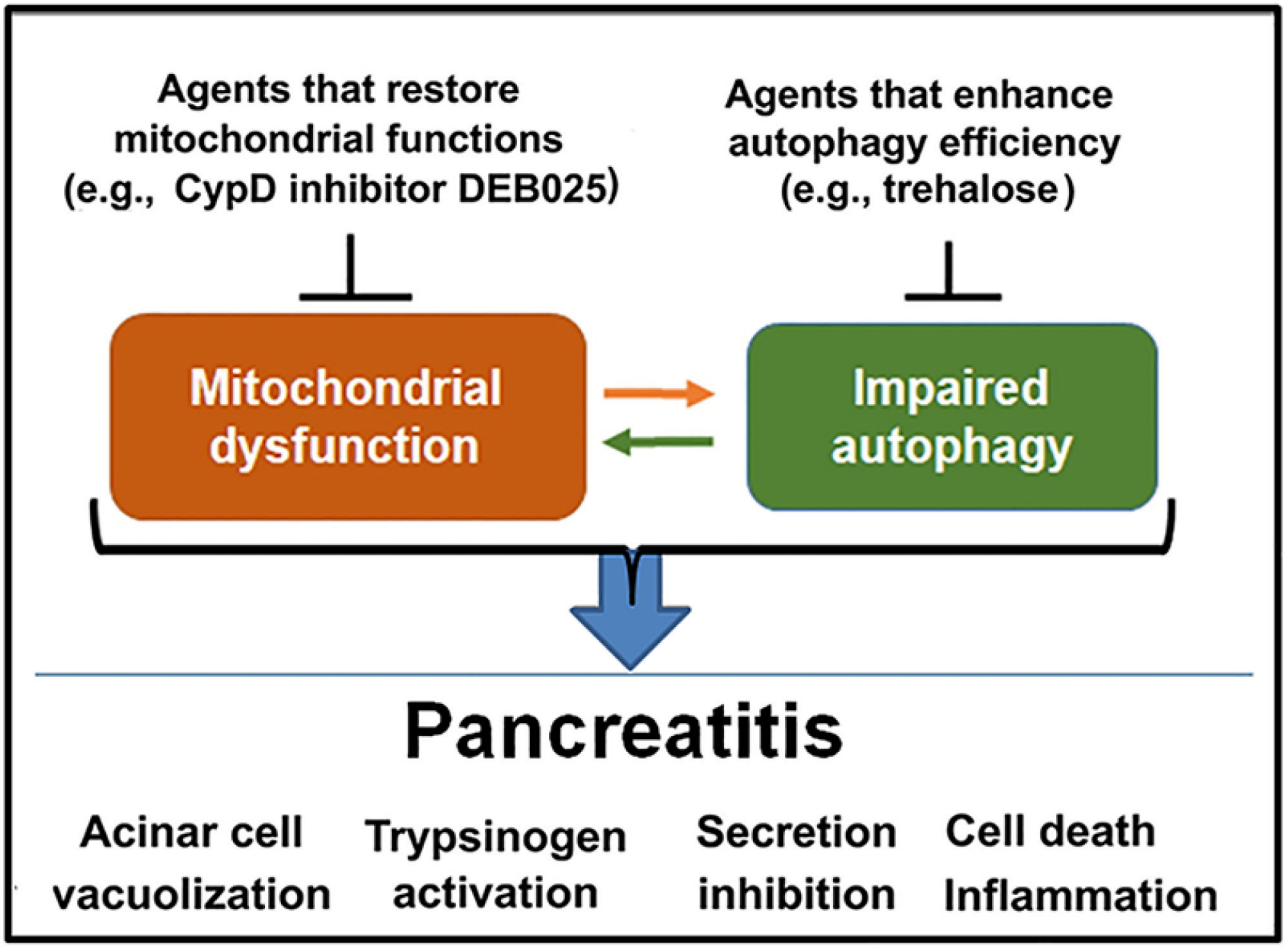

FIGURE 5.
Potential therapeutic approaches to restore acinar cell organellar homeostasis for pancreatitis treatment.