Published in final edited form as:

Pancreas. 2019 April ; 48(4): 459–470. doi:10.1097/MPA.0000000000001298.

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

Anna S. Gukovskaya, PhD^{*,†}, Fred S. Gorelick, MD^{‡,§}, Guy E. Groblewski, PhD^{||}, Olga A. Mareninova, PhD^{*,†}, Aurelia Lugea, PhD[¶], Laura Antonucci, PhD[#], Richard T. Waldron, PhD[¶], Aida Habtezion, MD, MSci^{**}, Michael Karin, PhD[#], Stephen J. Pandol, MD[¶], and Ilya Gukovsky, PhD^{*,†}

^{*}Department of Medicine, David Geffen School of Medicine, University of California at Los Angeles;

[†]Department of Medicine, West Los Angeles VA Healthcare Center, Los Angeles, CA;

[‡]Department of Cell Biology Yale University School of Medicine, New Haven, CT;

[§]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT;

^{||}Department of Nutritional Sciences, University of Wisconsin, Madison, WI;

[¶]Division of Digestive and Liver Diseases, Cedars-Sinai Medical Center, Los Angeles, CA;

[#]Laboratory of Gene Regulation and Signal Transduction, Departments of Pharmacology and Pathology, University of California San Diego School of Medicine, La Jolla, CA;

^{**}Division of Gastroenterology and Hepatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA.

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 Ca²⁺ signaling) in the acinar cell trigger the inflammatory and cell death

Address correspondence to: Anna S. Gukovskaya, PhD, Pancreatic Research Group, West Los Angeles Veterans Affairs Healthcare Center, 11301 Wilshire Boulevard, Building 258/340, Los Angeles, CA 90073 (agukovsk@ucla.edu). A.S.G. and F.S.G. share first authorship.

Disclosure: The authors declare no conflict of interest

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; Ca²⁺ 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 temporarily 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 Ca²⁺ 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 Ca²⁺ 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 degradation³³ (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 through IP3 and RyR-sensitive channels. This release causes a transient increase in free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) 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 $[\text{Ca}^{2+}]_i$, 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. $[\text{Ca}^{2+}]_i$ 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 IKK α (the inhibitor of the nuclear factor κB (I κB) 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 reported⁸⁸ 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 Ca²⁺ 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 Ca²⁺

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 MPTP 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 data¹⁰³ 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, IKK α 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 IKK α 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 endosomal⁶⁹ 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 been 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 Ca²⁺ 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 diseases¹³¹ 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 inhibitors¹³³ 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 $[\text{Ca}^{2+}]_i$ 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}

ACKNOWLEDGMENT

Studies from our groups discussed in this review were supported, fully or in part, by the NIH/NIDDK Program Project grant P01 DK098108 “Organelle Disorders in Pancreatitis” (References: 3–5, 7, 17, 55–61, 66, 68–70, 72, 74–76, 88, 90, 91, 98, 101, 103, 119–122, 126, 127, 129, 136–143).

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
[Ca²⁺]_i	free cytosolic Ca ²⁺ concentration
IKK	inhibitor of the nuclear factor κ B 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)

REFERENCES

1. Peery AF, Crockett SD, Murphy CC, et al. Burden and cost of gastrointestinal, liver, and pancreatic diseases in the United States: update 2018. *Gastroenterology*. 2019;156:254–272 e11. [PubMed: 30315778]
2. Pandol SJ, Saluja AK, Imrie CW, et al. Acute pancreatitis: bench to the bedside. *Gastroenterology*. 2007;132:1127–1151. Erratum: *ibid*, 133:1056. [PubMed: 17383433]
3. Gukovskaya AS, Gukovsky I, Algul H, et al. Autophagy, inflammation, and immune dysfunction in the pathogenesis of pancreatitis. *Gastroenterology*. 2017;153:1212–1226. [PubMed: 28918190]
4. Gukovsky I, Li N, Todoric J, et al. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. *Gastroenterology*. 2013;144:1199–1209 e4. [PubMed: 23622129]
5. Szatmary P, Gukovsky I. The role of cytokines and inflammation in the genesis of experimental pancreatitis. In: Williams JA, ed. *Pancreatitis*. Mountain View, CA: Michigan Publishing; 2016:42–52.

6. Habtezion A Inflammation in acute and chronic pancreatitis. *Curr Opin Gastroenterol.* 2015;31:395–399. [PubMed: 26107390]
7. Messenger SW, Falkowski MA, Groblewski GE. Ca(2+)-regulated secretory granule exocytosis in pancreatic and parotid acinar cells. *Cell Calcium.* 2014;55:369–375. [PubMed: 24742357]
8. Williams JA. Regulation of acinar cell function in the pancreas. *Curr Opin Gastroenterol.* 2010;26:478–483. [PubMed: 20625287]
9. Palade G Intracellular aspects of the process of protein synthesis. *Science.* 1975;189:347–358. [PubMed: 1096303]
10. Williams JA. The nobel pancreas: a historical perspective. *Gastroenterology.* 2013;144:1166–1169. [PubMed: 23622125]
11. Murley A, Nunnari J. The emerging network of mitochondria-organelle contacts. *Mol Cell.* 2016;61:648–653. [PubMed: 26942669]
12. Senft D, Ronai ZA. UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem Sci.* 2015;40:141–148. [PubMed: 25656104]
13. Brodsky JL, Skach WR. Protein folding and quality control in the endoplasmic reticulum: Recent lessons from yeast and mammalian cell systems. *Curr Opin Cell Biol.* 2011;23:464–475. [PubMed: 21664808]
14. Lindholm D, Korhonen L, Eriksson O, et al. Recent insights into the role of Unfolded Protein Response in ER stress in health and disease. *Front Cell Dev Biol.* 2017;5:48. [PubMed: 28540288]
15. Schrader M, Godinho LF, Costello JL, et al. The different facets of organelle interplay-an overview of organelle interactions. *Front Cell Dev Biol.* 2015;3:56. [PubMed: 26442263]
16. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature.* 2016;529:326–335. [PubMed: 26791723]
17. Waldron J, Pandol S, Lugea A, et al. Endoplasmic reticulum stress and the unfolded protein response in exocrine pancreas physiology and pancreatitis. In: Williams JA, ed. *Pancreatitis.* Mountain View, CA: Michigan Publishing; 2016:88–96.
18. Petersen OH. Calcium signalling and secretory epithelia. *Cell Calcium.* 2014;55:282–289. [PubMed: 24508392]
19. Petersen OH, Courjaret R, Machaca K. Ca(2+) tunnelling through the ER lumen as a mechanism for delivering Ca(2+) entering via store-operated Ca(2+) channels to specific target sites. *J Physiol.* 2017;595:2999–3014. [PubMed: 28181236]
20. Marchi S, Bittremieux M, Missiroli S, et al. Endoplasmic reticulum-mitochondria communication through Ca(2+) signaling: the importance of mitochondria-associated membranes (MAMs). *Adv Exp Med Biol.* 2017;997:49–67. [PubMed: 28815521]
21. De Stefani D, Rizzuto R, Pozzan T. Enjoy the trip: calcium in mitochondria back and forth. *Annu Rev Biochem.* 2016;85:161–192. [PubMed: 27145841]
22. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. *Cell.* 2012;148:1145–1159. [PubMed: 22424226]
23. Spinelli JB, Haigis MC. The multifaceted contributions of mitochondria to cellular metabolism. *Nat Cell Biol.* 2018;20:745–754. [PubMed: 29950572]
24. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev.* 2007;87:99–163. [PubMed: 17237344]
25. Baines CP, Gutierrez-Aguilar M. The still uncertain identity of the channel-forming unit(s) of the mitochondrial permeability transition pore. *Cell Calcium.* 2018;73:121–130. [PubMed: 29793100]
26. Bernardi P, Rasola A, Forte M, et al. The mitochondrial permeability transition pore: channel formation by F-ATP synthase, integration in signal transduction, and role in pathophysiology. *Physiol Rev.* 2015;95:1111–1155. [PubMed: 26269524]
27. Lee H, Yoon Y. Mitochondrial fission and fusion. *Biochem Soc Trans.* 2016;44:1725–1735. [PubMed: 27913683]
28. Mishra P Interfaces between mitochondrial dynamics and disease. *Cell Calcium.* 2016;60:190–198. [PubMed: 27252081]
29. Hutagalung AH, Novick PJ. Role of Rab GTPases in membrane traffic and cell physiology. *Physiol Rev.* 2011;91:119–149. [PubMed: 21248164]

30. Rink J, Ghigo E, Kalaidzidis Y, et al. Rab conversion as a mechanism of progression from early to late endosomes. *Cell*. 2005;122:735–749. [PubMed: 16143105]
31. Stenmark H Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol*. 2009;10:513–525. [PubMed: 19603039]
32. Langemeyer L, Frohlich F, Ungermann C. Rab GTPase function in endosome and lysosome biogenesis. *Trends Cell Biol*. 2018;28:957–970. [PubMed: 30025982]
33. Settembre C, Fraldi A, Medina DL, et al. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat Rev Mol Cell Biol*. 2013;14:283–296. [PubMed: 23609508]
34. Coutinho MF, Prata MJ, Alves S. Mannose-6-phosphate pathway: a review on its role in lysosomal function and dysfunction. *Mol Genet Metab*. 2012;105:542–550. [PubMed: 22266136]
35. Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: new twists in the tale. *Nat Rev Mol Cell Biol*. 2003;4:202–212. [PubMed: 12612639]
36. Bonifacino JS, Neefjes J. Moving and positioning the endolysosomal system. *Curr Opin Cell Biol*. 2017;47:1–8. [PubMed: 28231489]
37. Braulke T, Bonifacino JS. Sorting of lysosomal proteins. *Biochim Biophys Acta*. 2009;1793:605–614. [PubMed: 19046998]
38. Staudt C, Puissant E, Boonen M. Subcellular trafficking of mammalian lysosomal proteins: an extended view. *Int J Mol Sci*. 2016;18.
39. Mindell JA. Lysosomal acidification mechanisms. *Annu Rev Physiol*. 2012;74:69–86. [PubMed: 22335796]
40. Rowan AD, Mason P, Mach L, et al. Rat procathepsin B. Proteolytic processing to the mature form in vitro. *J Biol Chem*. 1992;267:15993–15999. [PubMed: 1639824]
41. Eskelinen EL, Tanaka Y, Saftig P. At the acidic edge: emerging functions for lysosomal membrane proteins. *Trends Cell Biol*. 2003;13:137–145. [PubMed: 12628346]
42. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nat Rev Mol Cell Biol*. 2009;10:623–635. [PubMed: 19672277]
43. Ferguson SM. Beyond indigestion: emerging roles for lysosome-based signaling in human disease. *Curr Opin Cell Biol*. 2015;35:59–68. [PubMed: 25950843]
44. Settembre C, Di Malta C, Polito VA, et al. TFEB links autophagy to lysosomal biogenesis. *Science*. 2011;332:1429–1433. [PubMed: 21617040]
45. Gukovskaya AS, Gukovsky I. Autophagy and pancreatitis. *Am J Physiol Gastrointest Liver Physiol*. 2012;303:G993–1003. [PubMed: 22961802]
46. Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. *Antioxid Redox Signal*. 2014;20:460–473. [PubMed: 23725295]
47. Stolz A, Ernst A, Dikic I. Cargo recognition and trafficking in selective autophagy. *Nat Cell Biol*. 2014;16:495–501. [PubMed: 24875736]
48. Nguyen TN, Padman BS, Lazarou M. Deciphering the molecular signals of PINK1/Parkin mitophagy. *Trends Cell Biol*. 2016;26:733–744. [PubMed: 27291334]
49. Yule DI. Ca²⁺ signaling in pancreatic acinar cells. *Pancreapedia: Exocrine Pancreas Knowledge Base* DOI: 10.3998/panc.2015.24
50. Di Jeso B, Ulianich L, Pacifico F, et al. Folding of thyroglobulin in the calnexin/calreticulin pathway and its alteration by loss of Ca²⁺ from the endoplasmic reticulum. *Biochem J*. 2003;370:449–458. [PubMed: 12401114]
51. Jamieson JD, Palade GE. Intracellular transport of secretory proteins in the pancreatic exocrine cell. IV. Metabolic requirements. *J Cell Biol*. 1968;39:589–603. [PubMed: 5699933]
52. Voronina SG, Barrow SL, Simpson AW, et al. Dynamic changes in cytosolic and mitochondrial ATP levels in pancreatic acinar cells. *Gastroenterology*. 2010;138:1976–1987. [PubMed: 20102715]
53. Voronina SG, Barrow SL, Gerasimenko OV, et al. Effects of secretagogues and bile acids on mitochondrial membrane potential of pancreatic acinar cells: comparison of different modes of evaluating DeltaPsm. *J Biol Chem*. 2004;279:27327–27338. [PubMed: 15084611]
54. Odinokova IV, Sung KF, Mareninova OA, et al. Mechanisms regulating cytochrome c release in pancreatic mitochondria. *Gut*. 2009;58:431–442. [PubMed: 18596195]

55. Shalbueva N, Mareninova OA, Gerloff A, et al. Effects of oxidative alcohol metabolism on the mitochondrial permeability transition pore and necrosis in a mouse model of alcoholic pancreatitis. *Gastroenterology*. 2013;144:437–446 e6. [PubMed: 23103769]
56. Gukovskaya AS, Pandol SJ, Gukovsky I. New insights into the pathways initiating and driving pancreatitis. *Curr Opin Gastroenterol*. 2016;32:429–435. [PubMed: 27428704]
57. Gukovsky I, Gukovskaya AS. Impaired autophagy triggers chronic pancreatitis: lessons from pancreas-specific Atg5 knockout mice. *Gastroenterology*. 2015;148:501–505. [PubMed: 25613315]
58. Malla SR, Qin Y, Suriany S, et al. ATG5 deficiency worsens experimental pancreatitis in two genetic mouse models (Abstract). *Gastroenterology*. 2016;150:S142.
59. Malla SR, Ohmuraya M, Shalbueva N, et al. ATG5 deficiency worsens experimental pancreatitis. Manuscript in preparation.
60. Li N, Wu X, Holzer RG, et al. Loss of acinar cell IKKalpha triggers spontaneous pancreatitis in mice. *J Clin Invest*. 2013;123:2231–2243. [PubMed: 23563314]
61. Mareninova OA, Sandler M, Malla SR, et al. Lysosome associated membrane proteins maintain pancreatic acinar cell homeostasis: LAMP-2 deficient mice develop pancreatitis. *Cell Mol Gastroenterol Hepatol*. 2015;1:678–694. [PubMed: 26693174]
62. Oprins A, Rabouille C, Posthuma G, et al. The ER to Golgi interface is the major concentration site of secretory proteins in the exocrine pancreatic cell. *Traffic*. 2001;2:831–838. [PubMed: 11733050]
63. Klumperman J, Kuliawat R, Griffith JM, et al. Mannose 6-phosphate receptors are sorted from immature secretory granules via adaptor protein AP-1, clathrin, and syntaxin 6-positive vesicles. *J Cell Biol*. 1998;141:359–371. [PubMed: 9548715]
64. Arvan P, Castle D. Protein sorting and secretion granule formation in regulated secretory cells. *Trends Cell Biol*. 1992;2:327–331. [PubMed: 14731510]
65. Castle JD, Castle AM. Two regulated secretory pathways for newly synthesized parotid salivary proteins are distinguished by doses of secretagogues. *J Cell Sci*. 1996;109 (Pt 10):2591–2599. [PubMed: 8923220]
66. Messenger SW, Falkowski MA, Thomas DD, et al. Vesicle associated membrane protein 8 (VAMP8)-mediated zymogen granule exocytosis is dependent on endosomal trafficking via the constitutive-like secretory pathway. *J Biol Chem*. 2014;289:28040–28053. [PubMed: 25138214]
67. Grondin G, Beaudoin AR. Immunocytochemical and cytochemical demonstration of a novel selective lysosomal pathway (SLP) of secretion in the exocrine pancreas. *J Histochem Cytochem*. 1996;44:357–368. [PubMed: 8601695]
68. Messenger SW, Thomas DD, Falkowski MA, et al. Tumor protein D52 controls trafficking of an apical endolysosomal secretory pathway in pancreatic acinar cells. *Am J Physiol Gastrointest Liver Physiol*. 2013;305:G439–452. [PubMed: 23868405]
69. Messenger SW, Jones EK, Holthaus CL, et al. Acute acinar pancreatitis blocks vesicle-associated membrane protein 8 (VAMP8)-dependent secretion, resulting in intracellular trypsin accumulation. *J Biol Chem*. 2017;292:7828–7839. [PubMed: 28242757]
70. Shahheydari H, Frost S, Smith BJ, et al. Identification of PLP2 and RAB5C as novel TPD52 binding partners through yeast two-hybrid screening. *Mol Biol Rep*. 2014;41:4565–4572. [PubMed: 24604726]
71. Thomas DD, Weng N, Groblewski GE. Secretagogue-induced translocation of CRHSP-28 within an early apical endosomal compartment in acinar cells. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G253–263. [PubMed: 14977633]
72. Messenger SW, Thomas DD, Cooley MM, et al. Early to late endosome trafficking controls secretion and zymogen activation in rodent and human pancreatic acinar cells. *Cell Mol Gastroenterol Hepatol*. 2015;1:695–709. [PubMed: 26618189]
73. Weng N, Thomas DD, Groblewski GE. Pancreatic acinar cells express vesicle-associated membrane protein 2- and 8-specific populations of zymogen granules with distinct and overlapping roles in secretion. *J Biol Chem*. 2007;282:9635–9645. [PubMed: 17272274]
74. Gorelick FS, Lerch MM. Do animal models of acute pancreatitis reproduce human disease? *Cell Mol Gastroenterol Hepatol*. 2017;4:251–262. [PubMed: 28752114]

75. Lerch MM, Gorelick FS. Models of acute and chronic pancreatitis. *Gastroenterology*. 2013;144:1180–1193. [PubMed: 23622127]
76. Mareninova O, Orabi AI, Husain S, et al. Experimental acute pancreatitis: in vitro models. In: Williams JA, ed. *Pancreatitis*. Mountain View, CA: Michigan Publishing; 2016:3–14.
77. Balazs A, Hegyi P, Sahin-Toth M. Pathogenic cellular role of the p.L104P human cationic trypsinogen variant in chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol*. 2016;310:G477–486. [PubMed: 26822915]
78. Kubisch CH, Logsdon CD. Endoplasmic reticulum stress and the pancreatic acinar cell. *Expert Rev Gastroenterol Hepatol*. 2008;2:249–260. [PubMed: 19072360]
79. Kubisch CH, Sans MD, Arumugam T, et al. Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol*. 2006;291:G238–245. [PubMed: 16574987]
80. Logsdon CD, Ji B. The role of protein synthesis and digestive enzymes in acinar cell injury. *Nat Rev Gastroenterol Hepatol*. 2013;10:362–370. [PubMed: 23507798]
81. Lugea A, Tischler D, Nguyen J, et al. Adaptive unfolded protein response attenuates alcohol-induced pancreatic damage. *Gastroenterology*. 2011;140:987–997. [PubMed: 21111739]
82. Pandol SJ, Gorelick FS, Gerloff A, et al. Alcohol abuse, endoplasmic reticulum stress and pancreatitis. *Dig Dis*. 2010;28:776–782. [PubMed: 21525762]
83. Pandol SJ, Gorelick FS, Lugea A. Environmental and genetic stressors and the unfolded protein response in exocrine pancreatic function - a hypothesis. *Front Physiol*. 2011;2:8. [PubMed: 21483727]
84. Sah RP, Garg SK, Dixit AK, et al. Endoplasmic reticulum stress is chronically activated in chronic pancreatitis. *J Biol Chem*. 2014;289:27551–27561. [PubMed: 25077966]
85. Mounzer R, Whitcomb DC. Genetics of acute and chronic pancreatitis. *Curr Opin Gastroenterol*. 2013;29:544–551. [PubMed: 23872486]
86. Sahin-Toth M. Genetic risk in chronic pancreatitis: the misfolding-dependent pathway. *Curr Opin Gastroenterol*. 2017;33:390–395. [PubMed: 28650851]
87. Hegyi E, Sahin-Toth M. Human CPA1 mutation causes digestive enzyme misfolding and chronic pancreatitis in mice. *Gut*. 2018 7 25 pii: gutjnl-2018–315994. doi: 10.1136/gutjnl-2018-315994. [Epub ahead of print]
88. Lugea A, Gerloff A, Su HY, et al. The combination of alcohol and cigarette smoke induces endoplasmic reticulum stress and cell death in pancreatic acinar cells. *Gastroenterology*. 2017;153:1674–1686. [PubMed: 28847752]
89. Hess DA, Humphrey SE, Ishibashi J, et al. Extensive pancreas regeneration following acinar-specific disruption of Xbp1 in mice. *Gastroenterology*. 2011;141:1463–1472. [PubMed: 21704586]
90. Waldron RT, Su HY, Piplani H, et al. Ethanol induced disordering of pancreatic acinar cell endoplasmic reticulum: an ER stress/defective unfolded protein response model. *Cell Mol Gastroenterol Hepatol*. 2018;5:479–497. [PubMed: 29930975]
91. Setiawan VW, Monroe K, Lugea A, et al. Uniting epidemiology and experimental disease models for alcohol-related pancreatic disease. *Alcohol Res*. 2017;38:173–182. [PubMed: 28988572]
92. Yadav D, Hawes RH, Brand RE, et al. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. *Arch Intern Med*. 2009;169:1035–1045. [PubMed: 19506173]
93. Gerasimenko JV, Gerasimenko OV, Petersen OH. The role of Ca²⁺ in the pathophysiology of pancreatitis. *J Physiol*. 2014;592:269–280. [PubMed: 23897234]
94. Petersen OH, Tepikin AV. Polarized calcium signaling in exocrine gland cells. *Annu Rev Physiol*. 2008;70:273–299. [PubMed: 17850212]
95. Son A, Park S, Shin DM, et al. Orail and STIM1 in ER/PM junctions: roles in pancreatic cell function and dysfunction. *Am J Physiol Cell Physiol*. 2016;310:C414–422. [PubMed: 26739495]
96. Kim MS, Lee KP, Yang D, et al. Genetic and pharmacologic inhibition of the Ca²⁺ influx channel TRPC3 protects secretory epithelia from Ca²⁺-dependent toxicity. *Gastroenterology*. 2011;140:2107–2115, 2115 e1–4. [PubMed: 21354153]

97. Odinkova IV, Sung KF, Mareninova OA, et al. Mitochondrial mechanisms of death responses in pancreatitis. *J Gastroenterol Hepatol*. 2008;23 Suppl 1:S25–30. [PubMed: 18336659]
98. Mukherjee R, Mareninova OA, Odinkova IV, et al. Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP. *Gut*. 2016;65:1333–1346. [PubMed: 26071131]
99. Muili KA, Ahmad M, Orabi AI, et al. Pharmacological and genetic inhibition of calcineurin protects against carbachol-induced pathological zymogen activation and acinar cell injury. *Am J Physiol Gastrointest Liver Physiol*. 2012;302:G898–905. [PubMed: 22323127]
100. Orabi AI, Wen L, Javed TA, et al. Targeted inhibition of pancreatic acinar cell calcineurin is a novel strategy to prevent post-ERCP pancreatitis. *Cell Mol Gastroenterol Hepatol*. 2017;3:119–128. [PubMed: 28090570]
101. Biczko G, Vegh ET, Shalbueva N, et al. Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. *Gastroenterology*. 2018;154:689–703. [PubMed: 29074451]
102. Gukovsky I, Pandol SJ, Gukovskaya AS. Organellar dysfunction in the pathogenesis of pancreatitis. *Antioxid Redox Signal*. 2011;15:2699–2710. [PubMed: 21834686]
103. Mareninova O, Yakubov I, Gukovsky I, et al. Disordering of endo-lysosomal system in pancreatitis. (Abstract). *Pancreas*. 2018;47:1408; Manuscript in preparation.
104. Gukovsky I, Pandol SJ, Mareninova OA, et al. Impaired autophagy and organellar dysfunction in pancreatitis. *J Gastroenterol Hepatol*. 2012;27 Suppl 2:27–32. [PubMed: 22320913]
105. Mareninova OA, Hermann K, French SW, et al. Impaired autophagic flux mediates acinar cell vacuole formation and trypsinogen activation in rodent models of acute pancreatitis. *J Clin Invest*. 2009;119:3340–3355. [PubMed: 19805911]
106. Saluja A, Hashimoto S, Saluja M, et al. Subcellular redistribution of lysosomal enzymes during caerulein-induced pancreatitis. *Am J Physiol*. 1987;253:G508–516. [PubMed: 2821825]
107. Waterford SD, Kolodecik TR, Thrower EC, et al. Vacuolar ATPase regulates zymogen activation in pancreatic acini. *J Biol Chem*. 2005;280:5430–5434. [PubMed: 15582989]
108. Fortunato F, Burgers H, Bergmann F, et al. Impaired autolysosome formation correlates with Lamp-2 depletion: role of apoptosis, autophagy, and necrosis in pancreatitis. *Gastroenterology*. 2009;137:350–360, 360 e1–5. [PubMed: 19362087]
109. Arampatzidou M, Rehders M, Dauth S, et al. Imaging of protease functions--current guide to spotting cysteine cathepsins in classical and novel scenes of action in mammalian epithelial cells and tissues. *Ital J Anat Embryol*. 2011;116:1–19. [PubMed: 21898969]
110. Turk V, Stoka V, Vasiljeva O, et al. Cysteine cathepsins: from structure, function and regulation to new frontiers. *Biochim Biophys Acta*. 2012;1824:68–88. [PubMed: 22024571]
111. Takahashi K, Mashima H, Miura K, et al. Disruption of small GTPase Rab7 exacerbates the severity of acute pancreatitis in experimental mouse models. *Sci Rep*. 2017;7:2817. [PubMed: 28588238]
112. Adler G, Rohr G, Kern HF. Alteration of membrane fusion as a cause of acute pancreatitis in the rat. *Dig Dis Sci*. 1982;27:993–1002. [PubMed: 7140496]
113. Aho HJ, Nevalainen TJ, Havia VT, et al. Human acute pancreatitis: a light and electron microscopic study. *Acta Pathol Microbiol Immunol Scand A*. 1982;90:367–373. [PubMed: 7148455]
114. Brackett KA, Crockett A, Joffe SN. Ultrastructure of early development of acute pancreatitis in the rat. *Dig Dis Sci*. 1983;28:74–84. [PubMed: 6822184]
115. Helin H, Mero M, Markkula H, et al. Pancreatic acinar ultrastructure in human acute pancreatitis. *Virchows Arch A Pathol Anat Histol*. 1980;387:259–270. [PubMed: 7456314]
116. Koike H, Steer ML, Meldolesi J. Pancreatic effects of ethionine: blockade of exocytosis and appearance of crinophagy and autophagy precede cellular necrosis. *Am J Physiol*. 1982;242:G297–307. [PubMed: 7065251]
117. Niederau C, Grendell JH. Intracellular vacuoles in experimental acute pancreatitis in rats and mice are an acidified compartment. *J Clin Invest*. 1988;81:229–236. [PubMed: 3335639]

118. Diakopoulos KN, Lesina M, Wormann S, et al. Impaired autophagy induces chronic atrophic pancreatitis in mice via sex- and nutrition-dependent processes. *Gastroenterology*. 2015;148:626–638 e17. [PubMed: 25497209]
119. Antonucci L, Fagman JB, Kim JY, et al. Basal autophagy maintains pancreatic acinar cell homeostasis and protein synthesis and prevents ER stress. *Proc Natl Acad Sci U S A*. 2015;112:E6166–E6174. [PubMed: 26512112]
120. Vegh ET, Lotshaw E, Shalbueva N, et al. Defective lysosomal hydrolase trafficking causes spontaneous pancreatitis (Abstract). *Gastroenterology*. 2016;150:S143; Manuscript in preparation.
121. Mareninova OA, Jia W, Elperin J, et al. Effects of LC3 overexpression on pancreatic acinar cell homeostasis and pancreatitis responses. *FASEB J*. 2016;30(suppl 1):92012.abstr.
122. Mareninova OA, Jia W, Gretler SR, et al. Transgenic expression of GFP-LC3 perturbs autophagy in exocrine pancreas and acute pancreatitis in mice. Manuscript in preparation.
123. Molino D, Nascimbeni AC, Giordano F, et al. ER-driven membrane contact sites: Evolutionary conserved machineries for stress response and autophagy regulation? *Commun Integr Biol*. 2017;10:e1401699. [PubMed: 29259731]
124. Simmen T, Tagaya M. Organelle communication at membrane contact sites (MCS): from curiosity to center stage in cell biology and biomedical research. *Adv Exp Med Biol*. 2017;997:1–12. [PubMed: 28815518]
125. Dolai S, Liang T, Orabi AI, et al. Pancreatitis-induced depletion of syntaxin 2 promotes autophagy and increases basolateral exocytosis. *Gastroenterology*. 2018;154:1805–1821.e5. [PubMed: 29360461]
126. Lugea A, Waldron RT, Mareninova OA, et al. Human pancreatic acinar cells: proteomic characterization, physiologic responses, and organellar disorders in ex vivo pancreatitis. *Am J Pathol*. 2017;187:2726–2743. [PubMed: 28935577]
127. Lugea A, Waldron RT, Pandol SJ. Pancreatic adaptive responses in alcohol abuse: Role of the unfolded protein response. *Pancreatol*. 2015;15:S1–S5. [PubMed: 25736240]
128. Liang T, Dolai S, Xie L, et al. Ex vivo human pancreatic slice preparations offer a valuable model for studying pancreatic exocrine biology. *J Biol Chem*. 2017;292:5957–5969. [PubMed: 28242761]
129. Abu-El-Haija M, Gukovskaya AS, Andersen DK, et al. Accelerating the drug delivery pipeline for acute and chronic pancreatitis: summary of the working group on drug development and trials in acute pancreatitis at the National Institute of Diabetes and Digestive and Kidney Diseases workshop. *Pancreas*. 2018;47:1185–1192. [PubMed: 30325856]
130. Rubinshtein DC, Bento CF, Deretic V. Therapeutic targeting of autophagy in neurodegenerative and infectious diseases. *J Exp Med*. 2015;212:979–990. [PubMed: 26101267]
131. Hosseinpour-Moghaddam K, Caraglia M, Sahebkar A. Autophagy induction by trehalose: Molecular mechanisms and therapeutic impacts. *J Cell Physiol*. 2018;233:6524–6543. [PubMed: 29663416]
132. Shore ER, Awais M, Kershaw NM, et al. Small molecule inhibitors of Cyclophilin D to protect mitochondrial function as a potential treatment for acute pancreatitis. *J Med Chem*. 2016;59:2596–2611. [PubMed: 26950392]
133. Wen L, Voronina S, Javed MA, et al. Inhibitors of ORAI1 prevent cytosolic calcium-associated injury of human pancreatic acinar cells and acute pancreatitis in 3 mouse models. *Gastroenterology*. 2015;149:481–492.e7. [PubMed: 25917787]
134. Bustos V, Pulina MV, Bispo A, et al. Phosphorylated Presenilin 1 decreases beta-amyloid by facilitating autophagosome-lysosome fusion. *Proc Natl Acad Sci U S A*. 2017;114:7148–7153. [PubMed: 28533369]
135. Bustos V, Pulina MV, Kelahmetoglu Y, et al. Bidirectional regulation of Abeta levels by Presenilin 1. *Proc Natl Acad Sci U S A*. 2017;114:7142–7147. [PubMed: 28533411]
136. Habtezion A, Gukovskaya A, Pandol S. Acute pancreatitis: a multi-faceted set of organellar, cellular and organ interactions. *Gastroenterology*. 2019 1 17 pii: S0016–5085(19)30055–1. [Epub ahead of print].

137. Sakata K, Araki K, Nakano H, et al. Novel method to rescue a lethal phenotype through integration of target gene onto the X-chromosome. *Sci Rep.* 2016;6:37200. [PubMed: 27845447]
138. Sendler M, Weiss FU, Golchert J, et al. Cathepsin B-mediated activation of trypsinogen in endocytosing macrophages increases severity of pancreatitis in mice. *Gastroenterology.* 2018;154:704–718.e10. [PubMed: 29079517]
139. Su HY, Waldron RT, Gong R, et al. The Unfolded Protein Response plays a predominant homeostatic role in response to mitochondrial stress in pancreatic stellate cells. *PLoS One.* 2016;11:e0148999. [PubMed: 26849807]
140. Tokhtaeva E, Mareninova OA, Gukovskaya AS, et al. Analysis of N- and O-glycosylation of lysosomal glycoproteins. *Methods Mol Biol.* 2017;1594:35–42. [PubMed: 28456975]
141. Xue J, Sharma V, Hsieh MH, et al. Alternatively activated macrophages promote pancreatic fibrosis in chronic pancreatitis. *Nat Commun.* 2015;6:7158. [PubMed: 25981357]
142. Zhong Z, Sanchez-Lopez E, Karin M. Autophagy, inflammation, and immunity: A Troika governing cancer and its treatment. *Cell.* 2016;166:288–298. [PubMed: 27419869]
143. Zhong Z, Umemura A, Sanchez-Lopez E, et al. NF-kappaB restricts inflammasome activation via elimination of damaged mitochondria. *Cell.* 2016;164:896–910. [PubMed: 26919428]

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.