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New insights into the pathways initiating and driving pancreatitis

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Abstract

Purpose of review—In this article, we discuss recent studies that advance our understanding of molecular and cellular factors initiating and driving pancreatitis, with the emphasis on the role of acinar cell organelle disorders.

Recent findings—The central physiologic function of the pancreatic acinar cell – to synthesize, store, and secrete digestive enzymes – critically relies on coordinated actions of the endoplasmic reticulum (ER), the endolysosomal system, mitochondria, and autophagy. Recent studies begin to unravel the roles of these organelles' disordering in the mechanism of pancreatitis. Mice deficient in key autophagy mediators Atg5 or Atg7, or lysosome-associated membrane protein-2, exhibit dysregulation of multiple signaling and metabolic pathways in pancreatic acinar cells and develop spontaneous pancreatitis. Mitochondrial dysfunction caused by sustained opening of the permeability transition pore is shown to mediate pancreatitis in several clinically relevant experimental models, and its inhibition by pharmacologic or genetic means greatly reduces local and systemic pathologic responses. Experimental pancreatitis is also alleviated with inhibitors of ORAI1, a key component of the plasma membrane channel mediating pathologic rise in acinar cell cytosolic Ca2+. Pancreatitis-promoting mutations are increasingly associated with the ER stress. These findings suggest novel pathways and drug targets for pancreatitis treatment. In addition, the recent studies identify new mediators (e.g., neutrophil extracellular traps) of the inflammatory and other responses of pancreatitis.

Summary—The recent findings illuminate a critical role of organelles regulating the autophagic, endolysosomal, mitochondrial, and ER pathways in maintaining pancreatic acinar cell homeostasis and secretory function; provide compelling evidence that organelle disordering is a key pathogenic mechanism initiating and driving pancreatitis; and identify molecular and cellular factors that could be targeted to restore organellar functions and thus alleviate or treat pancreatitis.

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Conflicts of interest

There are no conflicts of interest.

Keywords

acute pancreatitis; autophagy; calcium; chronic pancreatitis; lysosomes; mitochondrial permeability transition pore

INTRODUCTION

Pancreatitis (acute, recurrent, and chronic) is a common disorder with significant morbidity and mortality. Its pathogenesis remains obscure, and no specific/effective treatment has been developed [1,2]. Here, we review recent studies that shed light on molecular and cellular factors initiating and driving pancreatitis, with particular emphasis on acinar cell organelle dysfunctions.

Historically, cellular organelles, such as mitochondria, endolysosomes, endoplasmic reticulum (ER), were regarded as isolated entities associated with distinct tasks, for example, ATP production or protein trafficking and degradation. This point of view has evolved toward a highly dynamic network of interacting compartments, which exchange signals and material to maintain and balance cellular homeostasis, metabolism, and survival. The central physiologic function of the pancreatic acinar cell – to synthesize, store, and secrete digestive enzymes – critically relies on coordinated actions of the ER, the endolysosomal system, mitochondria, and (as revealed more recently) autophagy. Previous studies indicated these pathways are perturbed in pancreatitis, but the role of organelle disordering, and the underlying mechanisms, remained largely unexplored until recently. Reports from the last year (Fig. 1) greatly advance our knowledge on molecular and cellular factors mediating the dysfunctions of key acinar cell organelles, and reveal their critical role in the pathogenesis of pancreatitis.

KEY POINTS

- The pancreatic acinar cell relies on coordinated actions of ER, the endolysosomal system, mitochondria, and autophagy to perform its secretory function.
- Disordering of these organelles, in particular those leading to autophagic/lysosomal and mitochondrial dysfunctions, is increasingly implicated in the pathogenic mechanism of pancreatitis, both acute and chronic.
- Recent studies reveal that genetic ablation of key mediators of autophagy or lysosomal function causes spontaneous pancreatitis in mice; whereas inhibition of the mitochondrial permeability transition or the pathologic Ca2+ influx greatly improves experimental pancreatitis.
- Identification of new molecular and cellular factors suggests therapeutic approaches aimed to restore organellar functions and thus treat or alleviate pancreatitis.

The recent studies also provide new mechanistic insights into the inflammatory and other responses of pancreatitis.

ORGANELLE DYSFUNCTION IN PANCREATITIS

Roles of autophagy and the endolysosomal system in pancreatitis

Accumulation of large vacuoles in acinar cells is a long-noted but poorly understood feature of both human and experimental pancreatitis. Recent studies [3,4,5■■] show that vacuolization is a result of impaired autophagic flux, which also mediates another signature response of pancreatitis, the increased intra-acinar trypsin activity. Autophagy (macroautophagy) is the principal cellular pathway for degradation and recycling of organelles, lipids, and long-lived proteins; it starts with sequestration of the material destined for degradation into autophagosomes, which then fuse with lysosomes forming the autolysosomes where cargo is degraded by lysosomal hydrolases such as cathepsins [4]. Studies published in 2015 [6■■,7■■] provide mechanistic insights into the role of autophagy in pancreas through detailed analyses of mice with pancreas-specific knockouts of key autophagy mediators, the autophagy-related proteins Atg5 [6■■] or Atg7 [7■■]. Blocking autophagosome formation by genetic ablation of either of these proteins triggered spontaneous pancreatitis, with fibrosis, inflammation, acinar-to-ductal metaplasia, and pancreas atrophy. Autophagy inhibition resulted in ER and oxidative stress, accumulation of dysfunctional mitochondria, disturbed metabolic pathways, and decreased protein synthesis.

Complementing the findings in Atg knockouts, lysosomal dysfunction is reported [5■■] to cause impaired autophagy and spontaneous pancreatitis. This study elucidates the role of lysosome-associated membrane proteins (LAMPs), which are critical for maintaining both the structure and function of lysosomes. The authors find dramatic decreases in pancreatic levels of LAMP-1 and LAMP-2 across various experimental models of nonalcoholic and alcoholic pancreatitis, because of LAMP degradation mediated by cathepsin B. LAMP-2 null mice gradually develop pancreatitis, starting with acinar cell vacuolization and progressing to severe pancreas damage characterized by trypsinogen activation, macrophage-driven inflammation, and acinar cell death. Moreover, amylase secretion, both basal and CCK-induced, is dysregulated in LAMP-2 deficient acinar cells [5■■].

Together, the recent findings [3,4,5■■–7■■,8–10] reveal the essential role of autophagic/lysosomal pathways in maintaining pancreatic acinar cell homeostasis and secretory function. Further, the development of spontaneous pancreatitis in Atg5, Atg7, and LAMP-2 knockouts indicates that defects in these pathways play a pathogenic role not only in acute pancreatitis but also chronic pancreatitis. Of note, human chronic pancreatitis has been associated with marked decreases in pancreatic LAMPs [5■■] and accumulation of the autophagy substrate p62/sequestosome-1 protein [9], suggesting novel molecular targets for pancreatitis treatment.

Further evidence on the important role of the endolysosomal system, and its derangement in pancreatitis, comes from a study [11■■] elucidating how the trafficking between early and late endosomes regulates secretion from rodent and human pancreatic acinar cells. In addition to the classic zymogen granule secretory pathway, acinar cells have a parallel,

'minor-regulated' pathway for zymogen secretion, which arises from immature secretory granules and trans-Golgi network, uses anterograde endosomal trafficking through early endosomes and recycling endosomes, and is regulated by the mediators of endosomal system, Rab5 and Rab11 (as well as Rab27A [12]). Previously, this group elucidated the role of vesicle associated membrane protein 8 in this pathway [13]. The present study [11■■] shows that high-dose CCK-8 and other toxins (ex-vivo pancreatitis model) inhibit early endosomes–late endosomes trafficking, resulting in decreased secretion, intra-acinar trypsin accumulation, and cellular damage, which were reversed with inhibition of the phosphoinositide kinase PIKfyve.

In addition to LAMP degradation and disturbed early endosomes–late endosomes trafficking, other manifestations of endolysosomal dysfunction have been noted in pancreatitis, such as aberrant processing and subcellular distribution of cathepsins [3,14], and the formation of abnormally large endocytic vacuoles [15]. However, elucidation of the mechanisms of acinar cell endolysosomal dysfunction, and how it leads to pancreatitis, is only starting.

Mitochondrial dysfunction in pancreatitis

Mitochondrial dysfunction is an early event in experimental pancreatitis; its main manifestation is the sustained opening of permeability transition pore (MPTP), a multiprotein nonspecific channel traversing both the inner and outer mitochondrial membranes [16]. In its 'open' conformation, MPTP allows unregulated entry of solutes less than 1500 Da (including water) into the matrix, resulting in mitochondrial depolarization. The MPTP backbone is organized around cyclophilin D; and inhibition of MPTP opening by genetic or pharmacologic knockdown of cyclophilin D is found [17■■] to abolish or greatly reduce all local (pancreatic), systemic, and distant (pulmonary) pathological responses in several clinically relevant experimental models of pancreatitis. The results suggest MPTP as a promising drug target for this disease.

However, the mechanisms that link mitochondrial dysfunction to disordering of other pathways and the 'classic' pancreatitis responses are poorly understood. The authors show [17■■] that persistent MPTP opening results in ATP depletion, a prerequisite for necrosis, and activation of phosphoglycerate mutase, an executioner of necrosis. Notably, almost 50 years ago Jamieson and Palade [18] found that mitochondrial inhibitors block post-ER trafficking and secretion of digestive enzymes, but the underlying mechanism has yet to be determined. The link between mitochondrial dysfunction and inflammation is also not clear; one possible mechanism is through excessive production of reactive oxygen species (ROS), which, in turn, may activate the key proinflammatory transcription factor nuclear factor kappa B (NF-κB). However, suppressing mitochondrial ROS with the specific mitochondrial antioxidant MitoQ did not ameliorate inflammation in cerulein-induced acute pancreatitis [19], suggesting an ROS-independent mechanism.

Endoplasmic reticulum stress and pancreatitis-promoting mutations

Sustained/pathologic ER stress is present in all experimental acute pancreatitis models [20], and was also shown [21] to play a key role in the mechanism of chronic pancreatitis in a

model where intra-acinar trypsinogen activation is prevented by genetic means. Furthermore, evidence indicates that hereditary pancreatitis caused by some mutations in human cationic trypsinogen (PRSS1), the physiologic trypsin inhibitor serine protease inhibitor Kazal-type 1 (SPINK1), and the trypsinogen-degrading enzyme chymotrypsinogen C (CTRC) are associated with protein misfolding resulting in the ER stress. For example, the PRSS1 mutation p.L104P (unique as it affects the substrate binding site) is found [22] to markedly reduce secretion of the mutated protein because of its retention and aggregation associated with ER stress. The results corroborate findings from this and other groups [23–25] on the role of ER stress responses in the mechanism of pancreatitis. Also of note are previous findings that alcohol abuse in animal models does not by itself lead to pancreatic disorder because the acinar cell uses adaptive/protective unfolded protein response to adjust to alcohol's effects [26,27]. The emerging findings suggest a common pathway involving ER stress for both mutational and environmental (alcohol) causes of pancreatitis [28].

As better methods, both molecular and epidemiologic, for evaluating genes–environment interactions develop, they will allow elucidation of key factors regulating these interplays in pancreatic diseases. An example of such an interplay is presented in a study [29] which used an extensive patients database to show that a common CTRC variant G60G acts as disease modifier promoting recurrent and chronic pancreatitis, especially in patients with pathogenic variants of cystic fibrosis conductance regulator (CFTR) or SPINK1, or smoking.

Interrelationships between acinar cell organellar dysfunctions

Recent studies are beginning to uncover interrelationships between disordering of lysosomal, autophagic, mitochondrial, and ER stress pathways in pancreatitis. For example, mitochondrial dysfunction mediates defective autophagy in cerulein-induced acute pancreatitis [17■■]; whereas defective autophagy promotes ER stress in a genetic model of pancreatitis triggered by inhibitor of kappa B kinase alpha genetic ablation [9]. The reports [6■■,7■■] provide direct evidence for the role of autophagy in maintaining normal unfolded protein response in acinar cells, indicating that ongoing autophagy is needed for continuous recycling of misfolded proteins, which spontaneously appear in the pancreatic acinar cell because of its very high protein synthesis rate; as well as for maintaining mitochondrial biogenesis, ATP production, and metabolism.

Intracellular calcium signaling and pancreatitis

Digestive enzyme secretion from the pancreatic acinar cell is mediated by oscillatory increases of cytosolic Ca2+ ([Ca2+]c) triggered by physiologic neurohumoral stimuli. The increases come from Ca2+ released from ER stores in response to the intracellular messenger inositol 1,4,5-trisphosphate (IP3), and are transient because the released Ca2+ is rapidly reuptaken into the stores [30–33]. In contrast, several acute pancreatitis triggers (bile salts, hyperstimulation with CCK-8 or cerulein, ethanol metabolites) cause massive and persistent Ca2+ release from ER stores resulting in their sustained Ca2+ depletion [34–36]. In this state, the acinar cell attempts to refill ER stores by Ca2+ entry through a plasma membrane store operated Ca2+ entry (SOCE) channel [37], with the Orai1 protein being a key component of the channel [38–40]. The SOCE channel activation causes sustained

[Ca2+]c increase, known to promote multiple pathologic events of pancreatitis [34–36,40–42].

Two recent reports [43■■,44] demonstrate approaches to inhibit the pathologic SOCE channel activation and thus attenuate experimental pancreatitis. One study [43■■] showed that two Orai1in-hibitors prevented the increase in [Ca2+]c and necrosis in mouse and human acinar cells caused by agents that prevent refilling of the ER Ca2+ stores. Furthermore, the Orai1 inhibitors attenuated acute pancreatitis responses in three dissimilar in-vivo models when given 1 h after the induction of pancreatitis, a clinically relevant design. Another study [44] used caffeine, a known inhibitor of IP3 receptor-mediated Ca2+ release from ER [45], to prevent sustained rises in [Ca2+]c and necrosis. Systemic administration of caffeine ameliorated pancreatitis responses in three experimental acute pancreatitis models. These results demonstrate that treatments that prevent the acinar cell toxicity because of pathologic rise of [Ca2+]c should be considered for acute pancreatitis treatment.

Another interesting report [46■], highlighting the role of Ca2+, showed that sweat chloride concentrations (a diagnostic test for cystic fibrosis) were increased in patients who acutely abused alcohol but not in healthy volunteers, indicating a cystic fibrosis type response to ethanol. The authors find that CFTR levels are lower in pancreata from acute pancreatitis and chronic pancreatitis patients compared with healthy persons; alcohol and fatty acids inhibit fluid and bicarbonate secretion and CFTR activity in pancreatic ductal cells; and these effects are mediated by sustained increases in [Ca2+]c, mitochondrial depolarization, and cellular ATP depletion. These events occur in the ductal cell illustrating that we have to consider the interplay between acinar and ductal cell responses in pancreatic diseases [47].

Acute pancreatitis is the most common iatrogenic complication of endoscopic retrograde cholangiopancreatography (ERCP), a widely used procedure that applies radiocontrast to visualize the pancreaticobiliary tree. A radiocontrast agent induced the pathologic [Ca2+]c response in mouse and human acinar cells [48■] leading to activation of the Ca2+-dependent phosphatase calcineurin and, in turn, NF-κB. Genetic or pharmacologic inhibition of calcineurin prevented NF-κB activation, inflammation, and acinar cell injury caused by the radio-contrast agent. The authors [48■] suggest using calcineurin inhibitors to prevent post-ERCP pancreatitis in patients.

Ca2+ and organellar damage in pancreatitis

Pathologic changes in [Ca2+]c are also found [49] to mediate the disordering of endosomal system in mouse acinar cells treated with cerulein or bile salt, as the Orai1 inhibitor GSK-7975A suppressed both SOCE and the formation of abnormally large endocytic vacuoles. The results expand the previous findings from the same group [15] identifying endocytic vacuoles as one site of intra-acinar trypsinogen activation. Persistent increases in [Ca2+]c also cause mitochondrial Ca2+ overload resulting in MPTP opening [17■■]. Thus, Ca2+ mediates both endosomal and mitochondrial dysfunctions in models of pancreatitis associated with pathologic increases in [Ca2+]c.

OTHER MOLECULAR/CELLULAR FACTORS IN PANCREATITIS

Inflammatory mediators

Several recent reports [50–53,54■,55,56■] establish new pathways and mediators of inflammation in pancreatitis. One study [50] finds a protective role for B-cell lymphoma 3-encoded protein (BCL3), a transcription factor that controls NF-κB activity, in the sterile inflammatory response of acute pancreatitis. Inflamed pancreatic tissues from acute pancreatitis patients display higher levels of BCL3 (as well as of activated NF-κB); pancreatic BCL3 is also increased in mouse models of acute pancreatitis induced by cerulein or bile salt; and BCL3 genetic ablation causes prolonged NF-κB activation and more severe inflammation and pancreas damage in these models. The authors posit that BCL3 upregulation reduces degradation of p50 homodimers known to inhibit NF-κB activation.

It is well established that neutrophils mediate the severity of experimental pancreatitis, but the underlying mechanisms are poorly understood [10]. Neutrophil extracellular traps (NETs) could be one such key mechanism [54■,55,56■]. NETs are extra-cellular structures composed of chromatin fibers coated with histones and granule proteins, produced by neutrophils to combat microbes during infection; NETs are also implicated in sterile inflammation [57]. The authors show [54■] that NETs are formed in experimental acute pancreatitis, and patients with severe acute pancreatitis have elevated blood levels of NET components. Administration of DNase I (to deplete NETs) reduced neutrophil infiltration and pancreas and lung damage. Interestingly, the addition of NETs and histones to acinar cells caused trypsinogen activation [54■], providing a possible mechanism for the surprising finding from years ago [58] that infiltrating neutrophils mediate intra-acinar trypsinogen activation. Another study [56■] showed that NETs aggregate and occlude pancreatic ducts in humans and mice, thus driving pancreatic inflammation. The effect is abrogated in mice deficient in a key enzyme mediating the extrusion of decondensed neutrophil chromatin. Moreover, pancreatic juice is found to instigate neutrophil chromatin extrusion [56■].

A recent review [53] summarizes the data on the role of peroxisome proliferator-activated receptors (nuclear receptors that control many inflammatory and metabolic pathways) in acute pancreatitis.

These studies provide new mechanistic insights into the inflammatory response of pancreatitis [10,52].

Triglycerides and pancreatic lipases

A series of reports are bringing attention to the role of serum triglycerides and pancreatic lipases in the mechanism of pancreatitis. Severe hypertriglyceridemia is a known cause for pancreatitis; but analysis of a cohort of prospectively enrolled acute pancreatitis patients [59] finds that even mild or moderate increases in serum triglycerides independently and proportionally correlate with aggravated course and persistent organ failure in pancreatitis regardless of cause. These findings are complemented by reports addressing the mechanisms of pancreatitis associated with pancreatic lipases, triglyceride hydrolysis, and necrosis [60,61■,62]. The data show exacerbation of pancreatitis by unsaturated fatty acids generated by pancreatic lipase-mediated visceral fat lipolysis. Most interesting is the finding that

obesity converted a mouse model of mild acute pancreatitis to severe pancreatitis, with peripancreatic fat necrosis and multisystem organ failure, and that inhibition of pancreatic lipase activity ameliorated these effects.

Simvastatin and pancreatitis

A retrospective analysis of nearly 4 million patients enrolled in Southern California Kaiser Permanente aimed to determine the effect of simvastatin use on the incidence of acute pancreatitis [63■]. In a multivariate analysis, simvastatin was independently associated with reduced risk of pancreatitis, after adjusting for age, sex, race/ethnicity, gallstone disorders, and other factors. It will be important to elucidate the mechanism of this effect, and how it is related to pathways discussed above.

CONCLUSION

The studies discussed in this review (Fig. 1) greatly advance our understanding of the critical role of organelles regulating the autophagic, endolysosomal, mitochondrial, and ER pathways in maintaining pancreatic acinar cell homeostasis and secretory function, and provide compelling evidence that organelle disordering is a key pathogenic mechanism initiating and driving pancreatitis. New mechanistic insights have been also gained on the inflammatory and other responses of pancreatitis.

Despite the recent progress, detailed analysis of these pathways in pancreas has just started, and there is much to be learned. Important questions (and future research directions) are about the interrelationships between the organelles' dysfunctions; how exactly they lead to downstream 'classic' pancreatitis responses such as inflammation and cell death; and, conversely, how the inflammatory and other responses affect acinar cell organellar damage.

The recent findings not only help elucidate the pathogenic mechanism of pancreatitis but also identify molecular and cellular factors that could be targeted to restore organellar functions and thus treat or alleviate pancreatitis.

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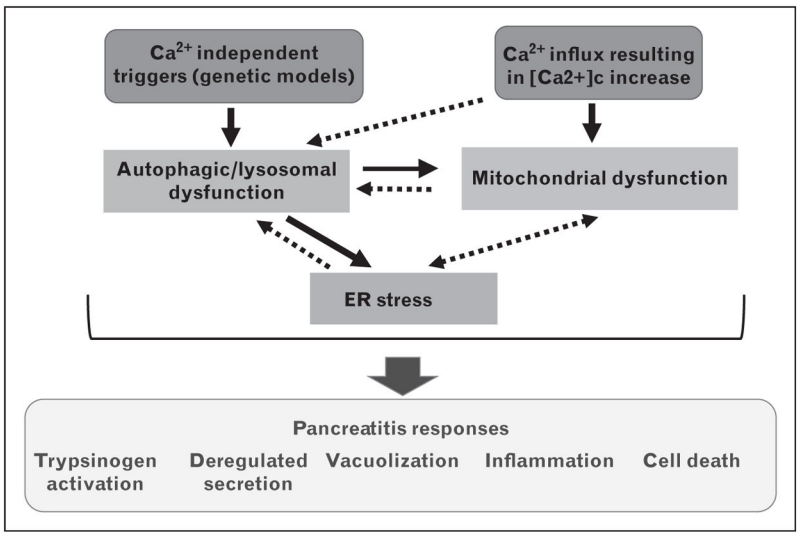

FIGURE 1.

Dysfunction of acinar cell organellar machinery initiates/drives pancreatitis. Calcium-dependent and independent pathways within the acinar cell can initiate pancreatitis by triggering defective autophagy as well as mitochondrial dysfunction. Autophagy and mitochondrial function are also linked, with defects in one pathway affecting the other. Solid lines indicate pathways operating in pancreas, based on the published results. Dashed lines indicate pathways that are likely to be involved, but not yet proven in the pancreas.