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Autophagy and Pancreas Disease

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Key Words
Autophagy · Acute pancreatitis · Cell death

Abstract
Autophagy is an evolutionarily preserved degradation process of cytoplasm cellular constituents which has been known for its roles in protecting cells against stresses such as starvation and in eliminating defective cellular constituents including subcellular structures. It is essentially a form of self-cannibalism, hence the name that means 'self-eating' in which the cell breaks down its own components. By mostly morphological studies, autophagy has been linked to a variety of pathological processes such as neurodegenerative diseases and tumorigenesis, which highlights its biological and medical importance. However, whether autophagy protects from or causes disease is unclear. Autophagic morphology was described in human pancreatitis by Helin et al. in 1980. Actually, acute pancreatitis is one of the earlier pathological processes where autophagy has been described in a human tissue. Autophagy, autodigestion and cell death are early cellular events in acute pancreatitis. The aim of this review is to introduce a description of the autophagic process and to discuss the possible role of autophagy in acute pancreatitis.
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The Autophagic Process

Autophagy is the cell's major regulated mechanism for degrading long-lived proteins and the only known pathway for degrading organelles. During autophagy, an isolation membrane forms as a pre-autophagosomal structure (phagophore), invaginates and sequesters cytoplasm constituents including mitochondria, endoplasmic reticulum and ribosomes. The edges of the membrane fuse to form a double structure, known as the autophagosome or autophagic vesicle. The outer membrane of the autophagosome fuses with the lysosome to deliver the inner membranous vesicle to the lumen of the degradative compartment (fig. 1). Degradation of the sequestered material generates nucleotides, amino acids, and free fatty acids that are recycled for macromolecular synthesis and ATP generation. Autophagy occurs at basal levels in several tissues and contributes to the routine turnover of cytoplasm components. Also, autophagy is involved in development, differentiation and tissue remodeling. In addition, autophagy is rapidly up-regulated during starvation or grow-factors withdrawal [reviewed in 1–4].

Autophagy is mediated by a set of evolutionarily conserved gene products (termed the Atg proteins) originally discovered in yeast. Beclin1 and LC3 are well-known mammalian autophagy-related proteins participating in autophagosome formation [1, 2]. Studies on the yeast Atg components have provided some insight into the molecular basis for the autophagic process; however, there are many questions that remain to be addressed on autopha-

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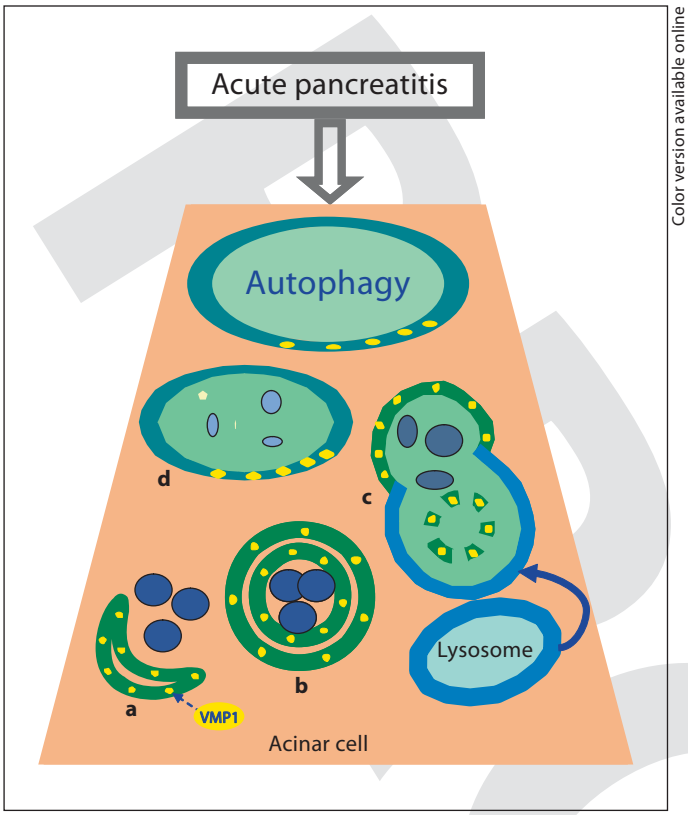

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Fig. 1. Autophagic process. Pancreatitis induces autophagy in acinar cell (**a**); cytosolic material is sequestered by an expanding membrane sac (cup-shaped structure), the phagophore, **b** resulting in the formation of a double-membrane vesicle, the autophagosome; **c** the outer membrane of the autophagosome subsequently fuses with a lysosome, exposing the inner single membrane of the autophagosome to lysosomal hydrolases; **d** the cargo-containing membrane compartment is then lysed, and the contents are degraded.

gy mechanism in mammalian cells [4]. The pancreatitis-associated protein named vacuole membrane protein 1 (VMP1) is a trans-membrane protein with no known homologues in yeast. VMP1 transcript is highly activated in acinar cells early during experimental pancreatitis and its expression correlates with acinar cell vacuole formation [5, 6]. VMP1 is a novel Atg protein that triggers autophagosome formation and remains as an integrated autophagosomal membrane protein in mammalian cells [unpubl. data]. Therefore, VMP1 expression is an early molecular event in acute pancreatitis whose expression induces autophagy and may be involved in the cellular response to disease.

Autophagic Cell Death, Another Way to Die

Classically, cell death is divided into two major types – necrosis and apoptosis. Necrosis has been traditionally thought to be a passive form of cell death, being the end point of a bioenergetic catastrophe resulting from ATP depletion to a level incompatible with cell survival [3]. Apoptosis or programmed cell death (PCD) is a cell-intrinsic mechanism for suicide that is regulated by a variety of cellular signaling pathways. Over the past few years the idea that cells can commit suicide by mechanisms other than apoptosis has been gaining momentum. The visualization of autophagosomes in dying cells has led to consider that autophagy is a non-apoptotic form of PCD. Now, the term 'autophagic cell death' describes a form of PCD morphologically distinct from apoptosis [2]. In classical apoptosis, or type I PCD, there is early collapse of cytoskeleton elements but preservation of organelles until late in the process. In contrast, in autophagic or type II PCD, there is early degradation of organelles but preservation of cytoskeleton elements until late stages. Whereas apoptotic cell death is caspase-dependent and characterized by internucleosomal DNA cleavage, in autophagic cell death caspase activation and DNA fragmentation occur very late. Another feature that distinguishes apoptosis from autophagy is the source of lysosomal enzymes used for dying cells' degradation. Apoptosis uses phagocytic cell lysosomes, whereas autophagy uses cell endogenous lysosomal machinery. In contrast with necrosis, both apoptotic and autophagic cell death processes develop without plasma membrane breakdown and characterize by the lack of tissue inflammatory response [3]. Autophagic cell death seems to occur primarily when developmental program or homeostatic processes require massive cell elimination [1]. For most of the disease-associated cell deaths that are presumed to be autophagic, the evidence of its role is only correlative [2]. Morphological studies cannot prove a causative relationship between the autophagic process and cell death. One question that arises is whether autophagic activity in dying cells, during a pathological process, is the cause of cell death or is actually an attempt to prevent it.

Autophagy and Pancreatitis: Self-Eating, Self-Digestion or Self-Killing?

The presence of autophagy has been described in dying acinar cells from human and experimental pancreatitis. Helin et al. [7] described the ultrastructural altera-

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tions in pancreatic acini from 6 patients operated for acute necrotizing pancreatitis. They studied by electronic microscopy those areas of pancreatic parenchyma that show edematous inflammation under light microscopy. Their findings in acinar cells included changes in zymogen granules and an increased autophagocytosis indicated by several autophagic vesicles. Cell death is a key factor in pancreas disease. In experimental models of acute pancreatitis, acinar cells have been shown to die through both necrosis and apoptosis. The severity of pancreatitis directly correlates with the extent of necrosis and inversely with that of apoptosis [8]. New insights into the autophagic process and its participation in PCD would lead us to think that autophagy could be an alternative mechanism of acinar cell self-killing during acute pancreatitis. Autophagic morphological features were described in caerulein-induced pancreatitis in 1984 by Watanabe et al. [■■] and in 1985 by Adler et al. [9]. These works were followed by a series of well-known studies that allow us to learn the early cellular events during acute pancreatitis and led to the conclusion that the initiation of the disease occurs within the acinar cell. Autophagy, autodigestion and cell death are early cellular events in the pathophysiology of acute pancreatitis [8]. At the acinar cell level, autophagy could be an execution mechanism or a survival strategy which the cell switches on in response to disease (fig. 2).

Autophagic features, alterations in zymogen granules and zymogen activation (self-digestion) were linked to intracytoplasm vacuole formation. Although lysosomal enzymes are considered to be involved in zymogen activation, the exact mechanism responsible for the activation remains the subject of much research effort and not a little debate. Autophagy, as a self-eating mechanism, is the only cellular pathway able to degrade organelles. It is tempting to think that autophagy could be involved in pancreas self-digestion. The activation of zymogen could be a result of zymogen granule lysosomal degradation by an autophagic process. In fact, one of the first proposed activation mechanisms was crinophagy, which was defined as the discharge of zymogen granules into lysosomes [8]. Although both pathways involve endogenous lysosomal enzymes, we do not know with certainty whether crinophagy and autophagy are morphological evidence of the same mechanism. The development of various transgenic and knockout mouse strains have delivered interesting results that we will briefly analyze in order to have an initial approach in the discussion of the role of autophagy in acute pancreatitis.

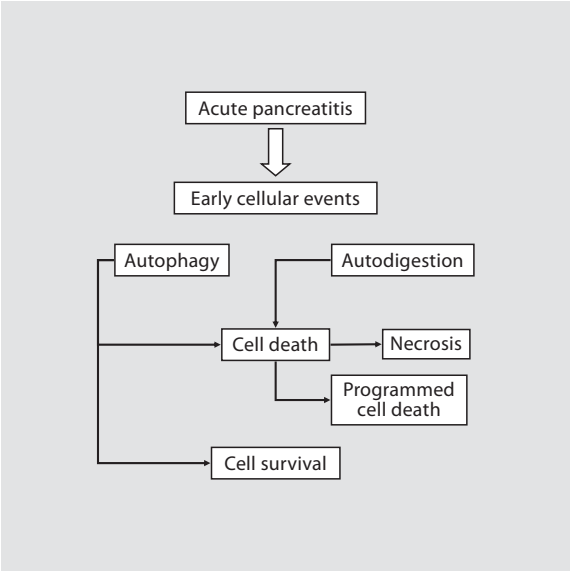

Fig. 2. Autophagy, autodigestion and cell death are early cellular events in acute pancreatitis. While autodigestion leads to cell death, autophagy would result in PCD or cell survival.

GFP-LC3-Transgenic Mouse [10]. In GFP-LC3 mice the expression and localization of the autophagosome-associated protein LC3 can be monitored by the detection of the green fluorescence protein. The in vivo analysis of autophagy in response to nutrient starvation in these mice revealed the massive induction of autophagy in pancreas tissue, which correlates with the loss of zymogen granules. However, the autophagic pancreas of starved mice did not present any other morphological alteration resembling pancreatitis. These observations suggest that autophagy would not be a pathogenic event in acute pancreatitis and let us speculate that autophagy does not lead to autodigestion.

CTSB$^{-/-}$Mouse [11]. The lysosomal cysteine proteinase cathepsin B (CTSB) was considered to play an essential role in the intracellular activation of trypsinogen. Caerulein-induced acute pancreatitis in CTSB$^{-/-}$ mice showed a significant reduction in the premature intrapancreatic zymogen activation and necrotic cell death. But neither the quality nor the frequency of autophagic features differed in the course of pancreatitis between CTSB$^{-/-}$ and CTSB$^{+/+}$ animals. Thus, the lack of CTSB does not affect the autophagic process of experimental pancreatitis. Instead, it prevents necrotic cell death. We can conclude that premature zymogen activation would lead to cell death, but it may not lead to autophagy.

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SPINK3$^{-/-}$ Mouse [12]. Intracellular trypsin activity is predominantly controlled by serine protease inhibitor Kazal type 1 (SPINK1), which is also known as pancreatic secretory trypsin inhibitor. SPINK1 is synthesized in pancreas acinar cells and binds to trypsin to prevent further activation of pancreatic enzymes. Surprisingly, trypsin was not significantly activated in SPINK3$^{-/-}$ mice. Instead, rapid cell death occurred in the pancreas and duodenum within a few days after birth leading to mouse death around 15 days after birth. By morphological and molecular studies authors showed that acinar cells, which lack SPINK activity, developed extensive autophagic process. Therefore, the lack of SPINK expression promotes autophagic cell death without previous zymogen activation. One possible explanation of the onset of autophagy but not autodigestion in pancreas of SPINK3$^{-/-}$ mice comes from the structural similarities between SPINK and epidermal grow factor. During pancreas development, SPINK may function as a growth factor for acinar cells, and the lack of growth factor may trigger the autophagic process. These results indicate that autophagy may be related to cell death during pancreas development, but once more, autophagy was not related to pancreas autodigestion.

Conclusions

Results from genetically modified models summarized above support the idea that self-digestion and self-eating are independent processes, since the induction of autophagy did not induce autodigestion, and the inhibition of autodigestion did not inhibit autophagy. Moreover, while inhibition of cathepsin B inhibited necrotic cell death but not autophagy, starvation-induced autophagy neither promoted acute pancreatitis nor triggered cell death. These observations suggest that although autophagic features are present as early as 30 min after pancreatitis induction, autophagy might not trigger the pathological process. Interestingly, the lack of SPINK, which surprisingly promotes massive cell death during development, did not trigger autodigestion. Nevertheless, although autophagy was described in human pancreatitis 30 years ago, the role of autophagy in pancreatitis has not been elucidated. Studies focusing on the autophagy-related molecular mechanisms within the acinar cell would be a strategy to describe the actual role of the autophagic process during acute pancreatitis.

Acknowledgments

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