Gastroenterology 2015;148:626–638

BASIC AND TRANSLATIONAL—PANCREAS

Impaired Autophagy Induces Chronic Atrophic Pancreatitis in Mice via Sex- and Nutrition-Dependent Processes

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BACKGROUND & AIMS: Little is known about the mechanisms of the progressive tissue destruction, inflammation, and fibrosis that occur during development of chronic pancreatitis. Autophagy is involved in multiple degenerative and inflammatory diseases, including pancreatitis, and requires the protein autophagy related 5 (ATG5). We created mice with defects in autophagy to determine its role in pancreatitis. **METHODS:** We created mice with pancreas-specific disruption of *Atg5* (*Ptf1a-Creex1;Atg5F/F* mice) and compared them to control mice. Pancreata were collected and histology, immunohistochemistry, transcriptome, and metabolome analyses were performed. ATG5-deficient mice were placed on diets containing 25% palm oil and compared with those on a standard diet. Another set of mice received the antioxidant N-acetylcysteine. Pancreatic tissues were collected from 8 patients with chronic pancreatitis (CP) and compared with pancreata from ATG5-deficient mice. **RESULTS:** Mice with pancreas-specific disruption of *Atg5* developed atrophic CP, independent of β-cell function; a greater proportion of male mice developed CP than female mice. Pancreata from ATG5-deficient mice had signs of inflammation, necrosis, acinar-to-ductal metaplasia, and acinar-cell hypertrophy; this led to tissue atrophy and degeneration. Based on transcriptome and metabolome analyses, ATG5-deficient mice produced higher levels of reactive oxygen species than control mice, and had insufficient activation of glutamate-dependent metabolism. Pancreata from these mice had reduced autophagy, increased levels of p62, and increases in endoplasmic reticulum stress and mitochondrial damage, compared with tissues from control mice; p62 signaling to Nqo1 and p53 was also activated. Dietary antioxidants, especially in combination with palm oil–derived fatty acids, blocked progression to CP and pancreatic acinar atrophy. Tissues from patients with CP had many histologic similarities to those from ATG5-deficient mice. **CONCLUSIONS:** Mice with pancreas-specific disruption of Atg5 develop a form of CP similar to that of humans. CP development appears to involve defects in autophagy, glutamate-dependent metabolism, and increased production of reactive oxygen species. These mice might be used to identify therapeutic targets for CP.

Keywords: Pathogenesis; Autophagosome; Signal Transduction; Lipidation.

Chronic pancreatitis (CP) has a prevalence of about 50/100,000 individuals.[1] Recurrent acute pancreatitis (AP) may lead to CP, but CP may also occur unrelated to AP.[1] In addition, CP is reported to increase the risk of pancreatic cancer.[1] Common risk factors for CP are alcohol, smoking, and male sex.[1] Histopathologically, CP is characterized by progressive inflammation, fibrosis, and necrosis, leading to acinar as well as endocrine cell atrophy.[2]

Chronic inflammatory diseases are associated with many metabolic changes. On the cellular level, proinflammatory cytokines, for example, tumor necrosis factor$-\alpha$ and interleukin 6, have been shown to stimulate multiple metabolic pathways, including gluconeogenesis/glycolysis, proteolysis, and lipolysis, in order to satisfy increased energetic

Abbreviations used in this paper: **AP, acute pancreatitis; CL, cardiolipin; CP, chronic pancreatitis; ER, endoplasmic reticulum; GLDH, glutamate dehydrogenase; GSSG, glutathione disulfide; POD, palm oil diet; ROS, reactive oxygen species.**

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2014.12.003

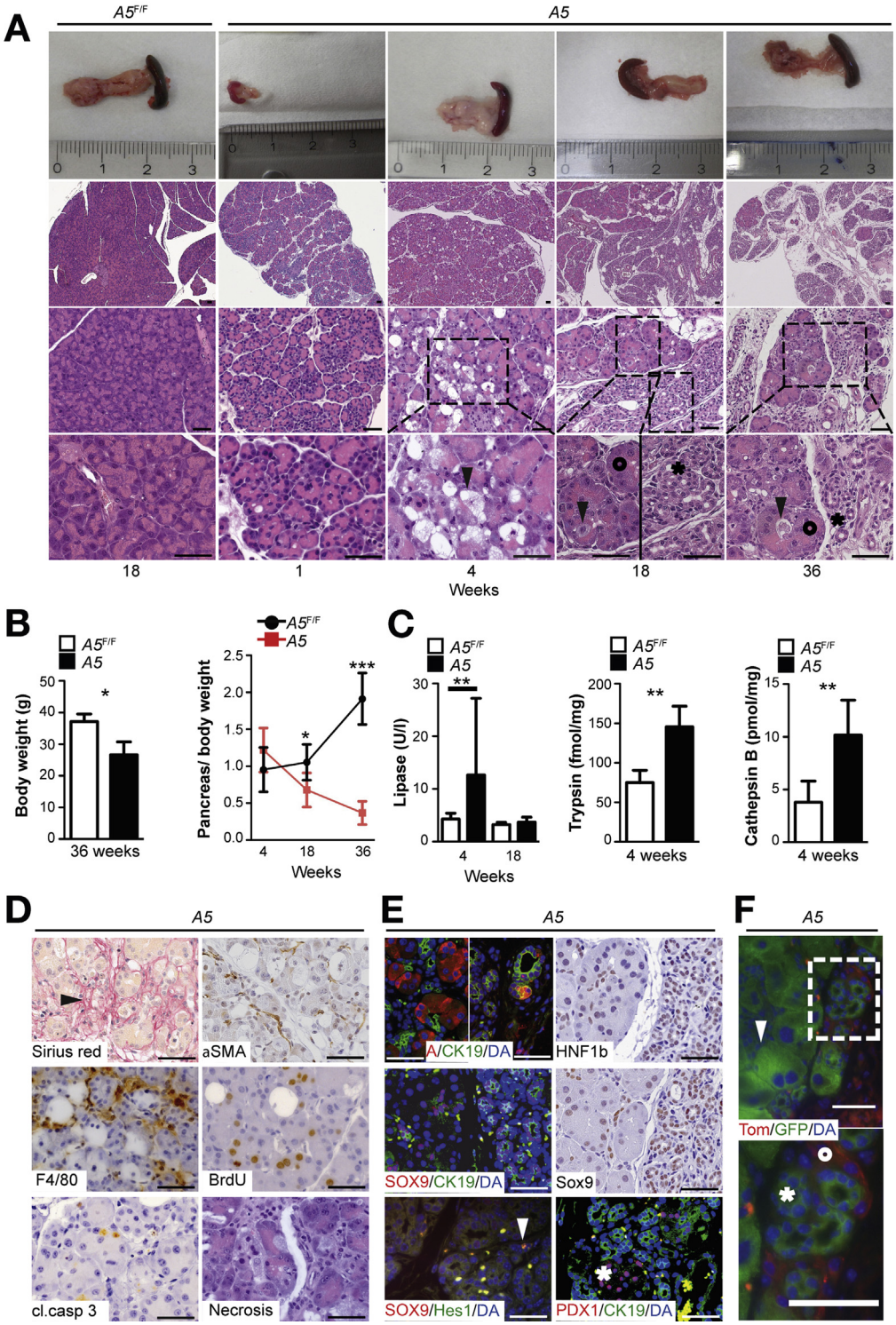

Figure 1. Pancreas-specific deletion of *Atg5* induces pancreatic changes reminiscent of CP. (*A*) Morphology of pancreata from *Atg5* deficient (*A5*) compared with control mice ($A5^{F/F}$); *arrowhead*, *asterisk*, and *circle* indicate vacuolization, duct-like structures, and hypertrophic acinar cells, respectively. (*B*) Body weight and pancreas/body weight of male $A5^{F/F}$ and *A5* mice ($n \geq 3$). (*C*) Serum lipase activities in *A5* compared with $A5^{F/F}$ mice at 4 ($n = 10$ per group) and 18 ($n = 8$ *A5*, $n = 3$ $A5^{F/F}$) weeks of age; amounts of activated trypsin and cathepsin B in the pancreas of *A5* mice as compared with controls ($n \geq 6$). $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. (*D*) Analyses for fibrosis (sirius red, *black arrowhead*), pancreatic stellate cell activation (α-smooth muscle actin [αSMA]), proliferation (bromodeoxyuridine [BrdU]), inflammation (F4/80), apoptosis (cleaved caspase 3 [cl. casp 3]), and necrosis (H&E) in *A5* pancreas. (*E*) Immunofluorescence and immunohistochemical staining of pancreata from *A5* mice (A, amylase); *white arrowhead* and *asterisk* indicate SOX9/Hes1-positive cell and PDX1-positive/CK19-negative islet, respectively; nuclei are stained with 4′,6-diamidino-2-phenylindole (DA). (*F*) Lineage tracing in Tomato (Tom)-green fluorescent protein (GFP)–expressing A5 mice; white arrowhead, asterisk and circle indicate GFP-positive acinar cell, GFP-positive duct-like cells and Tom-positive stromal cells; *box* in the *top* is magnified in the *bottom frame*; nuclei are stained with DA. *Scale bars* = 50 μm.