Organellar Dysfunction in Pancreatitis

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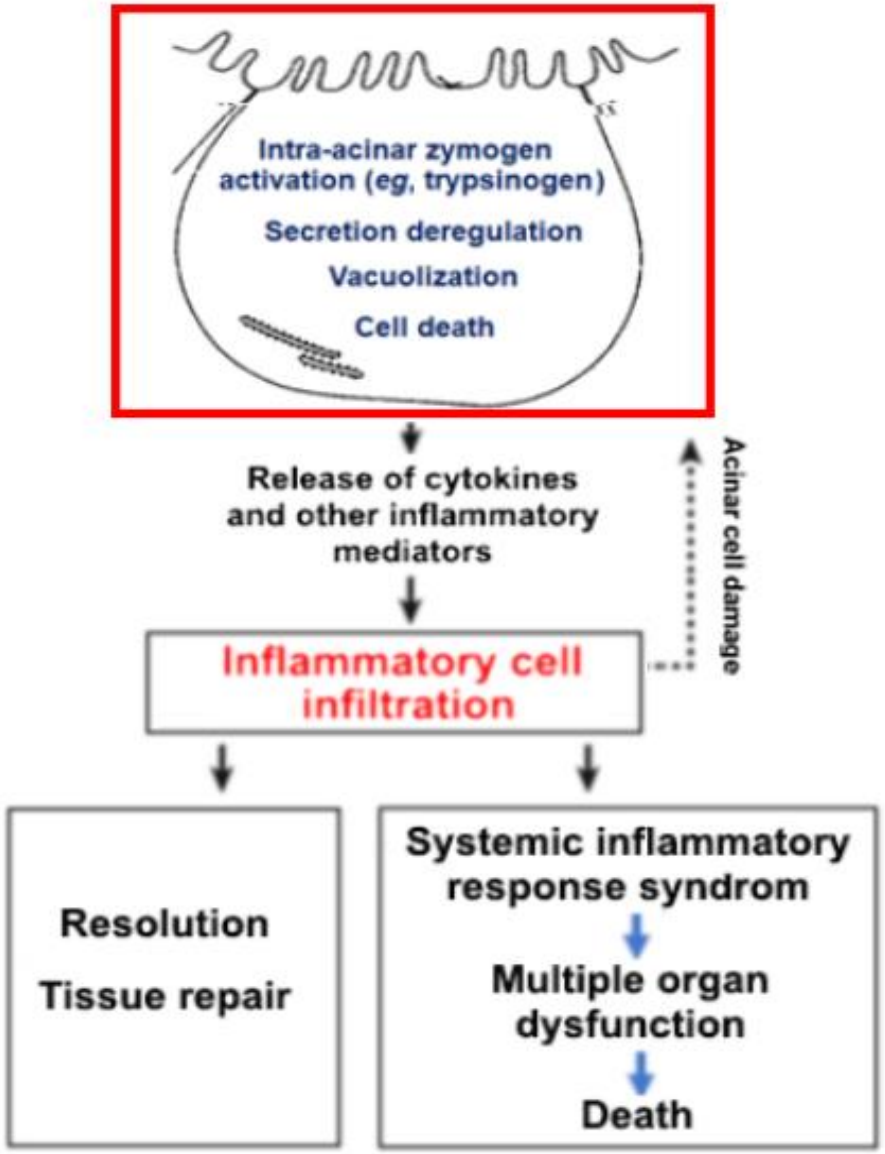
PancreasFest 2019

Pittsburgh July 26, 2019

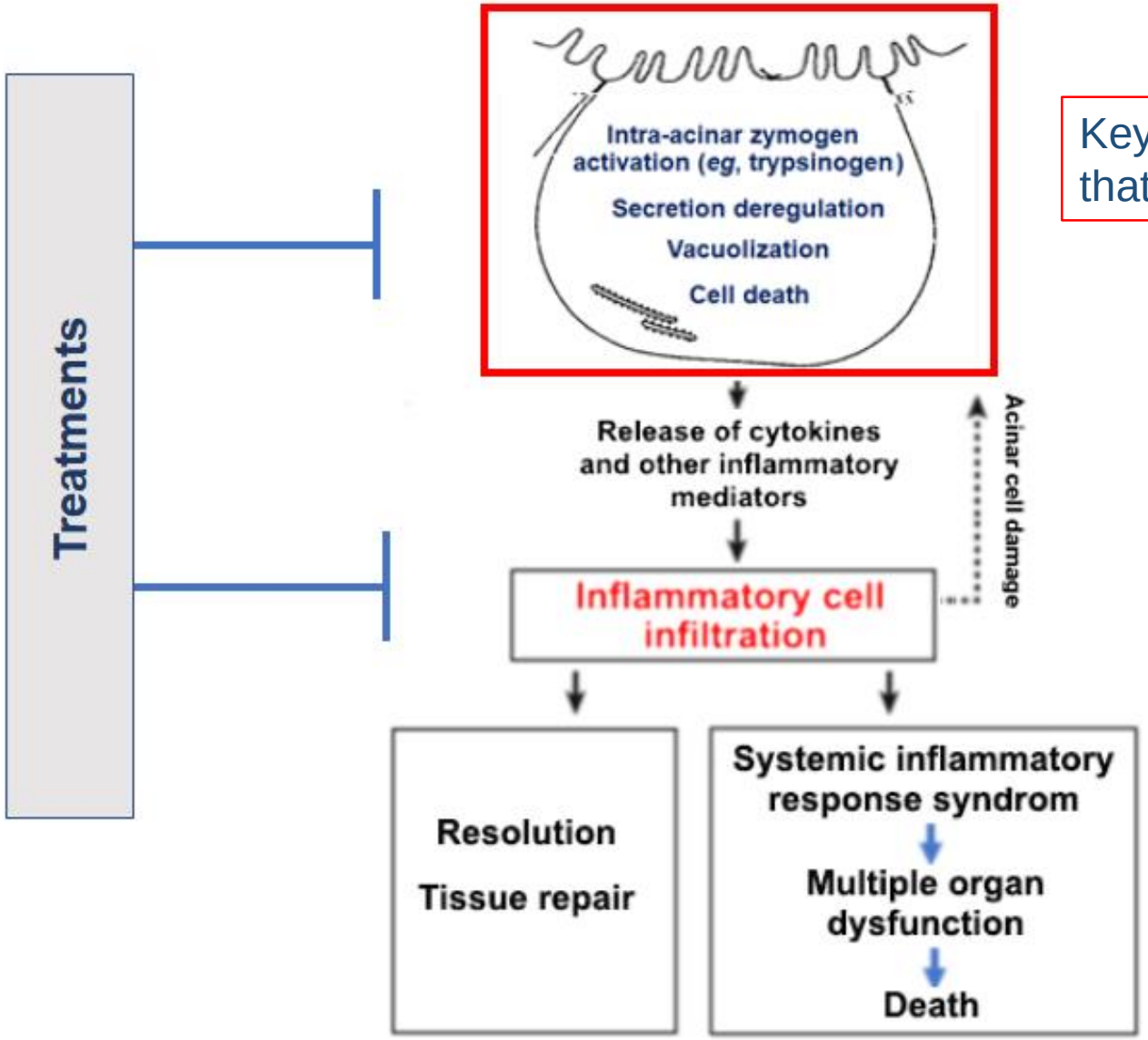
AP is initiated by injured acinar cells, leading to inflammation and necrosis

Key intra-acinar pathologies that initiate pancreatitis



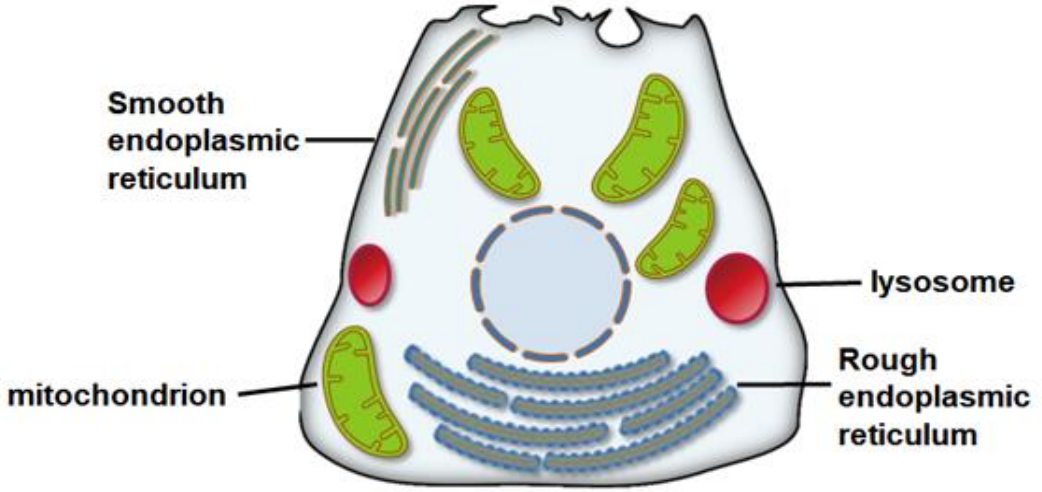
- One cause of non-resolving inflammation in AP could be unremitting acinar cell injury, which perpetuates the inflammatory response
- Pathogenic mechanisms of acinar cell injury that initiate AP remain poorly understood
- One reason is that the molecular mechanisms maintaining acinar cell homeostasis were largely unknown until recently

AP is initiated by injured acinar cells, leading to inflammation and necrosis



Key intra-acinar pathologies that initiate pancreatitis

Current understanding of functions of cellular organelles



ER

- Protein synthesis
- Protein folding
- Post-translational protein modification
- Protein degradation
- Ca^{2+} homeostasis
- Lipid synthesis
- Autophagy

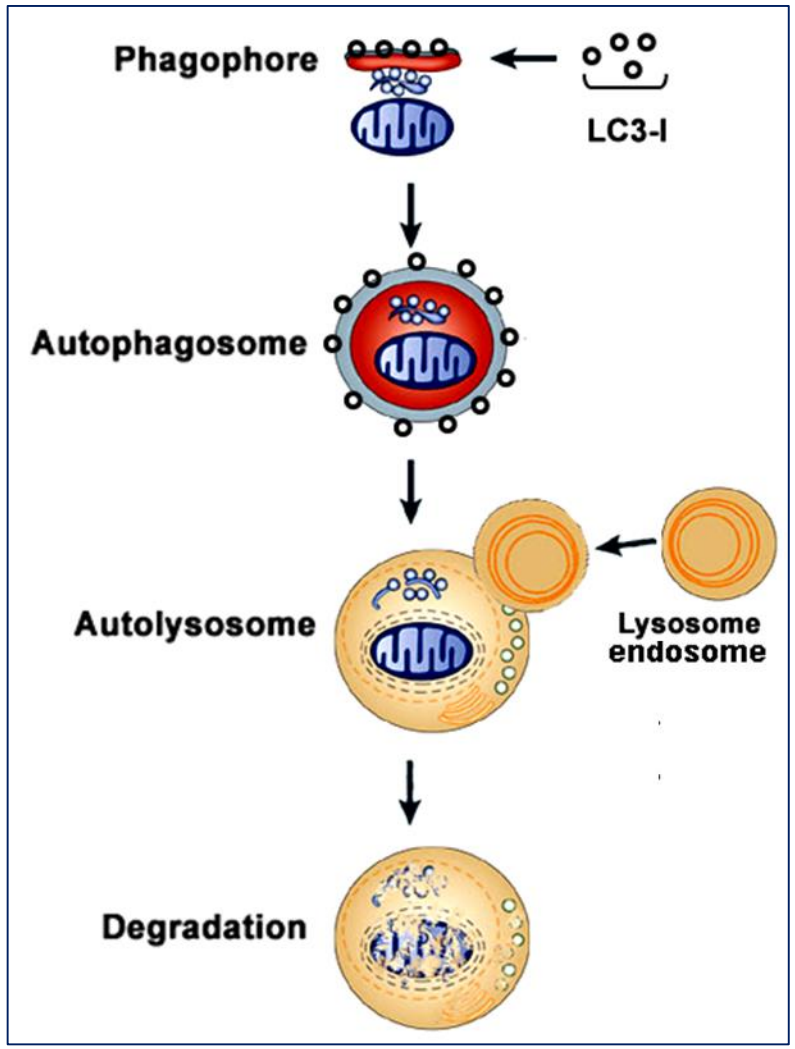
Mitochondria

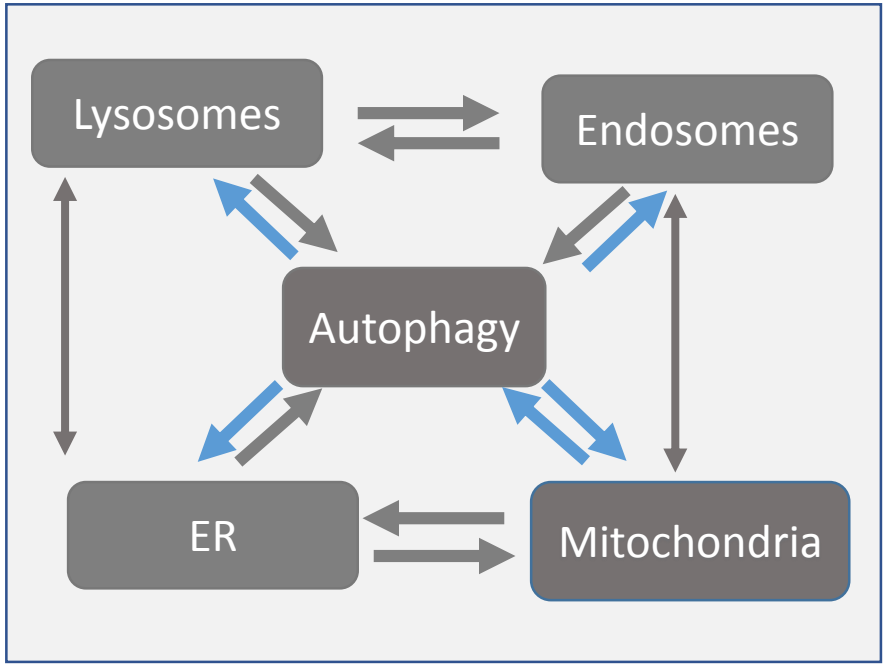
- Energy production-ATP
- Survival
- Metabolism
- Ca^{2+} homeostasis
- Reactive oxygen species production
- Autophagy

Lysosomes Endosomes

- Nutrient sensing
- Protein trafficking
- Protein degradation
- Lipid degradation
- Endocytosis
- Exocytosis
- Autophagy

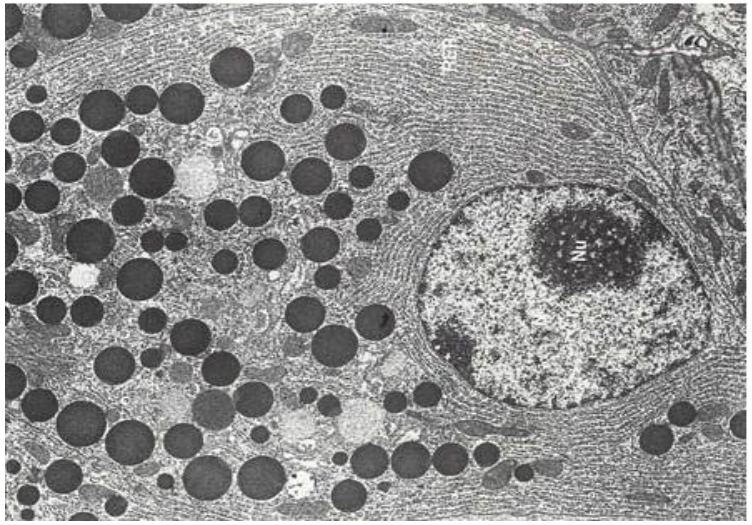
Autophagy degrades and recycles damaged, dysfunctional or unneeded organelles and thus controls cellular homeostasis

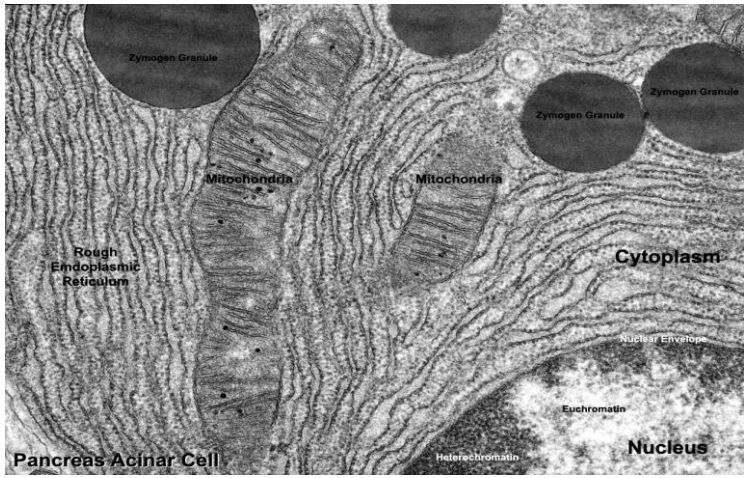




Acinar cell secretory function relies on coordinated action of cytoplasmic organelles: the endoplasmic reticulum, Golgi, mitochondria, and autophagy

Pancreas





Mitochondria

Endoplasmic reticulum

Golgi apparatus

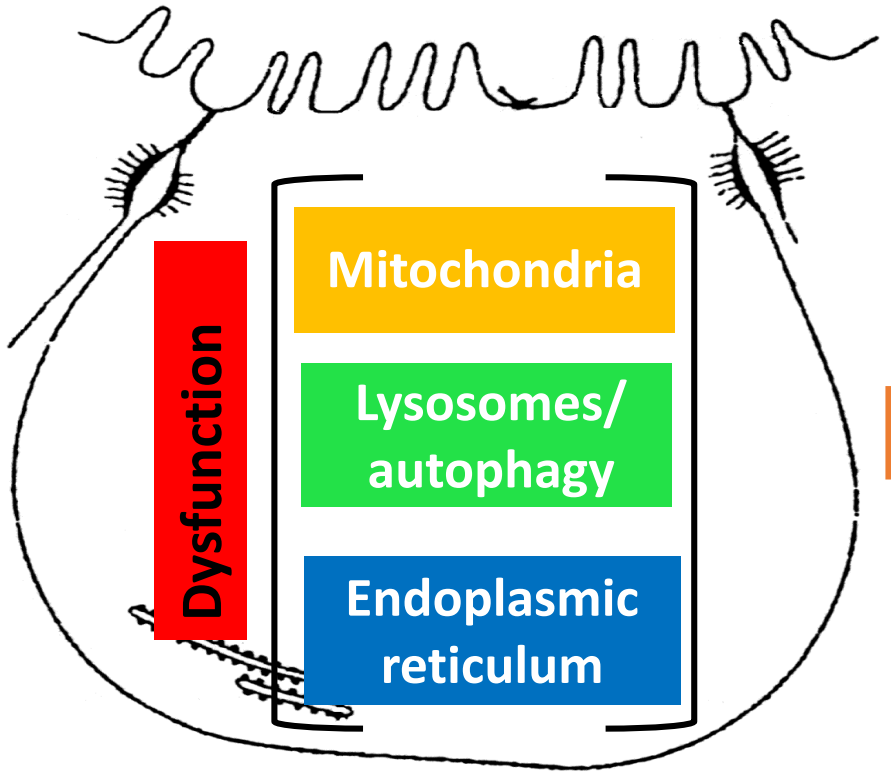
Endosomes Lysosomes

Secretory granules

Autophagic degradation

Exocytosis

Hypothesis: Disordering of acinar cell organellar machinery initiates/drives pancreatitis

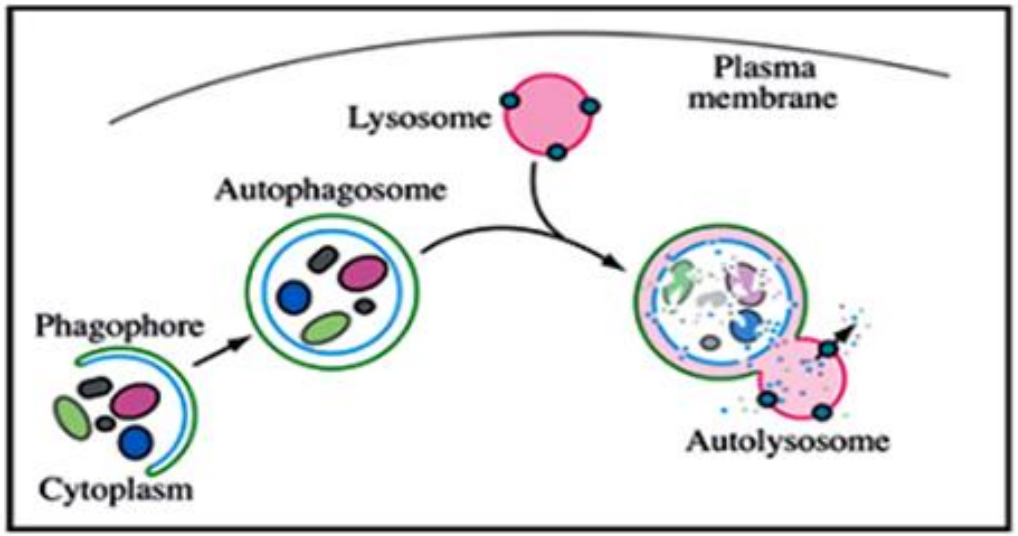


PANCREATITIS

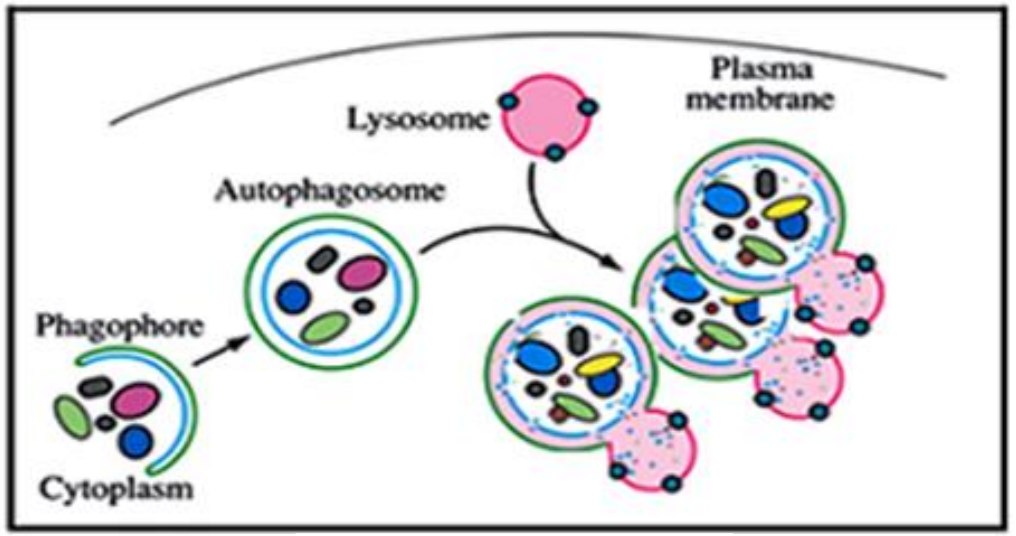
- Acinar cell death
- Inflammation
- Vacuolization
- Trypsinogen activation

Autophagy is impaired in pancreatitis

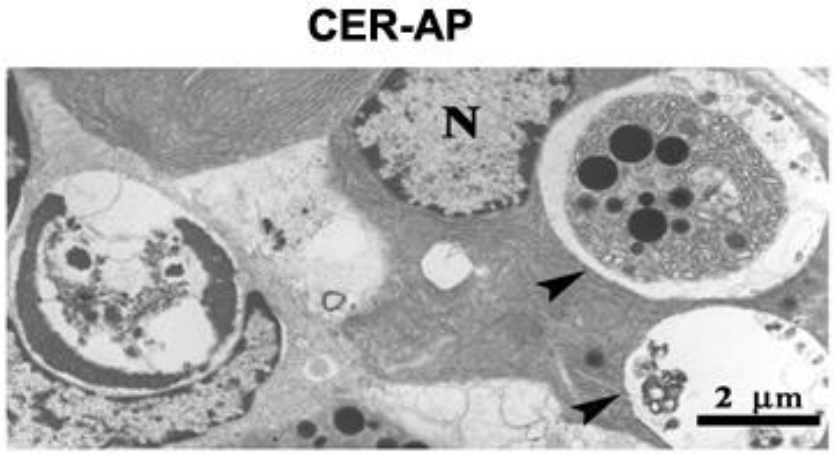
Normal pancreas



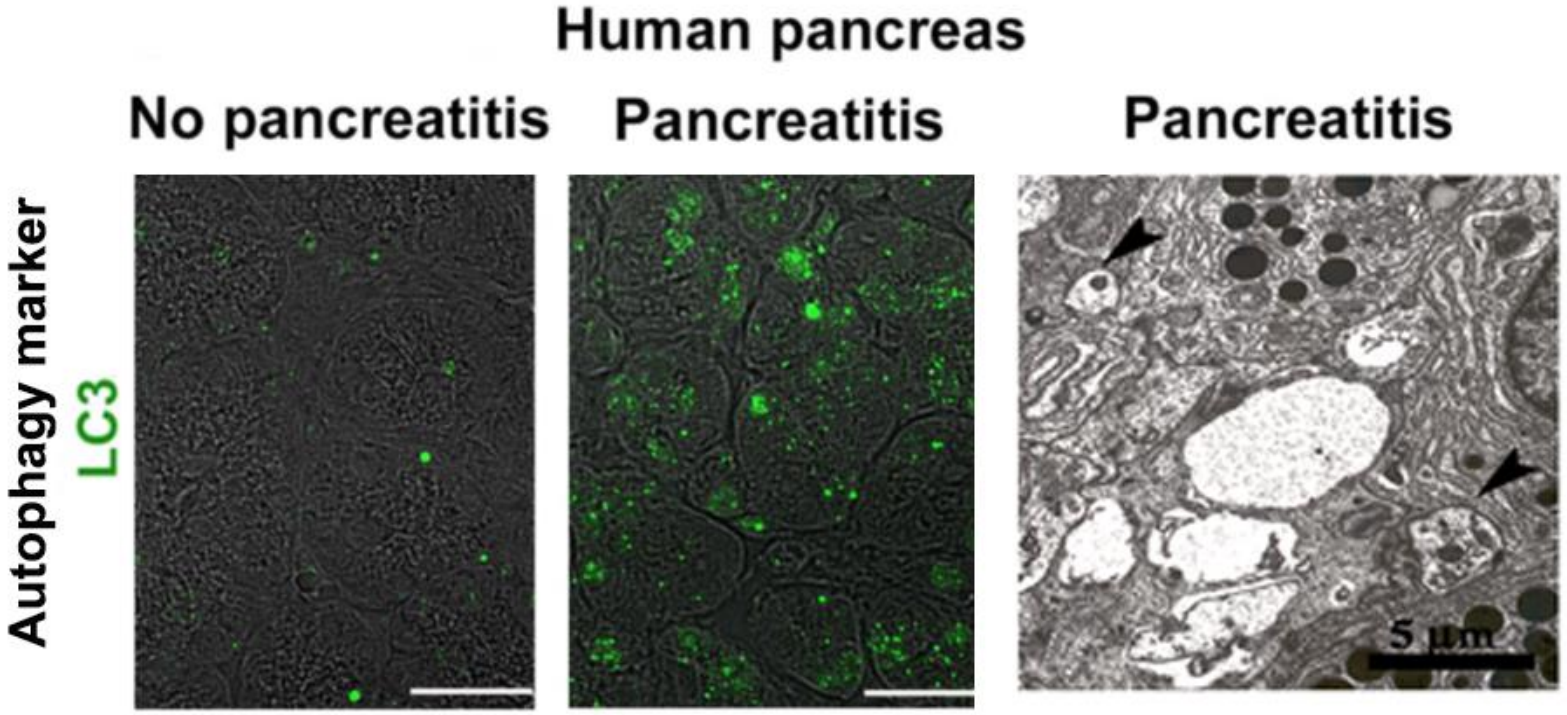
Pancreatitis



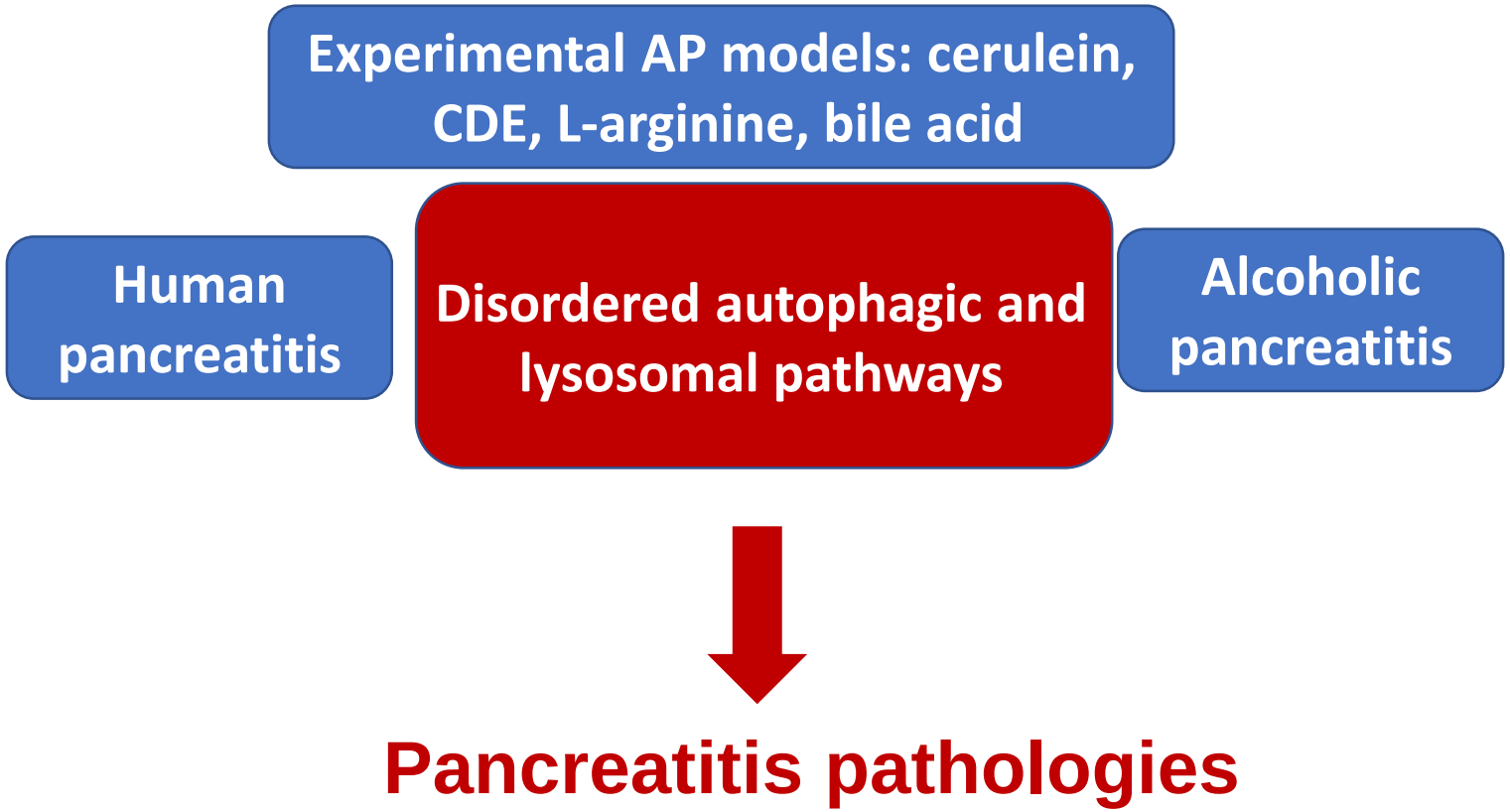
Autophagy dysfunction is prominent in experimental and genetic models of pancreatitis, and in human disease



OA Mareninova et al.
J Clin Invest 2009



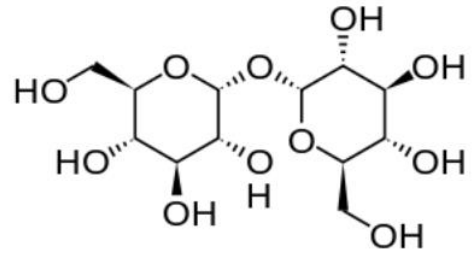
Impaired autophagy mediates pancreatitis

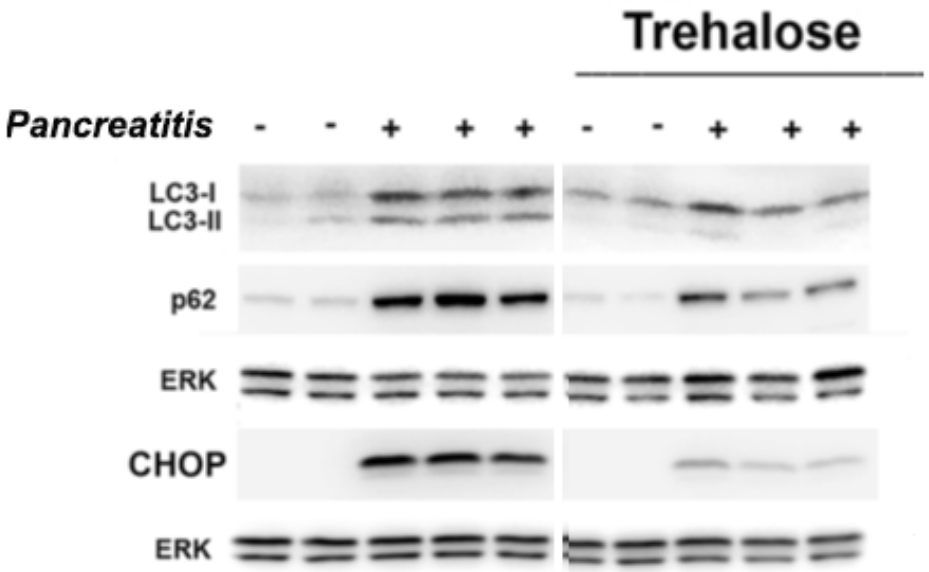


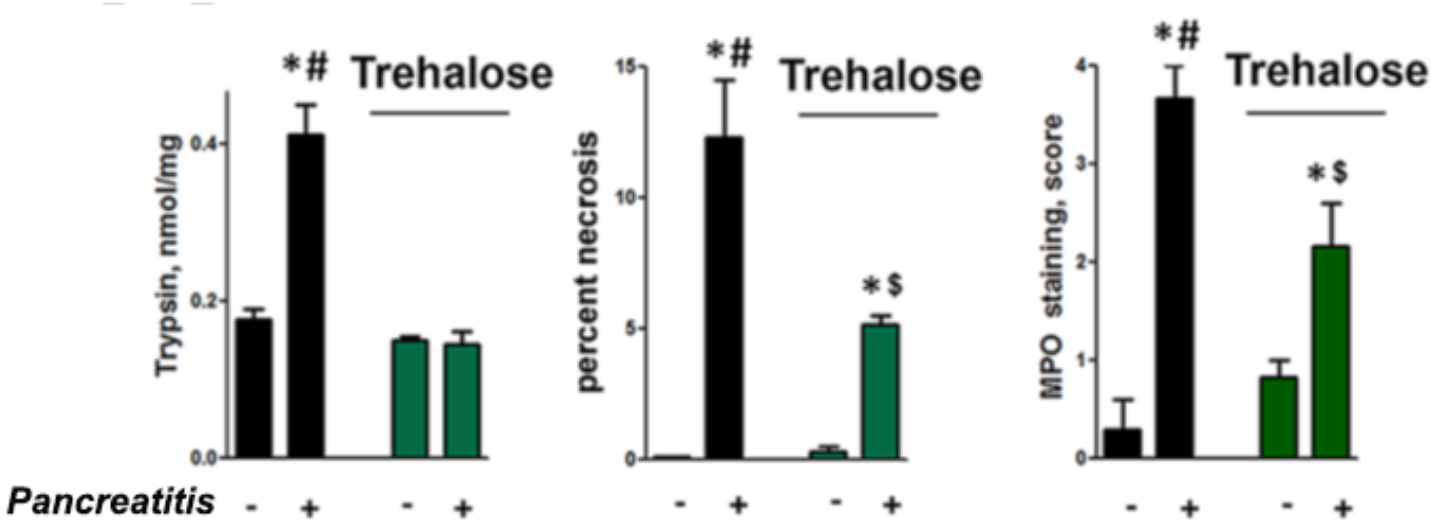
Enhancing autophagic efficiency with trehalose prevents or greatly alleviates pancreatitis responses

Trehalose → **Autophagic efficiency** ⊣ **Pancreatitis**

Trehalose







G Biczo et al. *Gastroenterology* 2018

Autophagy blockade in pancreas by genetic means causes spontaneous pancreatitis

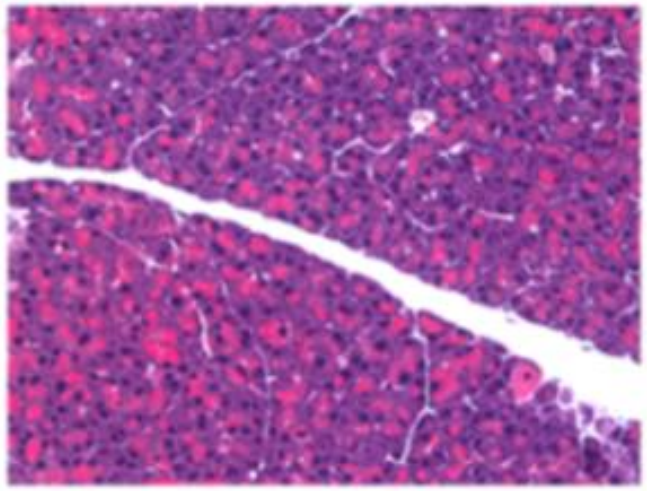
- $Atg7^{\Delta pan}$
- $Atg5^{\Delta pan}$
- $IKK\alpha^{\Delta pan}$
- $LAMP2^{-/-}$
- $GNPTab^{-/-}$
- $TFEB^{\Delta pan}$

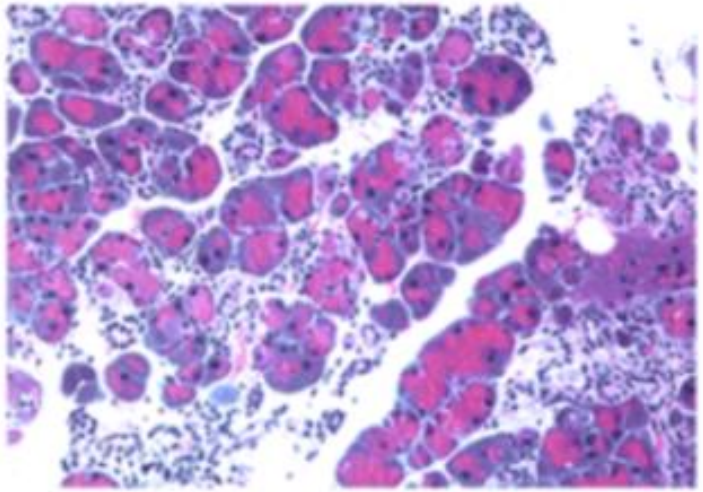
⇨ **Autophagy blockade** ⇨ **PANCREATITIS**

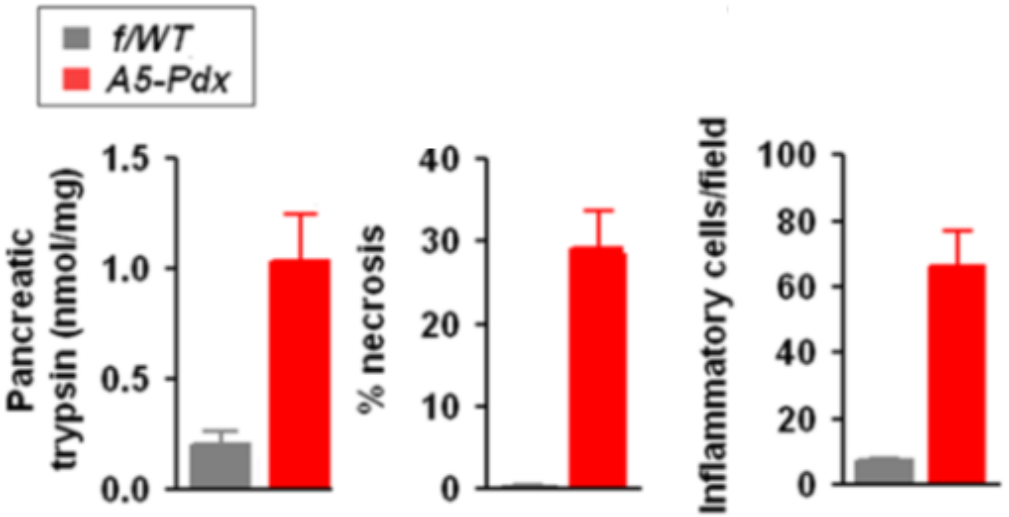
Trypsinogen activation, inflammation, necrosis, fibrosis

f/WT (control)

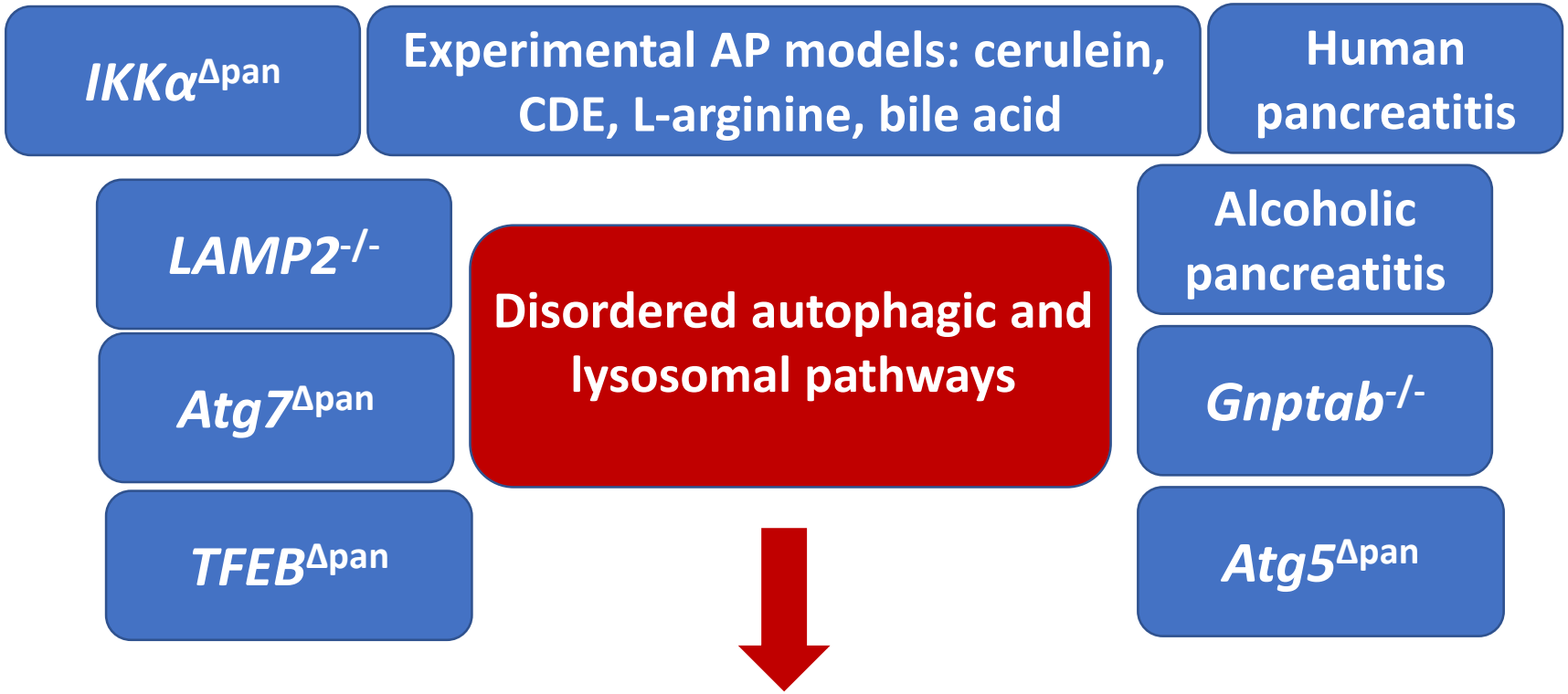
Pancreas-specific ablation of autophagy mediator Atg5 (*A5-Pdx*)







Impaired autophagy mediates pancreatitis



Mutations in regulatory elements of the *ATG5* gene in humans are associated with recurrent acute pancreatitis

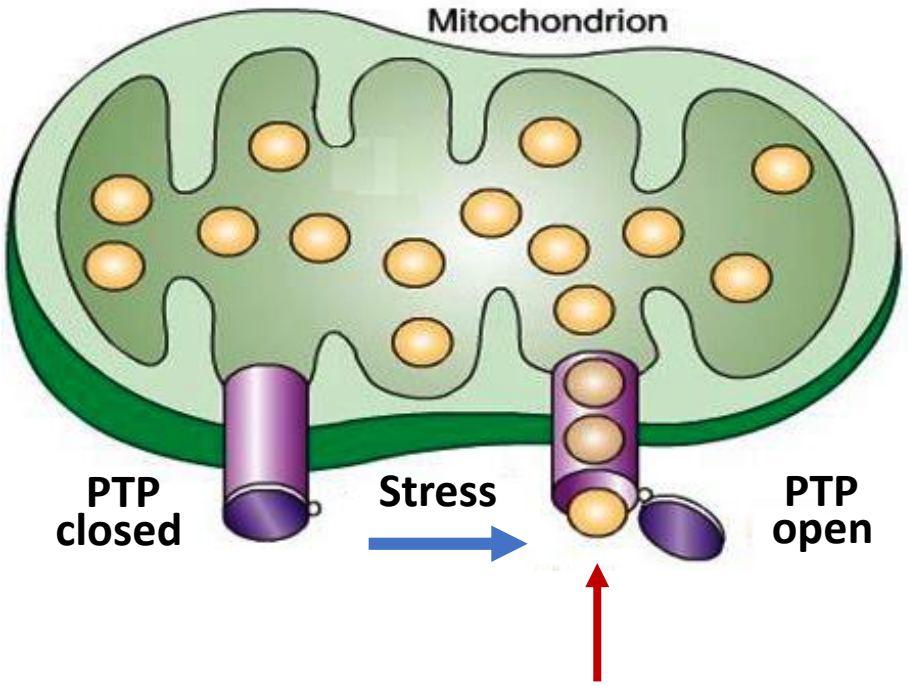
- A genetic association study was conducted using previously genotyped SNPs from the well phenotyped NAPS2 cohort (1217 total samples)
- SNPs within 50 kb flanking the ATG5 gene were compared for possible association with pancreatitis, and pancreatitis subtypes (RAP vs CP; alcohol, smoking)

Three linked SNPs (LD >= 0.8) in *ATG5* were significantly associated with RAP, when controlled for alcohol, smoking and pain.

All SNPs were located in non-coding regions and altered known nucleotide binding sites for enhancer histone marks in pancreatic tissue and transcription factors (TFs).

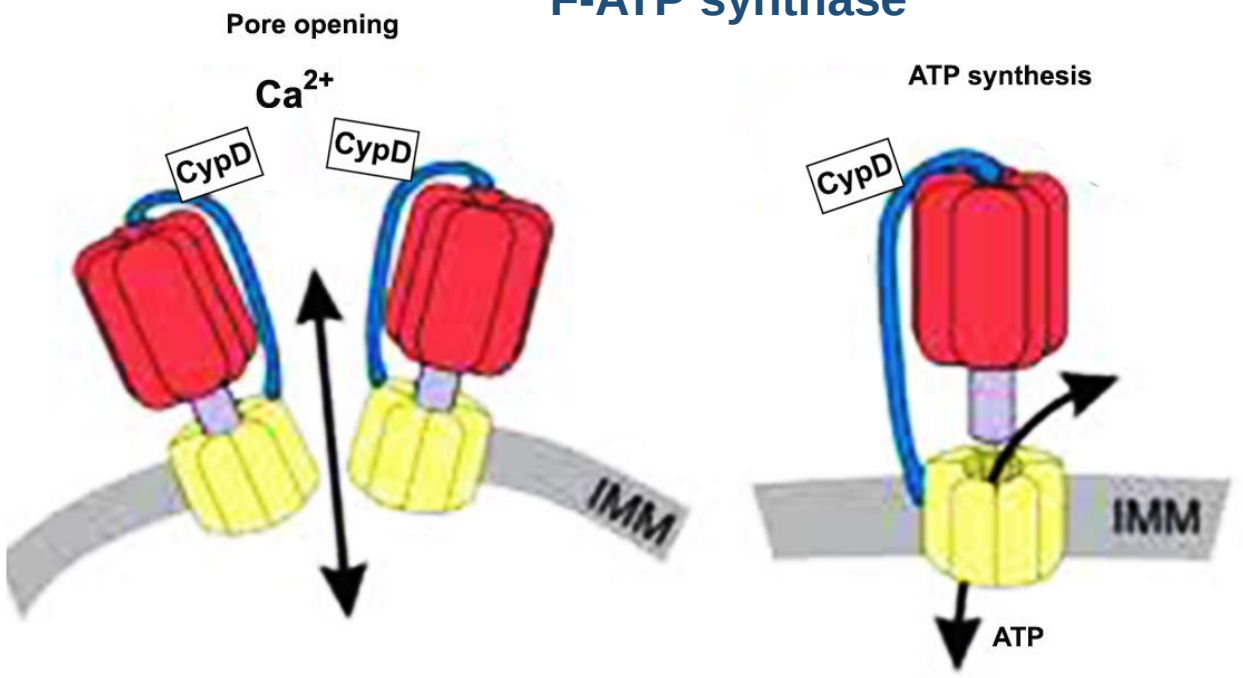
Adapted from presentation by Dr. David Whitcomb at the DDW-2019: **"Mutations in regulatory elements of the ATG5 gene in humans are associated with recurrent acute pancreatitis: functional validation using pancreas-specific ATG5 knockout mice."** Tanvi Nagpal,[1] Celeste Shelton,[1] Phil Greer,[1] Brandon Blobner,[1] Ilya Gukovsky,[2] Anna S. Gukovskaya,[2] David Whitcomb.[1]

Permeability transition pore (PTP) is a non-selective channel in mitochondrial membrane regulated by cyclophilin D (CypD)



All molecules with molecular mass <1,500 Da including H_2O

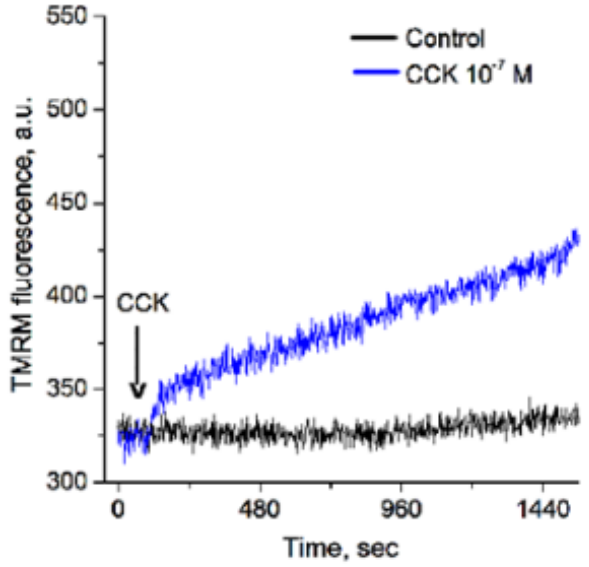
F-ATP synthase

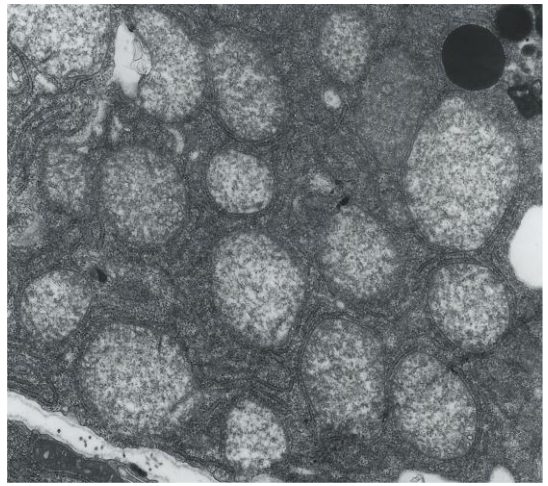


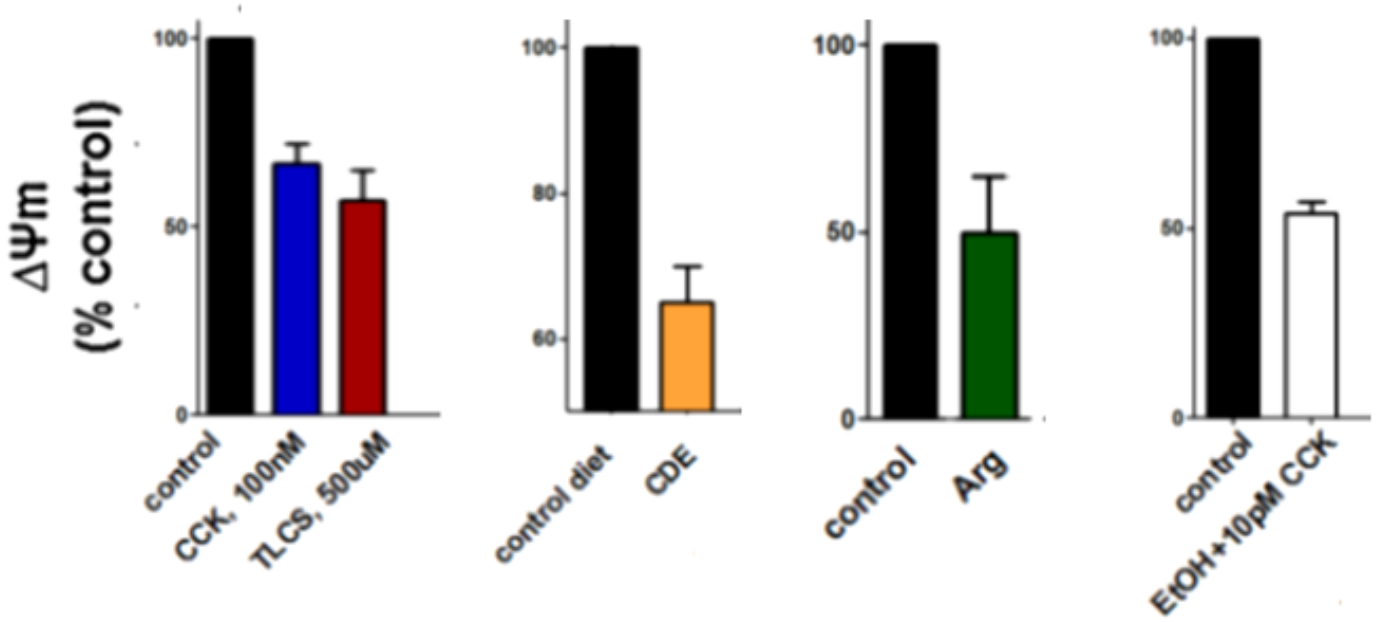
Consequences of PTP opening:

Mitochondria swelling
Loss of the mitochondrial membrane potential (ΔΨm) and the ability to generate ATP

Mitochondrial depolarization is an early common event in experimental pancreatitis

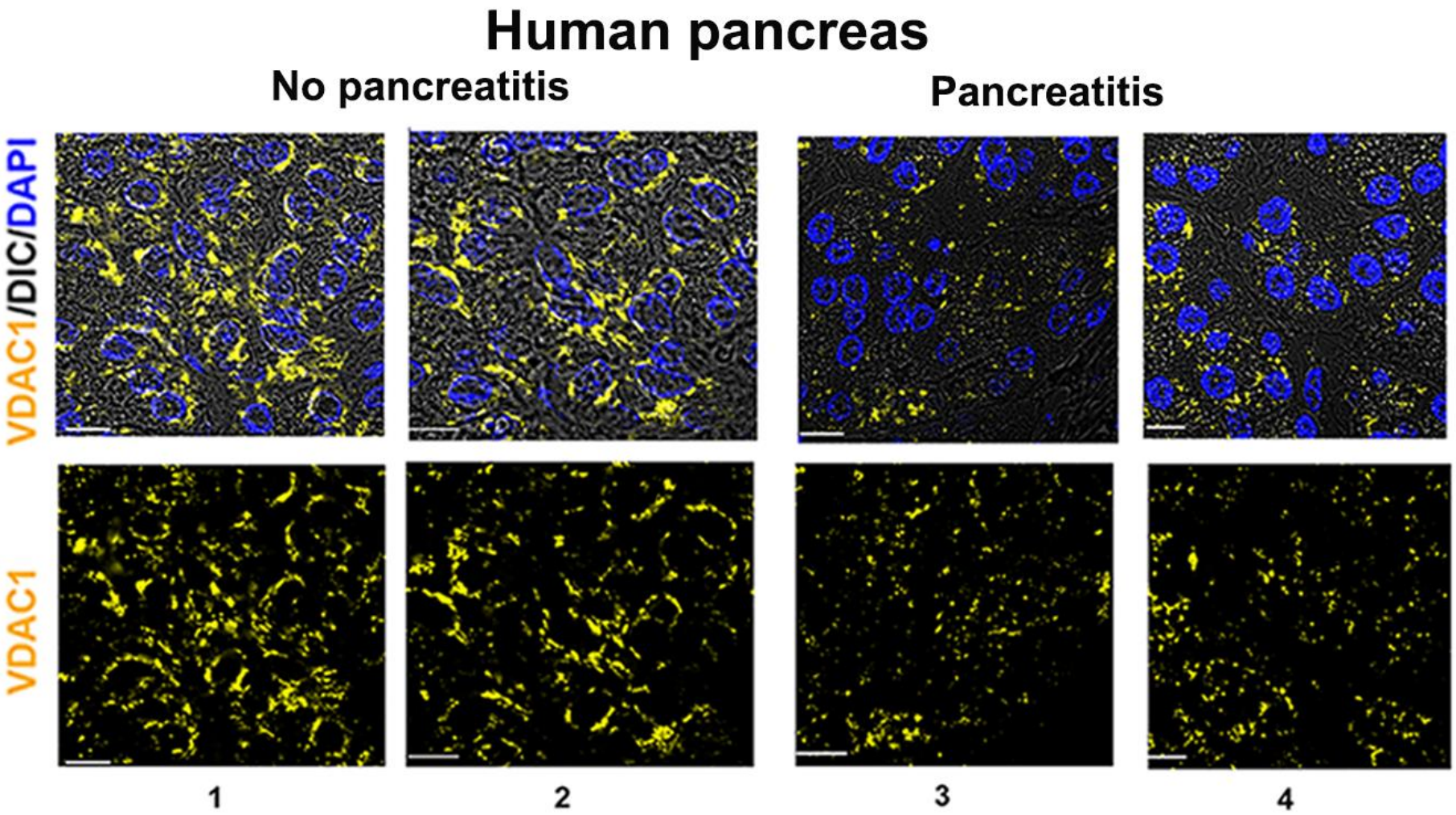






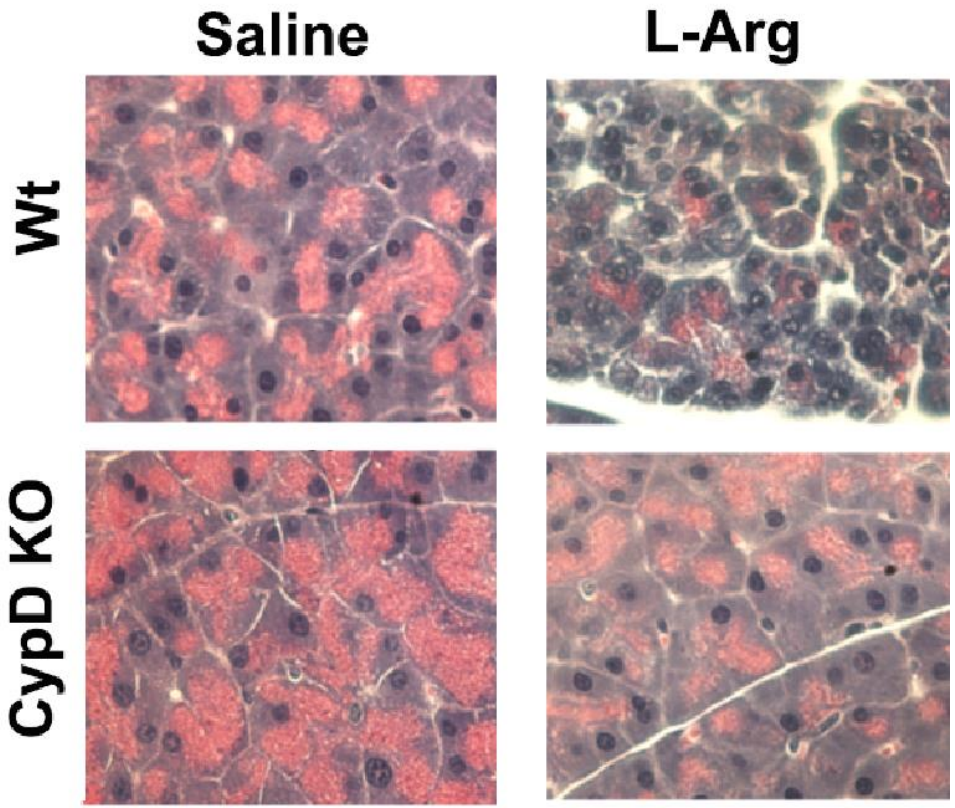
N Shalbueva et al. *Gastroenterology* 2013
R Mukherjee*, OA Mareninova* et al. *Gut* 2016
G Biczo et al. *Gastroenterology* 2018

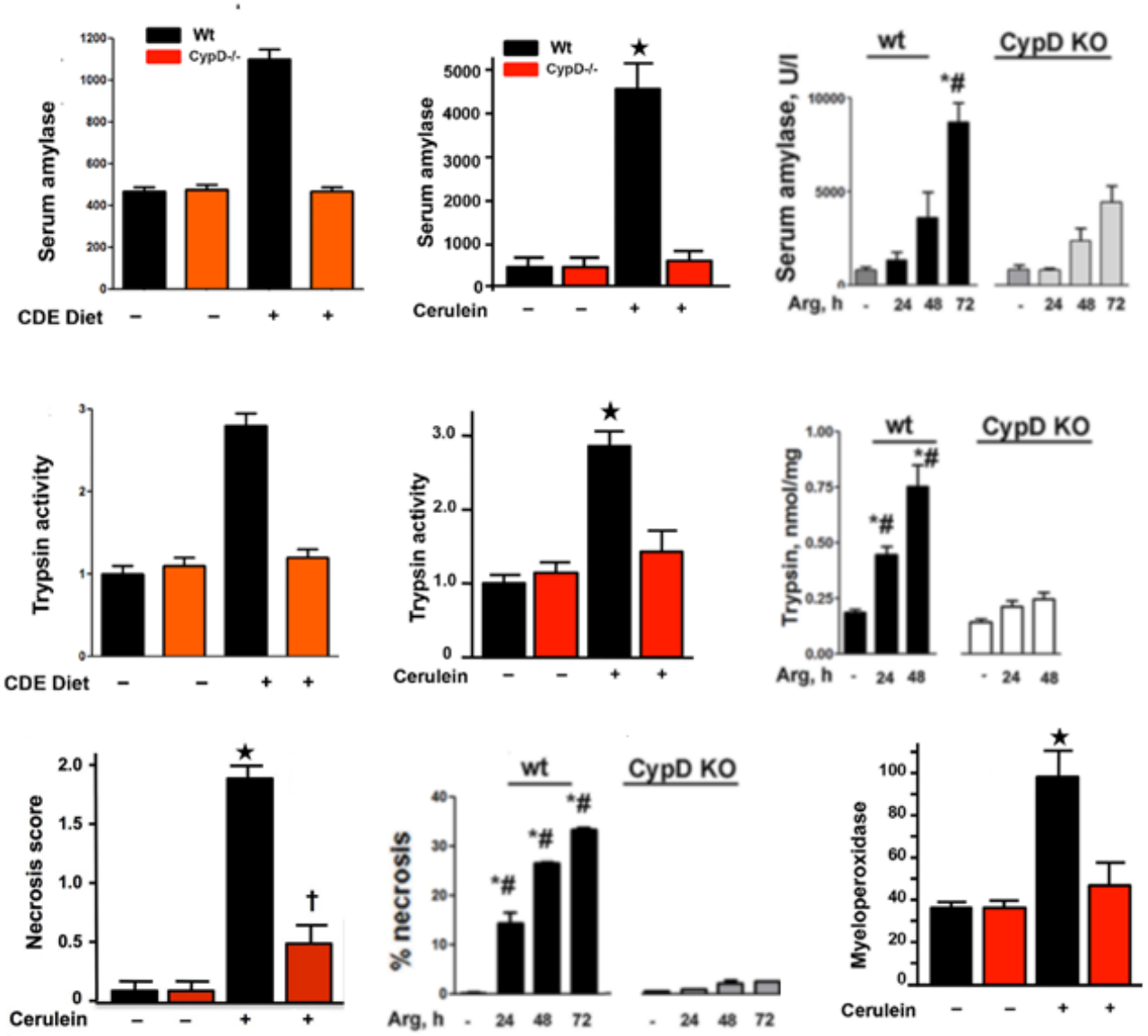
Mitochondrial fragmentation in human pancreatitis



G Biczo et al. *Gastroenterology* 2018

Genetic ablation of CypD greatly improves experimental pancreatitis





R Mukherjee*, OA Mareninova* et al. *Gut* 2016
G Biczo et al. *Gastroenterology* 2018

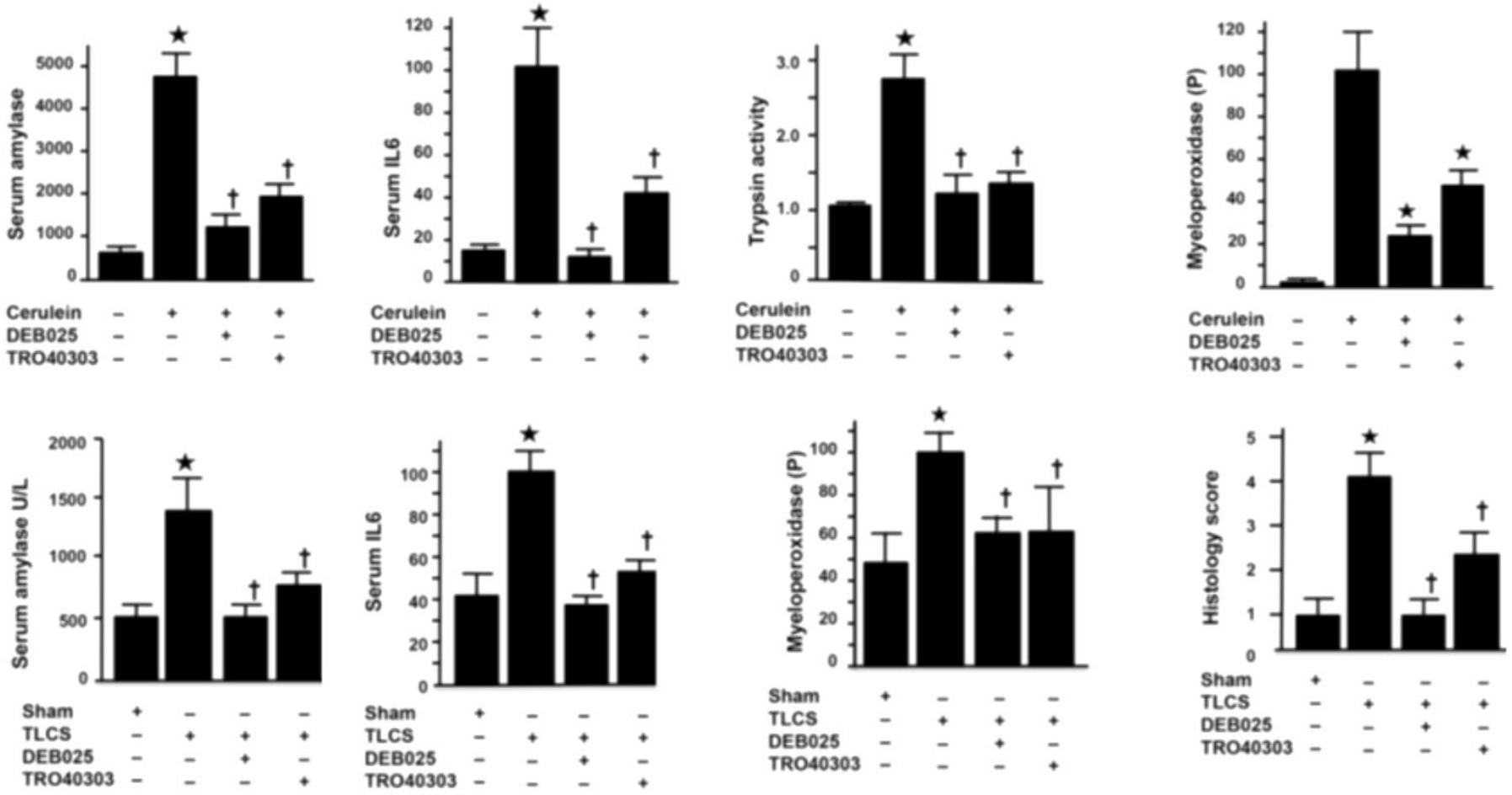
Pharmacologic approaches to prevent or inhibit PTP opening ameliorate experimental pancreatitis

CypD inhibitors

DEB025 TR040303

CypD-dependent mitochondrial dysfunction

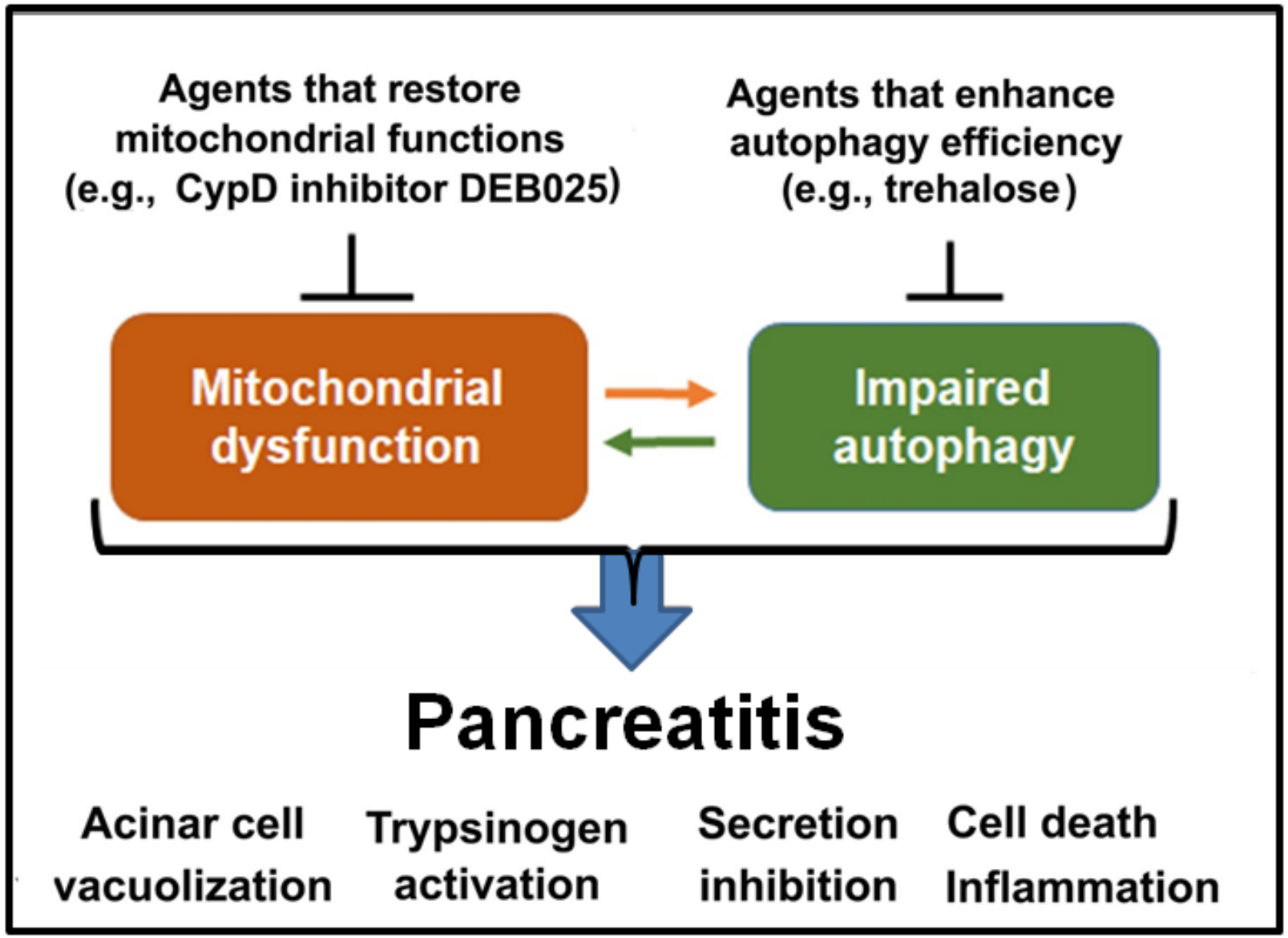
Pancreatitis

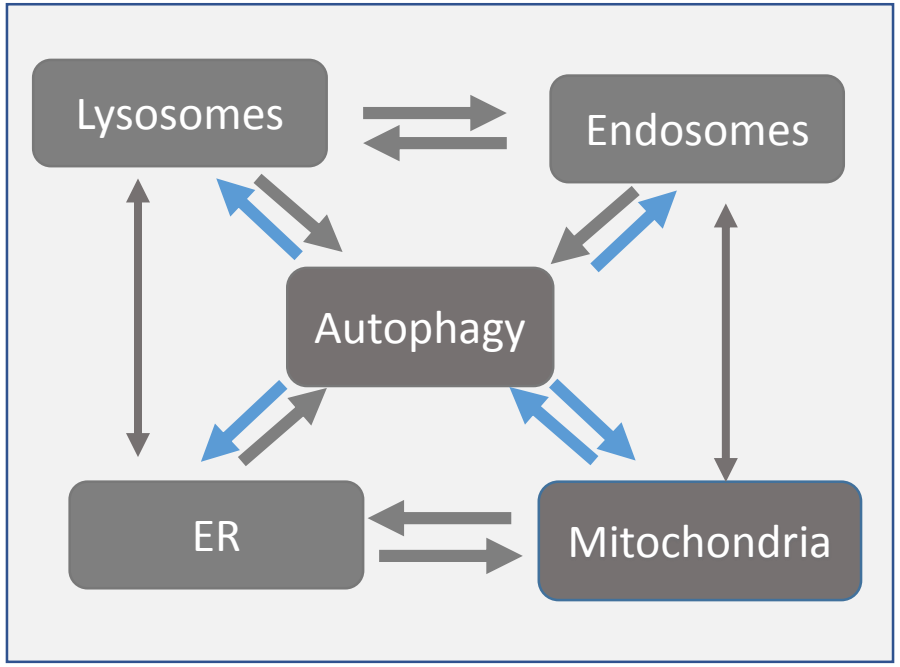


R Mukherjee*, OA Mareninova* et al. *Gut* 2016

Potential targets for AP treatments

Impaired autophagy	Associated with experimental and genetic pancreatitis models (total, 12 dissimilar models)	Associated with human disease	Genetic approaches to block autophagy caused spontaneous pancreatitis in 6 mouse models	Enhancing autophagy efficiency with a pharmacologic agent alleviated pancreatitis responses in 2 experimental mouse models of AP
Mitochondrial dysfunction	Associated with experimental and genetic AP models (total, 9 dissimilar models	Associated with human disease	Genetic approaches to restore mitochondrial function ameliorated AP in all models tested	Pharmacologic approaches to restore mitochondrial function ameliorated AP in 4 experimental mouse models


Agents that restore mitochondrial functions (e.g., CypD inhibitor DEB025)
Agents that enhance autophagy efficiency (e.g., trehalose)
Mitochondrial dysfunction
Impaired autophagy
Pancreatitis
Acinar cell vacuolization
Trypsinogen activation
Secretion inhibition
Cell death Inflammation



The results provide strong evidence that:

- *Functional organellar network is critical for acinar cell homeostasis*
- *Organelles in exocrine pancreas form an inter-connected system, so that disordering of a particular type of organelle results in failure of the whole network*
- *Disordering of organellar network is a key pathologic event across various models of experimental pancreatitis*
- *Failure/dysfunction of acinar cell organellar network causes spontaneous pancreatitis*

REVIEW ARTICLE

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

Abstract: Acute pancreatitis (AP) is a potentially lethal inflammatory disease that lacks specific therapy. Damaged pancreatic acinar cells are believed to be the site of AP initiation. The primary function of these cells

reticulum; $[Ca^{2+}]i$ - free cytosolic Ca^{2+} concentration; IKK - inhibitor of the nuclear factor κB kinase; MSP - minor secretory pathway;

(*Pancreas* 2019;48: 459–470)

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PRG

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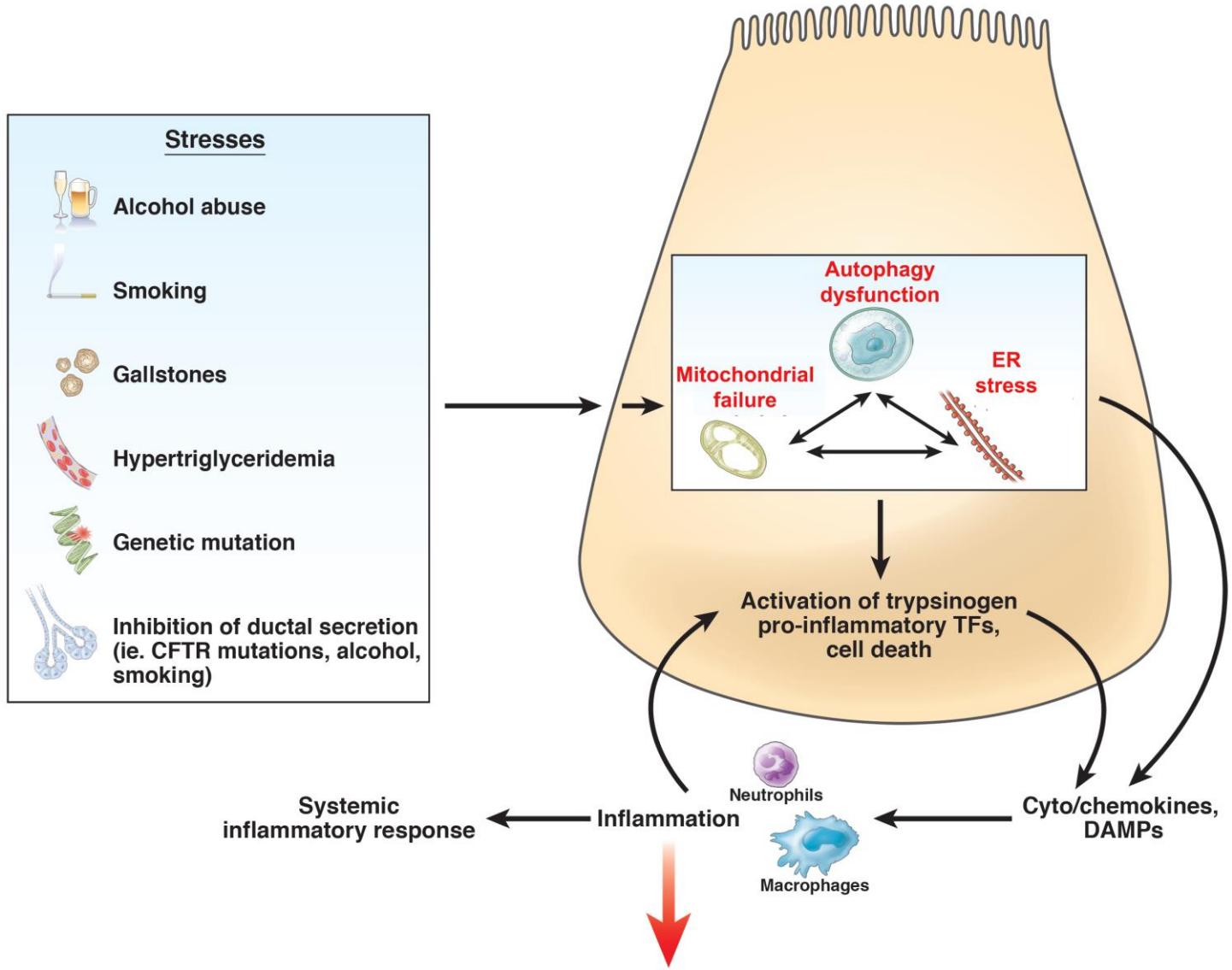
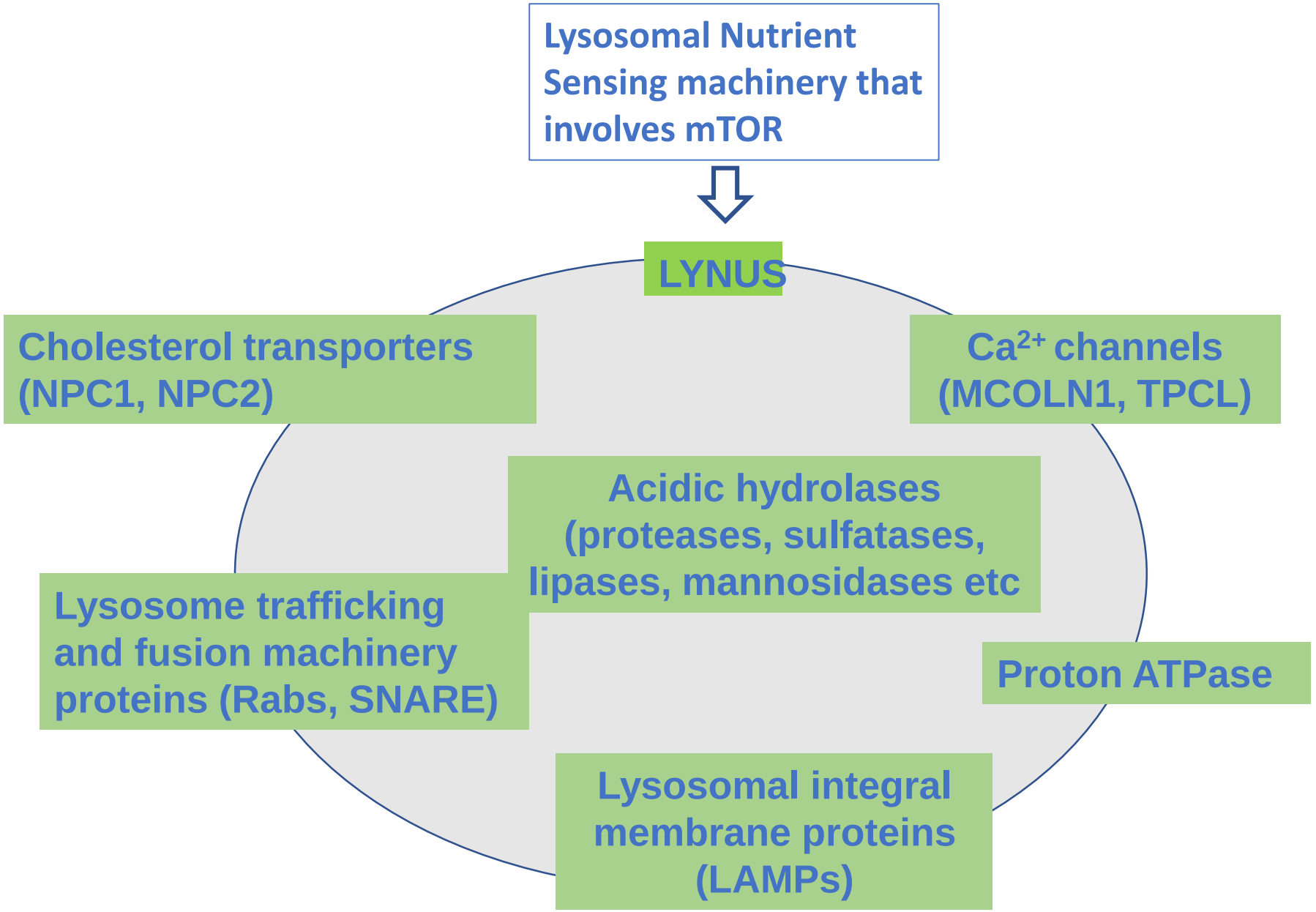
- One cause of non-resolving inflammation in AP could be unremitting acinar cell injury, which perpetuates the inflammatory response
- Pathogenic mechanisms of acinar cell injury that initiate AP remain poorly understood
- One reason is that the molecular mechanisms maintaining acinar cell homeostasis were largely unknown until recently

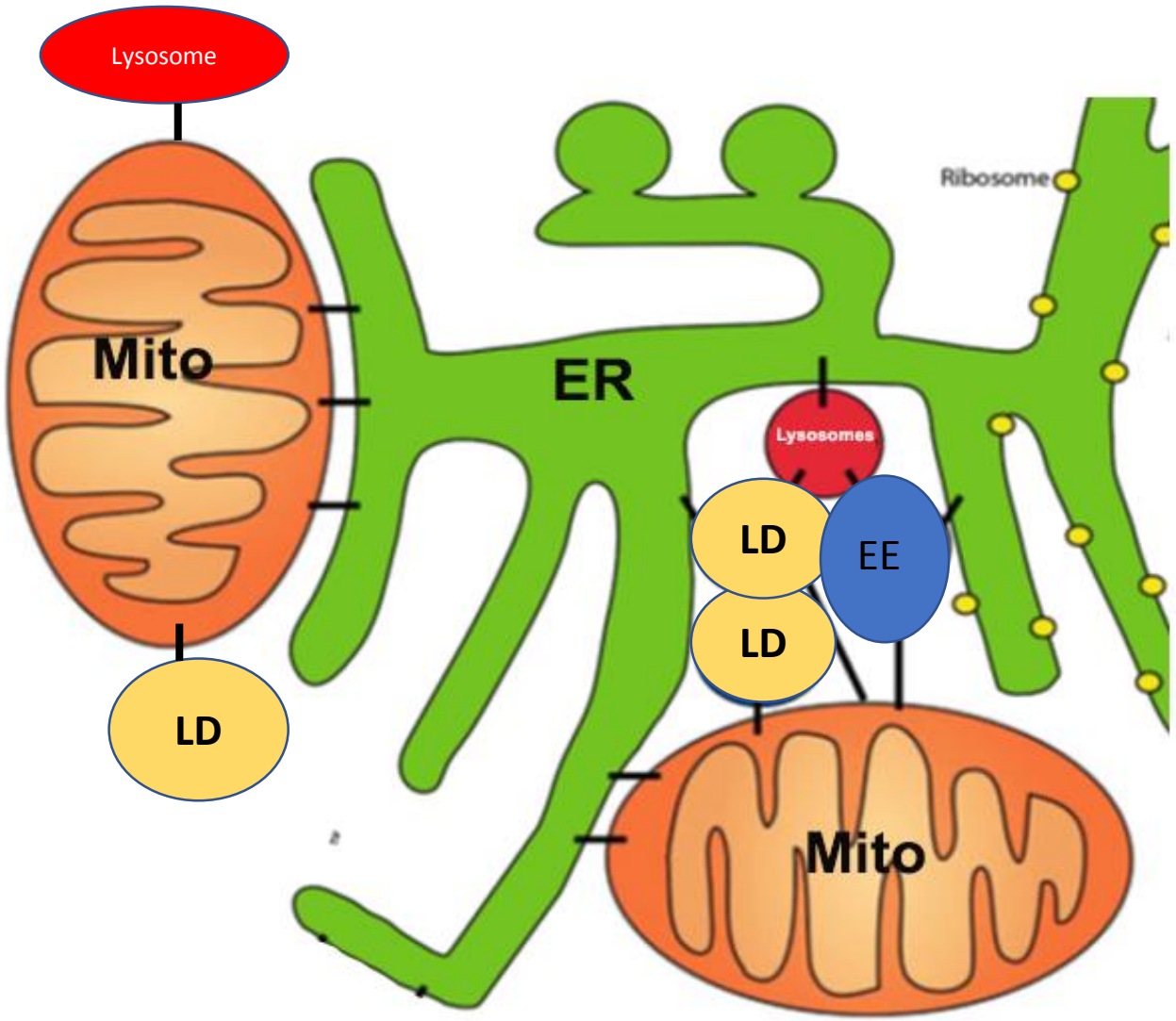
- Not long ago, we thought that cytosolic proteins, such as kinases, mediate most cellular processes, whereas cytoplasmic organelles only play limited and supportive roles
- During the last 2 decades, there was tremendous progress in our understanding of the functions of cytoplasmic organelles


Stresses
Alcohol abuse
Smoking
Gallstones
Hypertriglyceridemia
Genetic mutation
Inhibition of ductal secretion (ie. CFTR mutations, alcohol, smoking)
Autophagy dysfunction
Mitochondrial failure
ER stress
Activation of trypsinogen pro-inflammatory TFs, cell death
Neutrophils
Macrophages
Inflammation
Systemic inflammatory response
Cyto/chemokines, DAMPs

Current understanding of the lysosome structure

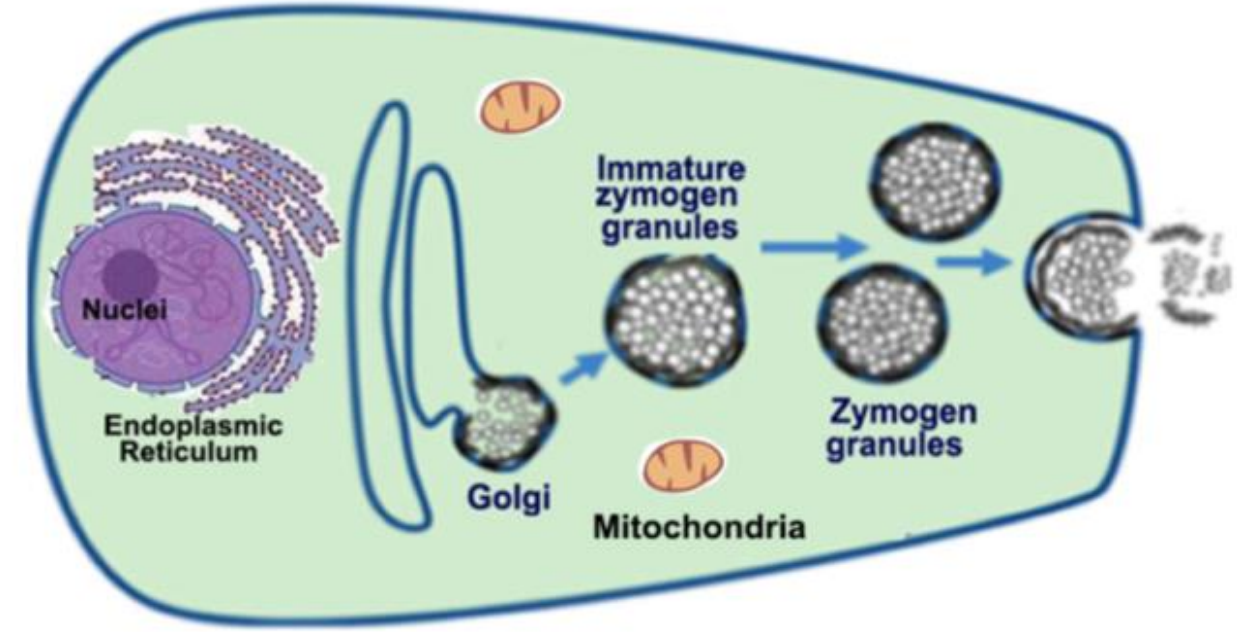


Organelles exchange information through contacts membrane/membrane contacts



ER Endoplasmic reticulum
Mito, Mitochondria
LD, Lipid droplets
E**E,** Early endosomes

Secretory function of acinar cells relies on coordinated action of cytoplasmic organelles: the endoplasmic reticulum, Golgi, mitochondria



Endoplasmic reticulum	Golgi, endo-lysosomes	Mitochondria
Protein synthesis	Protein trafficking	Energy production

Acinar cell relies on cytoplasmic organelles to coordinate secretion according to cell demands.