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Advances in Acute and Chronic Pancreatitis: From Development to Inflammation and Repair

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The conference “Advances in Acute and Chronic Pancreatitis: From Development to Inflammation and Repair” was held June 25–26, 2012, on the NIH campus, in Bethesda, Maryland (<http://www2.niddk.nih.gov/News/Calendar/Pancreatitis2012.htm>).

The conference covered the regulation of pancreatic development, stem cells, acute and chronic pancreatitis, and cancer.

Pancreatic Development and Regulation of Organogenesis

After a superb introductory lecture by Al Lowenfels (New York Medical College) on the history and epidemiology of pancreatitis research, the first session of the conference provided an overview of the molecular signals that guide the development of pancreatic

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Conflicts of interest

The authors disclose no conflicts.

exocrine cells and how changes in developmental pathways contribute to pancreatitis. Maiké Sander (UCSD) reported on the role of the transcription factor Sox9 in formation of exocrine cells. Mice in which elements of the Sox9 promoter regulate expression of tamoxifen-inducible Cre recombinase (*Sox9CreERT2*) allow for lineage tracing and gene manipulation during development and in the adult pancreas.¹ Although Sox9 is expressed throughout the pancreas epithelium during early stages of organogenesis, it is confined to pancreatic ductal cells in the adult pancreas, excluding acinar and centroacinar cells (CACs). Interestingly, Sox9 is reactivated in acinar cells that undergo de-differentiation after induction of pancreatitis with cerulein. In mice that express oncogenic Kras, de-differentiated acinar cells undergo permanent neoplastic transformation, developing into pancreatic intraepithelial neoplasia (PanINs) and eventually pancreatic adenocarcinomas. Sander's findings show that Sox9 is required for acinar to ductal metaplasia. Importantly, elimination of Sox9 from acinar, but not duct cells or CACs, prevented formation of PanINs. These data indicate that acinar cells are the main progenitors of Kras-induced neoplasia.

Ray MacDonald (UT Southwestern Medical Center) continued the discussion of acinar plasticity, demonstrating that a distinct set of transcription factors (Ptf1a, Rbpjl, Nr5a2, Gata4, and Foxa2) are required not only for acinar cell formation, but also maintenance of the differentiated state in the mature pancreas. Transcriptional network analysis revealed that these factors regulate expression of acinar-specific genes and can activate their own transcription to propagate the entire network. It will be important to determine whether these transcription factors also control acinar stability, and if defects in their function increase susceptibility to pancreatitis or adenocarcinoma.

Steven Leach (Johns Hopkins University) reported that CACs function as facultative stem cells within the adult pancreas. CACs are located in a unique position, at the interface between ductal and acinar cells. They express markers of stem cells, including aldehyde dehydrogenase (ALDH)1, and can be labeled with the substrate aldefluor. Sorted ALDH1-positive cells develop into pancreato-spheres that can differentiate into cells that express markers of acinar, duct, and endocrine cells.² Furthermore, ALDH1-positive cells expand during pancreatitis.²

Exocrine Pancreatic Stem and Progenitor Cells

Derek van der Kooy (University of Toronto) described pancreas-derived multipotent cells,³ which are rare (1/5000 pancreas cells) and form spheres in vitro. They can differentiate into multiple lineages, including several endocrine cell types and neurons. der Kooy used a combination of lineage tracing and reporter-based assays to show that most pancreas-derived multipotent cells are derived from insulin-expressing cells and not from neural crest cells. They showed that cells with these properties could exist in vivo, because they observed labeled insulin-positive cells that gave rise to other lineages (including acinar cells and neurons) over a long period. Similar results were obtained when human islets were dissociated and cells were cultured at clonal density in vitro.

Roland Schmid (Technische Universitat, Munchen) reported that daily administration of cerulein to mice induces pancreatitis with 50% mortality. *IL-6^{-/-}* mice all survive cerulein

administration, and it seems that interleukin (IL)-6 involves trans-signaling (and activation of Stat3) in the pancreas. Serum levels in IL-6 in patients with pancreatitis correlate with disease severity, so IL-6 might be used to treat or protect patients against acute pancreatitis.

Charles Murtaugh (University of Utah) also discussed mechanisms of pancreatic injury and recovery, focusing on the roles of Wnt signaling and β -catenin. Pancreatic regeneration is significantly impaired when β -catenin, which is activated by Wnt signaling, is missing from acinar cells—loss of β -catenin disrupts acinar cell proliferation during the recovery phase. To determine whether activity of β -catenin during pancreatic regeneration requires Wnt signaling (or some other signaling pathway) Murtaugh's group generated mice with pancreas-specific deletion of Porcupine (*Porcn*) acyl-transferase, which is required to produce intact Wnt ligands. Surprisingly, the *Porcn*-mutant mice developed pancreatic steatosis over the first 3–6 months of life; the group is studying the origins of the new pancreatic adipocytes and their effects on pancreatic regeneration.

Francisco (Paco) Real (Spanish National Cancer Research Centre) presented data on the role of *Nr5a2*, an orphan nuclear receptor regulated by *HNF1 α* ⁴ in acinar homeostasis and progression of pancreatic cancer. *Nr5a2*^{-/-} mice develop more severe pancreatitis than wild-type mice; *Nr5a2* haploinsufficiency also accelerates the formation of premalignant PanIN lesions in mice that express oncogenic *Kras*. Moreover, variants of *Nr5a2* that increase the risk of pancreatic cancer were identified in a recent genome-wide association study. This transcription factor might therefore be involved in development of pancreatitis and PanINs, along with progression of pancreatic cancer.

Pancreatitis and the Microenvironment

Stephen Pandol (UCLA) discussed mechanisms underlying pancreatitis. Feeding ethanol to mice and rats can lead to disorders that in some ways resemble alcoholic pancreatitis. Intragastric ethanol feeding induces endoplasmic reticulum stress and abnormal unfolded protein response in the pancreas. Autophagy and reactive oxygen species are abnormally up-regulated. Pancreatitis in mice and humans is accompanied by a disruption in the protein production mechanisms, and by altered proteomic profiles in tissue, serum, and pancreatic juice, as well as in archival tissue. John Williams (University of Michigan) discussed the proteomics techniques that have been used to characterize the changes that occur during development of pancreatitis.^{5–7} These techniques might be used in diagnosis, to determine response to therapy, and compare the nature of pancreatitis-induced damage in animal models and humans.

Craig Logsdon (MD Anderson Cancer Center) spoke about mice that express an active form of PACE-trypsin in the pancreas do not have any phenotype, but they develop pancreatitis more rapidly than wild-type mice when fed alcohol.⁸ Expression of the oncogenic form of *Kras* in the pancreas also causes chronic pancreatitis that eventually progresses to cancer.^{9,10}

The repair process after pancreatitis involves the interaction of different cell types, including infiltrating immune cells, according to Matthias Hebrok (UCSF). *Rag1*^{-/-} mice, which do not produce B or T cells, have severe impairment in the repair process after pancreatitis; 40% of the pancreas still comprises acinar-ductal metaplasia, rather than recovered acinar

clusters, even several weeks after the initial injury. This defect can be corrected by replacement of B cells or regulatory T cells, but not by other T-cell subsets. The inability of *Rag1*^{-/-} mice to repair the pancreas after pancreatitis is likely owing, at least in part, to the abnormal persistence of macrophages within the pancreas.

Genetics of Pancreatitis

Variants in *PRSS1*, which encodes a cationic trypsinogen, are linked to hereditary pancreatitis.¹¹ Specific variants of *PRSS1* alter chymotrypsin C-dependent regulation of trypsinogen activation, increasing autoactivation and levels of trypsin.¹² Furthermore, variants in chymotrypsin C that prevent its degradation of trypsinogen and trypsin increase risk for chronic pancreatitis,¹³ as described by Miklos Sahin-Toth (Boston University).

Peter Durie (University of Toronto) spoke about the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel, has also been linked with recurrent acute and chronic pancreatitis. A conceptual model has shown an inverse relationship between the degree of CFTR dysfunction and the risk of pancreatitis.¹⁴ However, the lack of an absolute correlation between CFTR variants and development of pancreatitis indicates that other genes affect risk.

Studies of rare childhood diseases have provided insights into the pathogenesis of pancreatitis and exocrine pancreatic function. Shwachman-Diamond syndrome, an autosomal-recessive disorder, is caused by variants of *SBDS* that lead to pancreatic acinar cell hypoplasia and bone marrow and skeletal abnormalities.¹⁵ Little is known about the function of *SBDS*, which seems to be involved in ribosome function and RNA metabolism.¹⁶

Limitations to epidemiologic and genetic analyses have posed challenges to identification of genetic factors associated with pancreatitis. Most research has involved candidate gene studies with an element of ascertainment bias, selection of appropriate controls, and accurate phenotype definition, in particular with regard to disease severity. Next-generation sequencing approaches, which are less expensive and faster, are now being used, but require intense informatic analyses of large datasets.

Acute Pancreatitis

In rodent models of pancreatitis, autoactivation of trypsinogen does not cause disease; however, cathepsin-B-induced trypsinogen activation seems to be important for disease induction. Marcus Lerch (University of Greifswald, Germany) presented data that other cathepsins, such as cathepsin-L, and other serine proteases, such as elastase, seem to have different and sometimes opposing functions in acinar cells.¹⁷ Further research is needed to determine the sequence of activation events inside the pancreas, identify the subcellular compartment(s) in which protease activation begins and occurs, and determine whether and how individual proteases contribute to acinar cell injury, necrosis, apoptosis, and autophagy.

Bechien Wu (Kaiser Permanente, Los Angeles Medical Center) discussed treatment and prevention of acute pancreatitis. He suggested that development of evidence-based therapies

for acute pancreatitis have lagged behind advances in our understanding of the pathophysiology of this disease. There is a lack of evidence about optimization of supportive care and there is no pharmacologic therapy that has been shown to affect disease progression. He discussed several potential reasons for the lack of progress in development of treatments for acute pancreatitis. These include a lack of sustained effort to transition basic science findings into clinical trials, a lack of industry-sponsored clinical trials for this disease, an emphasis on patients with severe disease or rare outcomes (mortality, organ failure), and a lack of appropriate pre-clinical models for testing potential therapeutic agents. Reagents that looked promising in experimental models have not been found to be effective in patients.

Wu suggested the following for the development of therapeutics for acute pancreatitis:

1. Collaboration between basic science investigators and clinical researchers to develop more appropriate animal models of disease.
2. Expansion of approach to clinical trials: Consider a 2-tiered approach with first-line therapy applied to all patients with acute pancreatitis in the early phase of disease.
3. Identification and measurement of clinically meaningful outcomes for patients, based on duration of pain and symptoms.
4. Establishment of standardized criteria for grading the quality of preclinical data, to facilitate transition of most promising agents to clinical trials.
5. Emphasis on further testing of agents that have already been approved by the US Food and Drug Administration for use in humans.

Chronic Pancreatitis

Dhiraj Yadav (University of Pittsburgh) proposed increasing our understanding of the effects of amount and patterns of alcohol consumption on disease risk, interactions between smoking and alcohol, effectiveness of counseling in encouraging abstinence from alcohol, factors associated with progression to chronic pancreatitis, and factors that predict response to abstinence. Likewise, the effects of smoking (and patterns of smoking) on pancreatic function, the interactions between smoking and other factors, and the effectiveness of smoking cessation in the development of chronic pancreatitis require further study. Part of this will be achieved through experimental animal models, which was reviewed by Fred Gorelick (Yale University). As noted by David Whitcomb (University of Pittsburgh), identification of more genetic variants associated with chronic pancreatitis will guide studies of epidemiology and risk factors.

Darwin Conwell (Brigham and Women's Hospital) concluded that studies are needed to identify better diagnostic methods, with focus on modeling and simulation, combinations of risk factors (genetic and environmental), and individual diagnostic features of imaging and pancreatic function tests.

There was general discussion about diagnosis of chronic pancreatitis. Because patients can have histologic features of chronic pancreatitis (fibrosis and chronic inflammation) without

actually having the disease, diagnostic strategies are affected. A substantial amount of pancreatic damage must accumulate before it can be consistently detected by computed tomography, magnetic resonance imaging, or endoscopic ultrasonography. It is a challenge to diagnose patients with early-stage disease or differentiate it from bland pancreatic fibrosis. Patients are first assessed for presenting features and risk factors for chronic pancreatitis, and then undergo computed tomography or magnetic resonance imaging analysis. Some centers use secretin to enhance pancreatic duct visualization and assess qualitative pancreatic secretion, but the overall diagnostic utility of this technique requires further study. Tests of pancreatic function can also be performed, with either a traditional Dreiling tube or an endoscope, but these are used at only a few referral centers.

Inflammation and Pancreatic Neoplasia

After a talk on clinical needs by Michael Levy (Mayo Clinic), Jay Pasricha (Johns Hopkins University) spoke about pain as the most challenging symptom to manage in patients with chronic pancreatitis, and a main contributor to the burden of disease. Pancreatic pain signals are transmitted via primary afferent nociceptors and induced by inflammation, morphological changes in peripheral nerves, and damage to the tissue.^{18,19} Central sensitization also contributes to the pain associated with chronic pancreatitis. Continuous pain signals lead to molecular changes in spinal neurons that affect ion channels such as TRPV1 and TRPA1, voltage-dependent potassium channels, PAR-2, and neurotransmitters such as calcitonin gene-related peptide, brain-derived neurotrophic factor, and substance P. Some of this plasticity seems to be promoted by nerve growth factor. Although most of this research has been performed in animal models, similar observations have been made in tissue samples from patients, and might therefore lead to new therapeutic approaches.

Roger Liddle (Duke University) reported that, although primary nociceptors are best known for their role in processing noxious stimuli, they also participate in neurogenic inflammation (the local release of inflammatory mediators from afferent neurons), which involves some of the same molecules involved in nociception, such as TRPV1 and substance P. These neurotransmitters can significantly attenuate acute pancreatitis, as demonstrated by experimental blockade of afferent neurons by pharmacologic, operative, or genetic means. Not all neurogenic processes are pro-inflammatory; vagal counter-regulatory reflexes have been described that reduce pancreatitis.

Gustavo Ayala (University of Texas, Houston) described recent findings into the roles of nerves in cancer progression. Pancreatic cancer cells seem to migrate toward and invade nerves. There is some evidence that extrinsic nerves are important for systemic dissemination of cancer cells, and that neural invasion could be a prognostic factor. Neural factors might even affect tumor progression.²⁰ Increasing our understanding of these interactions could improve staging and treatment of pancreatic cancer.

Conclusion

This conference provided a state-of-the-art update and overview of acute and chronic pancreatitis, spanning developmental biology, genetics, and the microenvironment

(especially inflammatory cues, animal models, cell culture models) and clinical aspects (diagnosis, therapy). It is clear that the future holds promise in the employment of functional genomics for gene discovery that can be exploited for new diagnostics and risk stratification and new therapeutic targets, the latter exploiting specific molecular pathways.

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