The calcium binding protein S100A9 is essential for pancreatic leukocyte infiltration and induces disruption of cell-cell contacts.


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Leukocyte infiltration is an early and critical event in the development of acute pancreatitis. However, the mechanism of leukocyte transmigration into the pancreas and the function of leukocytes in initiating acute pancreatitis are still poorly understood. The authors studied the role of S100A9 (MRP14), a calcium binding protein specifically released by polymorph nuclear leukocytes (PMN), in the course of acute experimental pancreatitis. Acute pancreatitis was induced by repeated supramaximal caerulein injections in S100A9 deficient or S100A9 wild-type mice. The authors then determined S100A9 expression, trypsinogen activation peptide (TAP) levels, serum amylase and lipase activities, and tissue myeloperoxidase (MPO) activity. Cell-cell contact dissociation was analyzed in vitro with biovolume measurements of isolated acini after incubation with purified S100A8/A9 heterodimers, and in vivo as measurement of Evans Blue extravasation after intravenous application of S100A8/A9. Pancreatitis induced increased levels of S100A9 in the pancreas. However, infiltration of leukocytes and MPO activity in the lungs and pancreas during acute pancreatitis was decreased in S100A9-deficient mice and associated with significantly lower serum amylase and lipase activities as well as reduced intrapancreatic TAP-levels. Incubation of isolated pancreatic acini with purified S100A8/A9-heterodimers resulted in a rapid dissociation of acinar cell-cell contacts which was highly calcium-dependent. Consistent with these findings, in vivo application of S100A8/A9 in mice was in itself sufficient to induce pancreatic cell-cell contract dissociation as indicated by Evans Blue extravasation. These data show that the degree of intrapancreatic trypsinogen activation is influenced by the extent of leukocyte infiltration into the pancreas which, in turn, depends on the presence of S100A9 that is secreted from PMN. S100A9 directly affects leukocyte tissue invasion and mediates cell contact dissociation via its calcium binding properties.


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Pancreatitis-associated proteins (PAPs) are induced in acute pancreatitis and antisense-mediated gene knockdown of PAP decreased PAP gene expression and worsened pancreatitis. Here, the authors investigated the effect of a more stable inhibition of PAP using small-interference RNA gene knockdown in vitro and in an in vivo model of experimental pancreatitis. Pancreatitis-associated protein-specific siRNA was administered to AR42J cell cultures or rats induced with pancreatitis. Controls included administration of scrambled siRNA or vehicle alone. After 24 hours, cells and pancreata were harvested and assessed for PAP (PAP 1, PAP 2, PAP 3) gene expression and pancreatitis severity. In vitro, PAP protein,
and mRNA levels were reduced (PAP 1, 76%; PAP 2, 8%; PAP 3, 24%) in cells treated with PAP siRNA. In vivo, PAP 1, and PAP 3 expressions were reduced (PAP 1, 36%; PAP 3, 66%) in siRNA-treated rats; there was no difference in PAP 2 isoform mRNA expression and serum protein levels. Serum amylase and lipase levels decreased (equal to, or greater than, 50%) after administration of siRNA; interleukin (IL) 1beta, IL-4, and IL-6 increased, whereas C-reactive protein and tumor necrosis factor-alpha decreased when compared with vehicle control. Administration of PAP siRNA correlated with worsening histopathology. In conclusion, siRNA-mediated gene knockdown of PAP worsens pancreatitis. Differences in gene knockdown technology may provide different approaches to study gene function.


Pancreatitis and calcium signalling: report of an international workshop.

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“Pancreatitis and Calcium Signalling” was an international research workshop organized by the authors and held at the Liverpool Medical Institution, Liverpool, United Kingdom, from Sunday 12th to Tuesday 14th November 2006. The overall goal of the workshop was to review progress and explore new opportunities for understanding the mechanisms of acute pancreatitis with an emphasis on the role of pathological calcium signaling. The participants included those with significant interest and expertise in pancreatitis research and others who are in fields outside gastroenterology but with significant expertise in areas of cell biology relevant to pancreatitis. The workshop was designed to enhance interchange of ideas and collaborations, to engage and encourage younger researchers in the field, and promote biomedical research through the participating and supporting organizations and societies. The workshop was divided into 8 topic-oriented sessions. The sessions were: 1) Physiology and pathophysiology of calcium signaling; 2) Interacting signaling mechanisms; 3) Premature digestive enzyme activation; 4) Physiology Society Lecture: Aberrant Ca2+ signaling, bicarbonate secretion, and pancreatitis; 5) NFkappaB, cytokines, and immune mechanisms; 6) Mitochondrial injury; 7) Cell death pathways; and 8) Overview of areas for future research. In each session, speakers presented work appropriate to the topic followed by discussion of the material presented by the group. The publication of these proceedings is intended to provide a platform for enhancing research and therapeutic development for acute pancreatitis.


Inhibition of hydrogen sulfide synthesis attenuates chemokine production and protects mice against acute pancreatitis and associated lung injury.

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The present study investigated whether chemokines are involved in hydrogen sulfide (H2S)-associated pathogenesis of acute pancreatitis and associated lung injury. The authors have examined the effect of DL-propargylglycine, a cystathionine gamma-lyase inhibitor, on the synthesis of CC chemokine monocyte chemotactic protein 1, regulated upon activation, normal T-cell expressed, and secreted, and macrophage inflammatory protein-1alpha (MIP-1alpha), and CXC chemokine MIP-2 in an in vitro and in vivo model of cerulein-induced acute pancreatitis and associated lung injury. In addition, the pancreatic acinar cells were treated with H2S donor drug, sodium.
The expression of these chemokines in the pancreatic acini, pancreas, and lungs was determined by quantitative real-time reverse transcriptase polymerase chain reaction, enzyme-linked immunosorbent assay, and immunohistochemistry. After treatment with DL-propargylglycine, reverse transcriptase polymerase chain reaction, and enzyme-linked immunosorbent assay demonstrated down-regulation of cerulein-induced increase in monocyte chemotactic protein 1, MIP-1alpha, and MIP-2 expression but had no apparent effect on regulated upon activation, normal T-cell expressed, and secreted expression. In conclusion, these results suggest that the proinflammatory effect of H$_2$S may be mediated by chemokines.


**Expression of the Shwachman-Bodian-Diamond syndrome (SBDS) protein in human pancreatic cancer and chronic pancreatitis.**


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The Shwachman-Bodian-Diamond syndrome (SBDS) protein is a member of a highly conserved family which influences RNA activation and is associated with pancreatic, skeletal and bone marrow deficiencies, as well as hematological malignancies. In this study, the expression and localization of SBDS were investigated in normal human pancreatic tissues, chronic pancreatitis (CP) tissues, primary and metastatic pancreatic ductal adenocarcinoma (PDAC) tissues, as well as in cultured pancreatic cancer cell lines by immunohistochemistry, immunoblotting and immunocytochemistry. In the normal pancreas, SBDS was localized in the cytoplasm of islet cells and ductal cells. In CP tissues, SBDS was found in the cytoplasm of ductal cells, tubular complexes, stromal fibroblasts and in PanIN1-2 lesions. In PDAC tissues, SBDS exhibited cytoplasmic and occasionally nuclear localization in tubular complexes, PanIN1-3 lesions, cancer cells, and stromal fibroblasts. Different levels of SBDS protein were detected in cultured pancreatic cancer cell lines. SBDS is expressed in normal, CP, and PDAC tissues, as well as in pancreatic cancer cell lines. The different expression and localization patterns suggest a role of SBDS in the pathogenesis of, or response to, inflammatory and neoplastic pancreatic diseases.

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**EUS and/or EUS-guided FNA in patients with CT and/or magnetic resonance imaging findings of enlarged pancreatic head or dilated pancreatic duct with or without a dilated common bile duct.**

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EUS examination was performed by using a radial echoendoscope followed by a linear echoendoscope, if a focal pancreatic lesion was identified. Fine-needle aspirates were stained with Diff-Quik and Papanicolaou's methods, and were immediately assessed by an attending cytopathologist. The main outcome measurements were to assess the prevalence of pancreatic neoplasms and to evaluate the performance characteristics of EUS-FNA for identifying malignant neoplasm in this patient group. In 110 study patients, the final diagnosis included adenocarcinoma (n=7), pancreatic intraepithelial neoplasia (n=1), neuroendocrine tumor (n=1), tumor metastasis (n=1), and benign cyst (n=3). Thirty-two patients had EUS evidence of chronic pancreatitis, and, in the remaining 65 patients, the pancreas was normal. The
accuracy of EUS and EUS-FNA for diagnosing pancreatic neoplasm in these patients was 99.1%, with 88.8% sensitivity, 100% specificity, 99% negative predictive value, and 100% positive predictive value. This results of this retrospective study show that a pancreatic neoplasm is seen in a clinically significant number of patients with "enlarged HOP" or "dilated PD with or without a dilated CBD" but without obstructive jaundice. EUS-FNA seems highly accurate for diagnosing pancreatic neoplasm in these patients.

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Inhibition of insulin-like growth factor-I receptor (IGF-IR) using NVP-AEW541, a small molecule kinase inhibitor, reduces orthotopic pancreatic cancer growth and angiogenesis.


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The insulin-like growth factor-I receptor (IGF-IR) is frequently overexpressed and constitutively activated in pancreatic cancer, thus representing a promising target for therapy. The authors investigated the impact of a novel inhibitor of IGF-IR (NVP-AEW541) on signalling and growth of pancreatic cancer. Human pancreatic cancer cells and endothelial cells were employed, and effects of NVP-AEW541 on signalling pathways investigated by Western blotting. NVP-AEW541 diminished the activation of IGF-IR, IRS-1, Erk, Akt and STAT3. Furthermore, NVP-AEW541 reduced cancer cell proliferation and abrogated migratory effects of IGF-I. NVP-AEW541 elicited a direct effect on endothelial cells in terms of reducing endothelial cell migration. In vivo, treatment of mice with NVP-AEW541 significantly reduced orthotopic pancreatic tumour growth, vascularisation, and VEGF expression. Interestingly, NVP-AEW541 lowered serum levels of IGF-binding-protein-3 (IGFBP-3). In conclusion, the IGF-IR inhibitor NVP-AEW541 effectively disrupts IGF-I signalling and reduces pancreatic tumour growth. Hence, blocking IGF-IR could prove valuable for targeted therapy of pancreatic cancer.

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Proteomic analysis of differential proteins in pancreatic carcinomas: effects of MBD1 knock-down by stable RNA interference.


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Methyl-CpG binding domain protein 1 (MBD1), a suppressor of gene transcription, may be involved in inactivation of tumor suppressor genes during tumorigenesis. Over-expression of MBD1 has been reported in human pancreatic carcinomas. The authors established a MBD1-knock-down pancreatic cancer cell line (BxPC-3) using stable RNA interference, to compare the proteomic changes between control and MBD1-knock-down cells using two-dimensional gel electrophoresis and mass spectrometry. The authors identified five proteins that were up-regulated and nine proteins that were down-regulated. Most of the identified proteins are involved in tumorigenesis, some are prognostic biomarkers for human malignant tumors. The data of the present study suggest that these differential proteins may be associated with the function of MBD1, and provide some insight into the functional mechanism of MBD1 in the development of pancreatic cancer.
Predominant Ashkenazi BRCA1/2 mutations in families with pancreatic cancer.

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The present study aimed to identify pancreatic cancer patients who harbor a mutation in BRCA1/2 genes within hereditary breast-ovarian cancer families. History of cancer in 1,014 families that attended to the breast-ovarian oncogenetic clinic was evaluated. Twenty-three families with pancreatic cancer were studied. In nine families wherein the probands themselves presented with pancreatic cancer, two (22%) carried a BRCA mutation (185delAG in BRCA1 in one case and 6174delT in BRCA2 in the other). In 14 families, only a family history of pancreatic cancer was elicited. Of these, seven families segregated either the 185delAG (three families) or the 6174delT (three families) mutation; one family segregated both mutations, but the parental status was not studied. Pedigree analysis shows that four of the seven pancreatic cancer cases were obligatory carriers. In summary, from among 23 families with pancreatic cancer, 6 (26%) informative BRCA1/2 mutation carriers were identified, equally cosegregating the 185delAG or the 6174delT mutation. Yet, it is not fully elucidated whether the risk for pancreatic cancer attributed to BRCA1 is similar to the high risk conferred by BRCA2. In Ashkenazi Jews, mutations in BRCA1/2 may constitute a major cause for pancreatic cancer.