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Altered Gene Expression in Cerulein-Stimulated Pancreatic Acinar Cells: Pathologic Mechanism of Acute Pancreatitis

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Acute pancreatitis is a multifactorial disease associated with the premature activation of digestive enzymes. The genes expressed in pancreatic acinar cells determine the severity of the disease. The present study determined the differentially expressed genes in pancreatic acinar cells treated with cerulein as an *in vitro* model of acute pancreatitis. Pancreatic acinar AR42J cells were stimulated with 10^{-8} M cerulein for 4 h, and genes with altered expression were identified using a cDNA microarray for 4,000 rat genes and validated by real-time PCR. These genes showed a 2.5-fold or higher increase with cerulein: lithostatin, guanylate cyclase, myosin light chain kinase 2, cathepsin C, progestin-induced protein, and pancreatic trypsin 2. Stathin 1 and ribosomal protein S13 showed a 2.5-fold or higher decreases in expression. Real-time PCR analysis showed time-dependent alterations of these genes. Using commercially available antibodies specific for guanylate cyclase, myosin light chain kinase 2, and cathepsin C, a time-dependent increase in these proteins were observed by Western blotting. Thus, disturbances in proliferation, differentiation, cytoskeleton arrangement, enzyme activity, and secretion may be underlying mechanisms of acute pancreatitis.

Key Words: Cerulein, Pancreatitis, Acinar cells, DNA microarray

INTRODUCTION

Acute pancreatitis is a multifactorial disease associated with the release of digestive enzymes to the pancreatic interstitium and the systemic circulation, as well as with increased cytokine production and release (Schoenberg et al., 1990). Cerulein pancreatitis is one of the best-characterized animal models of experimental pancreatitis and exhibits biochemical, morphological, and pathophysiological similarities to various aspects of human pancreatitis (Willemer et al., 1992). Doses of CCK or cerulein, a cholecystokinin (CCK) analog, beyond those that cause the maximum pancreatic secretion of amylase and lipase (Jensen et al., 1989; Sato et al., 1989) result in pancreatitis. The disease is characterized by dysregulation of the production and secretion of digestive enzymes, particularly the inhibition of pancreatic secretion and an elevation in serum levels, as well as cytoplasmic vacuolization, the death of acinar cells, edema formation, and infiltration of inflammatory cells into the pancreas (Schoenberg et al., 1990; Lerch and Adler, 1995). The key events appear to be a premature, intra-pancreatic activation of digestive enzyme granules, but the earliest events that trigger acute pancreatitis are unclear.

Previously we showed that intravenous infusion of cerulein induces hyperamylasemia, inflammation, edema formation, and high production of lipid peroxide, an index of oxidative cell damage, in rat pancreas (Choi et al., 1985). Cytokine expression and secretory responses using CCK were determined in freshly isolated pancreatic acinar cells. Maximum stimulation of digestive enzymes and cytokines were achieved with 10^{-9} M CCK (Kim et al., 1996) and 10^{-8} M CCK (Yu et al., 2002; Yu et al., 2005; Ju et al., 2006; Yu et al., 2006), respectively.

Stress or injury in acinar cells induces the activation of a signaling mechanisms and intracellular activation of digestive enzymes. These early events are translated into long-term responses by the expression of specific genes; these genes determine the ultimate severity of pancreatitis. We previously reported that NF-κB, AP-1, and mitogen-activated protein kinase are activated early and induce the expression of cytokines in cerulean-stimulated pancreatic acinar cells (Lee et al., 2003; Ju et al., 2006). We previously reported that cerulein (10^{-8} M) induces the activation of Ras, NF-κB, AP-1, mitogen-activated protein kinase (p38, ERK, JNK), and JAK2/STAT3 to induce expression of cytokines (IL-6, IL-8, IL-1β, TGF-β) and vascular endothelial growth factor-D (VEGF-D) in pancreatic acinar AR42J cells (Yu et al., 2002; Lee et al., 2003; Yu et al., 2005; Ju et al., 2006; Yu et al., 2006; Lee et al., 2007; Yu et al., 2008). In addition, neutrophils activated pancreatic acinar cells to induce cytokine expression via NF-κB activation (Kim et al., 1999). Gene chip analysis using 8,000 genes for rat pancre-

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ABBREVIATIONS: CCK, cholecystokinin; GC, guanylate cyclase; MLCK, myosin light-chain kinase.

atic acinar cells isolated from *in vivo* pancreatitis animal models using cerulein and taurocholate administration showed fifteen differentially expressed genes, including the pro-inflammatory mediators, MCP-1, IL-6, and gro-α as well as the transcription factor, EGR-1 (Ji et al., 2003). Cerulein (Grady et al., 1996) and taurocholate (Kim et al., 2002) activate stress kinases, including Jun kinase.

Here we determined the gene expression changes after cerulein treatment of pancreatic acinar cells to understand of the pathophysiology of acute pancreatitis. Pancreatic acinar AR42J cells were stimulated with 10^{-8} M cerulein for 4 h. Alterations in gene expression were identified using a cDNA microarray for 4,000 rat genes and validated by real-time RT-PCR. Western blot analysis was performed to confirm changes in protein expression.

METHODS

Cell culture

Rat pancreatic acinar AR42J cells (pancreatoma, ATCC CRL 1492) were obtained from the American Type Culture Collection (Manassas, Virginia, USA) and cultured in Dulbecco's modified Eagle's medium (Sigma, St. Louis, Missouri, USA) supplemented with 10% fetal bovine serum (GIBCO-BRL, Grand Island, New York, USA) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) under 44 mM sodium bicarbonate and 10% CO_2 environment as recommended (Freshney et al., 1994).

Experimental protocol

Acinar cells were plated at 2×10^6/ml in a 100-mm culture plate (Falcon 3,047, Becton Dickinson Labware, Lincoln Park, New Jersey, USA) and allowed to attach for 12 h. The cells were treated with cerulein (10^{-8} M) and cultured for 4 h. The dose and duration of cerulein treatment induced activation of NF-κB and Janus kinase (Jak)/signal transducer and activator of transcription (Stat), inflammatory cytokine expression, and hypersecretion (Kim et al., 1996; Yu et al., 2002; Yu et al., 2005; Ju et al., 2006; Yu et al., 2006).

Probe preparation and cDNA microarray

Total RNA was prepared from cells stimulated with or without cerulein for 4 h by guanidine thiocyanate extraction method (Chomczynski and Sacchi, 1987). Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia Biotec UK Ltd, Buckinghamshire, UK) was incorporated when 50 μg total RNA was reverse transcribed into cDNA and primed with oligo (dT) primer. A cDNA probe from cells cultured without cerulein was incorporated with Cy3 while that from the cells with cerulein was incorporated with Cy5. Cy3- or Cy5-labeled cDNA probe was purified with Chroma-spin 100 columns (Clontech Laboratories, Inc., Palo Alto, California, USA) following the manufacturer's instructions. A rat gene chip (4,000 genes and 2 housekeeping genes; Geno Check, Ansan, Kyunggi-do, Korea. http://www.genocheck.com) cDNA microarray was prehybridized at room temperature for 2 h in prehybridization buffer (6× SSC, 0.2% SDS, 5× Denhardt's solution and 1 mg/ml salmon sperm DNA). Different fluorescent-labeled cDNA probes were mixed and applied on the microarray following incubation at 62°C for 16 h under humidified conditions. The fluorescent images of the hybridized microarray were scanned with a fluorescent laser confocal slide scanner (GMS 418 array, Wallac Laboratories, Atlanta, Georgia, USA). Images and quantitative gene expression levels were analyzed by ImaGene™ II (BioDiscovery, Inc., Marina de Rey, California, USA).

Real-time PCR analysis

Real-time PCR analysis was performed with a SYBR® Green Realtime PCR master mix kit (Toyobo, Osaka, Japan)

Table 1. Altered genes by cerulein

No.	Gene	Primer sequences[a]	Fold[b]
	Up-regulated genes		Cy5/Cy3
1	Regeneration protein, lithostatin (Pancreatic stone protein)	(F) ACACCTTGTATCTGTGCTCAATGTAG (R) CAAACTAAAGCTGTTTGCTGTCTGGTA	7.86
2	Guanylate cyclase 2C	(F) GTGACATTGTCGGTTTCACG (R) CAAGGCCATCTTGGAAATGT	6.13
3	Myosin light chain kinase 2	(F) CTGACAAGACGGACATGTGG (R) AAGTCTTTGGCCTCGTCTGA	5.71
4	Cathepsin C	(F) TCAGACCCCAATCCTGAGTC (R) AACGGAGGCAGTTTTCCTTT	3.76
5	Progestin-induced protein	(F) CTGGCAAAAACACAGAAGCA (R) AGCATCGGCATCTGAACTCT	3.35
6	Pancreatic trypsin 2	(F) GGAGGATACACCTGCCAAGA (R) TCCTATCGAAGTTGGGATGC	2.83
	Down-regulated genes		Cy3/Cy5
1	Stathin 1	(F) AAGGATCTTTCCCTGGAGGA (R) TTCTCCTCTGCCATTTTGCT	2.66
2	Ribosomal protein S13	(F) ACCGGCTGGCTCGATACTA (R) GCTTGTGTACGCAACAGCAT	2.50

[a]Gene sequences used as forward (F) and reverse (R) primers for real-time PCR, [b]fold is the ratio of Cy5/Cy3for up-regulated genes and Cy3/Cy5 for down-regulated genes.

using a Roche Light cycler (Roche Molecular Biochemicals, Mannheim, Germany). Two micrograms of total RNA were reverse transcribed using the M-MLV reverse transcription system (Promega, Madison, Wisconsin, USA) in 20 μl in a thermocycler (Applied Biosystems GeneAmp PCR System 9700, Foster City, USA). Then 1/10 volume of each RT reaction was amplified with SYBR Green master mix (Toyobo, Osaka, Japan) containing 10 μM of customized primers and GAPDH (Table 1); the reactions were measured in a Light Cycler real-time PCR detection system (Roche Molecular Biochemicals). PCR was conducted using the following cycling conditions: pre-incubation and denaturation at 95°C for 10 min, followed by amplification with 40 cycles of: denaturation at 95°C for 30 s with a thermal ramp rate of 20°C/s; annealing at 60°C for 5 s with a thermal ramp rate of 20°C/s; amplification at 72°C for 30 s with a thermal ramp rate of 20°C/s. The mRNA levels of target genes were normalized to GAPDH. The primers used in real-time PCR were listed in Table 1. The primers for GAPDH were forward, ACCACAGTCCATGCCATCAC and reverse, TCCACCACCCTGTTGCTGTA, giving a 460 bp PCR product.

Western blot analysis for guanylate cyclase, myosin light chain kinase 2, and cathepsin C

Cells were treated with cerulein (10^{-8} M) and cultured for 6 h. The cells were harvested and lysed in Tris-HCl buffer (pH 7.4) containing 0.5% Triton X-100 and a protease inhibitor cocktail (Boehringer-Mannheim, Indianapolis, Indiana, USA) for the determinations of guanylate cyclase, myosin light chain kinase 2, and cathepsin C. The protein concentration of each sample was determined by Bradford assay (Bio-Rad laboratories, Hercules, CA, USA). Protein (50 μg) was separated on 8~10% SDS-polyacrylamide gel electrophoresis under reducing conditions, and transferred onto nitrocellulose membranes (Amersham Inc., Arlington Heights, IL) by electroblotting. The transfer of protein and equality of loading in all lanes was verified using reversible staining with Ponceau S. The membranes were blocked using 5% nonfat dry milk in TBS-T (Tris-buffered saline and 0.15% Tween 20) for 3 h at room temperature. The proteins were detected with antibodies for guanylate cyclase (1 : 1,000; sc-34428), myosin light chain kinase (1 : 1,000; sc-12450), cathepsin C (1 : 1,000; sc-74590) and actin (1 : 1,000; sc-1615) (all from Santa Cruz Biotechnology, Santa Cruz, CA) diluted in TBS-T containing 5% dry milk, and incubated at 4°C overnight. After washing in TBS-T, the immunoreactive proteins were visualized using goat anti-rabbit and donkey anti-mouse secondary antibodies conjugated to horse radish peroxidase, followed by enhanced chemiluminescence (Amersham). Actin was used as a loading control.

RESULTS

cDNA microarray

To characterize changes in mRNA expression induced by cerulein, rat pancreatic acinar AR42J cells were stimulated with or without cerulein for 4 h, and then total RNA was extracted. cDNA prepared from total RNA were labeled with Cy5 fluorochrome (with cerulein, red) and Cy3 (without cerulein, green) (Fig. 1A) to indicate relative expression levels. A Cy5/Cy3 ratio of 1 indicates identical expression.

Up- and down-regulated genes

Most genes showed only small differences after cerulein stimulation, indicated by Cy5/Cy3 ratios between 2 and 0.5. We extracted genes with expression levels more than 2.5 fold higher or lower after cerulein (Table 1). Two housekeeping genes, GADPDH and tubulin, were used as internal controls to correct for mRNA abundance. These genes showed similar intensities of signals in hybridized microarray, and the mean of those control genes were used to normalize the target genes. Cerulein elevated the expression of lithostatin, guanylate cyclase, myosin light chain kinase 2, cathepsin C, progestin-induced protein, and pancreatic trypsin 2. Cerulein down-regulated stathin 1 and ribosomal protein S13. These genes have a variety of functions, including cell proliferation and differentiation (lithostatin, progestin-induced protein, stathin 1, guanylate cyclase 2, trypsin 2), carcinogenesis (lithostatin, progestin-

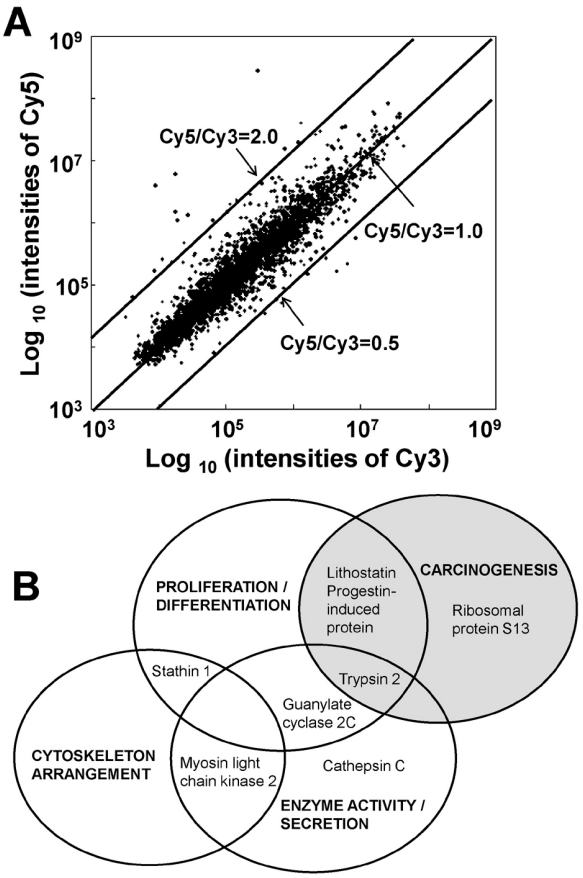

Fig. 1. A representative scatter plot of cDNA microarray analysis and modified Venn diagram according to gene function. (A) AR42J cells stimulated with cerulein (labeled with Cy5) or without cerulein (labeled with Cy3) were labeled and hybridized to the cDNA microarray. Cy5/Cy3 ratios indicate relative expression levels. (B) Venn diagram of genes shows functional overlap. Cerulein changed genes related to cell proliferation and differentiation, carcinogenesis, enzyme activity and secretion and cytoskeleton arrangement.

induced protein, ribosomal protein S13, trypsin 2), enzyme activity and secretion (myosin light chain kinase 2, cathepsin, trypsin 2, guanylate cyclase 2), and cytoskeleton arrangement (myosin light chain kinase 2, stathin 1) (Fig. 1B).

Real-time PCR analysis

To confirm these changes in gene expression, cells were stimulated with cerulein for up to 4 h. Real-time PCR analysis showed a time-dependent increase in 6 genes (lithostatin, guanylate cyclase, myosin light chain kinase 2, cathepsin C, progestin-induced protein, and pancreatic trypsin 2) and a time-dependent decrease in 2 genes (stathin 1 and ribosomal protein S13) (Fig. 2). At 4 h, cerulein increased mRNA levels of lithostatin, guanylate cyclase, and myosin light chain kinase 2 almost 10-fold, higher than in microarray analysis. Cerulein increased cathepsin C, progestin-induced protein, and pancreatic trypsin 2 about 2.5-fold. Cerulein decreased stathin 1 and ribosomal protein S13 levels about 2.5 fold, similar to changes in the microarray.

Western blot analysis of guanylate cyclase, myosin light chain kinase 2, and cathepsin C

To confirm changes in protein expression, Western blot analysis was performed using commercially available antibodies (Fig. 3). Cells were cultured in the presence of cerulein for 6 h, harvested, and lysed. Cerulein increased lev-

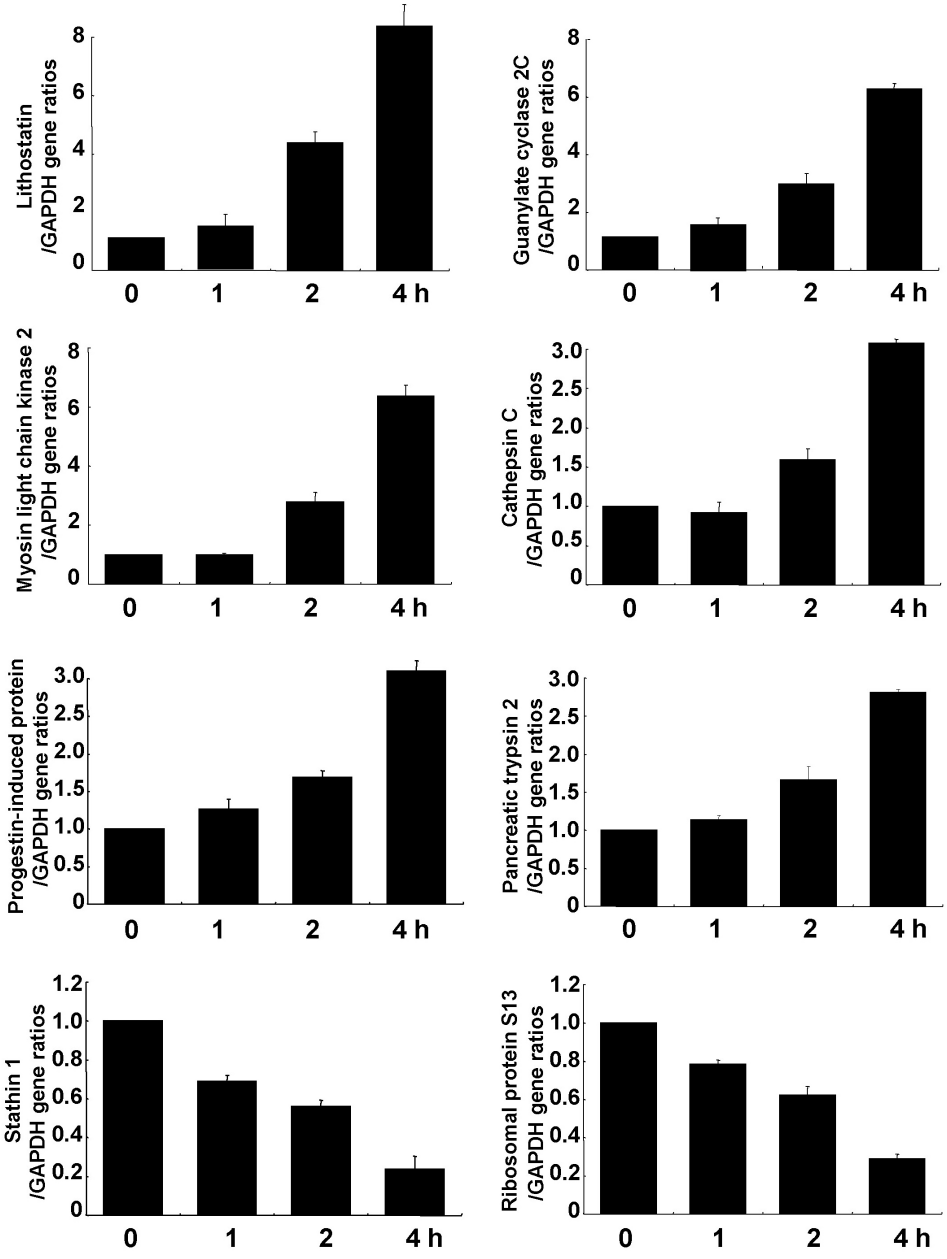

Fig. 2. Time-dependent mRNA expression after cerulein treatment for 8 genes. Relative mRNA expression in AR42J cells treated with cerulein (10^{-8} M) was assessed by real-time RT-PCR. The internal standard (GAPDH) was coamplified with each gene.

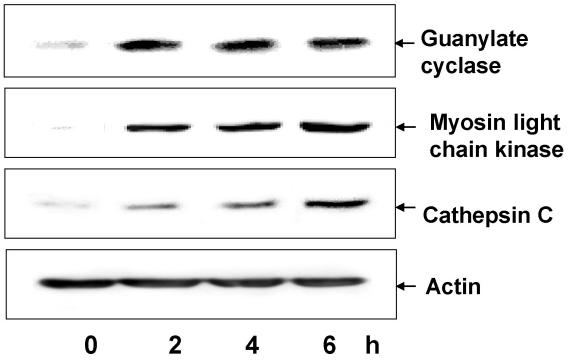

Fig. 3. Western blot analysis for guanylate cyclase, myosin light chain kinase 2, and cathepsin C. Cells were cultured with cerulein for 6 h, harvested, lysed, and extracted. Whole cell extracts (50 μg of protein/lane) were loaded, separated by 8~10% SDS-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membranes by electroblotting. The membranes were blocked with 5% nonfat dry milk in TBS-T. The proteins were detected with specific antibodies. After washing in TBS-T, the immunoreactive proteins were visualized using secondary antibodies conjugated to horseradish peroxidase, followed by enhanced chemiluminescence. Actin was used as a loading control.

els of guanylate cyclase, myosin light chain kinase 2, and cathepsin C, but did not change actin levels.

DISCUSSION

Doses of cerulein beyond those that cause the maximum pancreatic secretion of digestive enzymes results in pancreatitis (Jensen et al., 1989; Sato et al., 1989). The characteristic events of pancreatitis include the dysregulation of digestive enzyme production, cytoplasmic vacuolization, the death of acinar cells, edema formation, and an infiltration of inflammatory cells into the pancreas (Willemer et al., 1992; Lerch and Adler, 1995). The premature activation of digestive enzymes is indicated here as the up-regulation of cathepsin C and trypsin 2 (Willemer et al., 1992; Lerch and Adler, 1995). Cerulein-induced acute pancreatitis shows prominent interstitial edema and acinar cell vacuolization in rats (Zhou et al., 1994; Namkung et al., 2004), which was inhibited by a calcium channel blocker (Zhou et al., 1994) and a calpain I inhibitor (Virlos et al., 2004). Therefore, intracellular calcium and calpain activation may be involved in the pathogenesis of edema and vacuole formation in cerulein-induced pancreatitis.

The pancreas secretes primarily two types of metabolically important proteins: digestive enzymes such as amylase and lipase, and hormones, including insulin and glucagon. Lithostatin is the only protein secreted from the pancreas that has no known digestive or hormonal activity. Human lithostatin is a 144-residue protein that is identical to the reg protein, expressed in the endocrine compartment of the regenerating pancreas (Watanabe et al., 1990). It contains a trypsin-sensitive cleavage site that is conserved in several species. Tryptic cleavage produces the amino-terminal decapeptide and a carboxy-terminal peptide of 133 amino acid residues (Graf et al., 2001). The latter has a tendency to precipitate at neutral pH and is the predominant component of the protein matrix of pancreatic stones (calcium carbonate crystals). The physiological role of lithostatin is to stabilize pancreatic secretions that are saturated with calcium carbonate, as demonstrated through *in vitro* assays that show the inhibitory action of lithostatin against nucleation and growth of calcium carbonate crystals (Multigner et al., 1983). Thus, lithostatin is secreted into the pancreatic juice where it inhibits stone formation (Patard et al., 2003). Lithostatin was discovered in regenerating liver or regenerating islets in the pancreas, but not in normal tissues (Terazono et al., 1988). Lithostatin expression is low in the normal colon, but up-regulated in Crohn's diseases and ulcerative colitis (Hartupee et al., 2001) and colorectal tumors (Violette et al., 2003) as a prognostic indicator of tumor survival (Violette et al., 2003). Cerulein up-regulates lithostatin and may support regeneration and proliferation of pancreatic acinar cells, indicating a potential connection between pancreatitis and the development of pancreatic cancer.

Guanylate cyclase (GC) has two forms, soluble and particulate forms, and mediates cGMP production (Wedel and Garbers, 1997). Three isoforms of mammalian membrane GC (GC-A, GC-B, and GC-C) serve as receptors for natruretic peptides, heat-stable enterotoxin, and guanylin (Drewett and Garbers, 1994). Membrane GC is one polypeptide chain with high homology in the cytoplasmic domains but differences in extracellular ligand-binding domains (Drewett and Garbers, 1994). Little is known about intrinsic mechanisms of the regulation of particulate GC. Dephosphorylation (Potter and Garbers, 1992) and oligomerization (Lowe, 1992) of GC receptors, as well as association of GC with a regulatory phosphatase (Chinkers, 1994), regulate GC activity. Membrane GC may play a role in the physiology of the exocrine pancreas, particularly in regulating acinar cell growth (Seidler et al., 1989). CCK increases the accumulation of cGMP in pancreatic acinar cells, which activates cytosolic ADP-ribosyl cyclase activity and stimulates intracellular Ca^{2+} stores (Sternfeld et al., 2003). Therefore, increased GC expression may contribute to cell growth and exocrine function in acinar cells during pancreatitis.

Myosin light-chain kinase (MLCK), first purified from rat pancreas, phosphorylates two light chain subunits of myosin, a doublet with components of 18 and 20 kDa (Bissonnette et al., 1989). The enzyme is completely dependent on Ca^{2+} and calmodulin. Pancreatic MLCK may regulate myosin phosphorylation and enzyme secretion. Yoshida et al. (Yoshida et al., 2000) demonstrated that MLCK 4 is an important intracellular mediator during stimulus-secretion coupling of rat pancreatic acinar cells, whereas MLCK 2 has no effect on CCK-induced enzyme secretion. Therefore, MLCK 2 may contribute to cytoskeletal arrangement by mediating myosin phosphorylation and exocrine function by stimulating enzyme secretion in pancreatic acinar cells.

Acute pancreatitis increases intracellular chymotrypsin activity (Piotrowski et al., 2003). Two other enzymes with chymotrypsin-like activity, proteasome and lysosomal cathepsin A, exist in the pancreas (Piotrowski et al., 2003). Cathepsin C is a dipeptidyl peptide hydrolase acting on dipeptide esters and amides (Rojas-Espinosa et al., 1975). Cerulein-induced up-regulation of cathepsin C may hydrolyze pancreatic dipeptides and induce acinar cell damage during acute pancreatitis.

Progestin induces the differentiation of both endometrial stromal and epithelial cells, acting as the "differentiating" or "growth limiting" hormone in the endometrium (Bulun

et al., 2006). This progestin effect is mediated by progesterone receptors in stromal cells (Kurita et al., 2000). In contrast, progestins control mammary gland tumorigenesis after binding to progesterone receptors (Carnevale et al., 2007). The progesterone receptor functions either as a transcription factor or as a signaling activator in a breast cancer cell line (Carnevale et al., 2007). Progestin initiates Wnt-beta-catenin signaling for proliferation and differentiation in rat uterine stromal cells (Rider et al., 2006). A progesterone antagonist prevented BRCA1-mediated mammary tumorigenesis in mice, suggesting anti-progesterone treatment may be effect for breast cancer prevention in individuals with BRCA1 mutation (Poole et al., 2006). Treatment of progesterone stimulates cell proliferation within the islets of Langerhans in rats (Nieuwenhuizen et al., 1999). Therefore, cerulein-induced increases in progestin may increase cell proliferation and relate pancreatitis and pancreatic cancer.

The pancreas is an important endocrine and exocrine secretory organ in mammals. Many digestive enzymes are synthesized in pancreatic acinar cells (Gorelick and Otani, 1999). Under normal conditions, these enzymes remain inactive in isolated zymogen granules inside pancreatic acinar cells (Kassell and Kay, 1973) and only become active after entering the small intestine. The activation of a key enzyme in zymogen granules, trypsin, requires proteolytic activation by cleavage of the propeptide, which can be completed in the duodenum through activation by the brush border endoprotease, enteropeptidase (Kassell and Kay, 1973). This initial activation of trypsin can further activate trypsinogen into active trypsin and other zymogens, such as chymotrypsinogen, protelastase, and prophospholipase to their active states (Gorelick and Otani, 1999). During acute pancreatitis, these digestive enzymes are prematurely activated before leaving the pancreas and start digesting the pancreas to lead to acute pancreatitis (Steer, 1999). Lithostatin contains a trypsin-sensitive site, and up-regulated trypsin 2 may cleave lithostatin to tryptic cleavage products, including a carboxy-terminal peptide of 133 amino acids. In addition, trypsin is activated in pancreatic cancer cells (Chen et al., 2009) to stimulate growth and adhesiveness in an autocrine manner (Giancotti and Mainiero, 1994). The stage and type of carcinoma is related to the level of trypsin associated with cell invasion and extracellular matrix degradation (Koivunen et al., 1991; Walz and Fenton, 1994). Therefore, up-regulation of trypsin 2 in pancreatic acinar cells may contribute to the development of pancreatic cancer.

Stathmin, a major microtubule-destabilizing protein, is down-regulated by cerulein. In general, stathmin interacts directly with soluble tubulin to form a complex that sequesters free tubulin and impedes the polymerization of microtubules (Belmont and Mitchison, 1996). The depolymerizing activity of stathmin is turned off upon its phosphorylation during the onset of mitosis, leading to formation of the mitotic spindle. Conversely, reactivation of stathmin by dephosphorylation is necessary before the cells exit mitosis and enter a new interphase (Rubin and Atweh, 2004). In addition to its role in mitosis and cell cycle progression, stathmin is also involved in diverse cell functions, such as cell proliferation and differentiation (Larsson et al., 1995). Stathmin is expressed in actively proliferating cells (Iancu et al., 2001), including liver regeneration after partial hepatectomy (Koppel et al., 1993) and hepatic ischemia-reperfusion injury (Barone et al., 2005), whereas its expression is dramatically decreased upon the induction of differentiation and cessation of proliferation of leukemia cells (Melhem et al., 1991), and in the later stages of megakaryocyte maturation (Rubin et al., 2003). Stathmin is abundantly expressed in fetal liver, but dramatically decreased in adult liver (Bièche et al., 2003). Cerulein may induce differentiation and cessation of proliferation by decreasing stathin expression, but cerulein also increased lithostatin and progestin, two genes that increase cell proliferation, indicating an imbalance between cell proliferation and differentiation.

Ribosomal protein S13 is found in the head region of the small subunit, where it interacts with the central protuberance of the large ribosomal subunit and with the P site-bound tRNA through its extended C terminus (Cukras and Green, 2005; Noller et al., 2005). The bridging interactions between the large and small subunits are dynamic and are critical in the molecular motions of the translation cycle. S13 provides a direct link between the tRNA-binding site and the movements in the head of the small subunit seen during translocation, thereby providing signal transduction (Cukras and Green, 2005). The expression level of ribosomal protein S13 was lower in NK/T cell lymphoma than in normal lymphocytes, indicating that it plays a role in the development of the NK/T cell lymphoma (Yang et al., 2006). Cerulein decreases S13 expression, indicating disturbances in translation or signal transduction may be involved in the pathogenesis and/or development of pancreatitis.

In our previous studies, cerulein induced the expression of cytokines (IL-6, IL-8, IL-1β, TGF-β) and VEGF-D by the activation of NF-κB, AP-1, Mitogen-activated protein kinases, and Jak2/Stat3 in pancreatic acinar AR42J cells (Yu et al., 2002; Lee et al., 2003; Yu et al., 2005; Ju et al., 2006; Yu et al., 2006; Lee et al., 2007; Yu et al., 2008). Here, cerulein up-regulated 6 genes (lithostatin, guanylate cyclase, myosin light chain kinase 2, cathepsin C, progestin-induced protein, pancreatic trypsin 2) and down-regulated 2 genes (stathin 1, ribosomal protein S13) that are related to proliferation, differentiation, carcinogenesis, cytoskeletal arrangement, enzyme activity, and secretion. These changes may accompany inflammatory events. Since lithostatin, progestin-induced protein, trypsin, and ribosomal protein S13 are involved in carcinogenesis, the relationship between pancreatitis and the development of pancreatic cancer requires further study. Additional *in vivo* studies should also be performed for comparison to human pathophysiology.

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