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Induced thrombospondin expression in the mouse pancreas during pancreatic injury

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Abstract

Chronic pancreatitis is a disease characterized by pancreatic fibrogenesis in response to sustained or repetitive injury. Pancreatic stellate cells (PSC) are interstitial cells that produce excessive extracellular matrix components during the process of fibrogenesis and therefore play a central role in the pathogenesis of chronic pancreatitis. Because the matricellular proteins thrombospondin-1 (TSP-1) and TSP-2 have a role in regulating fibrogenesis in other tissues, the expression of these major TSP isoforms in the whole pancreas was measured in a mouse model of repetitive pancreatic injury. Specifically, mice were treated with cerulein, 50 μg/kg/h × 6 h with treatments repeated once or twice every 48 h. Expression was also evaluated in cultured PSC. PSC were isolated by outgrowth from normal mouse pancreas and expression of TSP-1 and TSP-2 was evaluated after serum-activation. The mRNA transcripts for TSP-1 and TSP-2 were increased, 16-fold and 87-fold respectively, in the pancreas in response to repetitive injury. In cultured PSC, these transcripts were also increased in response to serum and increases in mRNA were reflected by the secretion of TSP-1 and TSP-2 proteins by PSC into culture media. In summary, PSC may be an important source of both TSP-1 and TSP-2 in the pancreas in response to injury. These modulators of fibrogenesis could play a role in the development of pancreatic fibrosis that characterizes chronic pancreatitis.

Keywords: Pancreatic stellate cells; Thrombospondin-1; Thrombospondin-2; Cerulein; Chronic pancreatitis

1. Introduction

Chronic pancreatitis is a disorder characterized morphologically by the induction of fibrogenesis within the pancreatic parenchyma and epithelial atrophy caused by loss of acinar cell mass. Understanding the mechanisms by which sustained injury to pancreatic acinar and ductal cells leads to these abnormalities is now the focus of intense scrutiny. Pancreatic stellate cells (PSC) are mesenchymal cells interdigitated among acinar cells and are primarily responsible for the production of excess extracellular matrix materials in response to injury (Apte et al., 1998; Bachem et al., 1998), a process that ultimately leads to excess fibrosis. A variety of stimuli appear to be responsible for the activation of PSC to a cell phenotype with smooth muscle characteristics (Powell et al., 1999). The cytokine transforming growth factor-β_1 (TGFβ1) may play a central role in stimulating PSC to produce matrix (Vogelmann, Ruf, Wagner, Adler, &

Abbreviations: PSC, pancreatic stellate cells; TSP, thrombospondin; TGFβ, transforming growth factor beta; αSMA, α-smooth muscle actin; FCS, fetal calf serum

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Menke, 2001). Additionally, the presence of a fibrotic matrix milieu around PSC may also play a role in sustaining the activated phenotype.

The thrombospondins (TSPs) are secreted "matricellular" proteins that interact with extracellular matrix components and cytokines such as transforming growth factor-β1 to promote angiogenesis and possibly perpetuate a profibrogenic response (Adams & Lawler, 2004; Esemuede, Lee, Pierre-Paul, Sumpio, & Gahtan, 2004). Two thrombospondin isoforms, TSP-1 and TSP-2, have been studied in detail. Mice with loss of function of each of these isoforms have been examined in the basal state and have mild phenotypic alterations of skeletal and pulmonary abnormalities in the case of TSP-1 and diminished scarring in response to wound healing in the case of TSP-2 (Kyriakides, Zu, Smith et al., 1998; Lawler et al., 1998).

One mechanism by which TSP-1 and TSP-2 might regulate fibrogenesis is through the regulation of latent TGF-β1 activation that typically occurs in the extracellular milieu (Abdelouahed, Ludlow, Brunner, & Lawler, 2000; Schultz-Cherry, Ribeiro, Gentry, & Murphy-Ullrich, 1994. TSP-1 may activate latent TGF-β by binding to the latency associated peptide-latent TGF-β1binding protein (LAP-LTBP) complex, facilitating the release of active TGF-β from LAP (Abdelouahed et al., 2000). Specifically, the TSP-1 amino acid sequence $K^{412}RFK^{415}$ binds to the LAP sequence $L^{54}SKL^{57}$ to induce a conformational change of LAP, releasing active TGF-β1 (Ribeiro, Poczatek, Schultz-Cherry, Villain, & Murphy-Ullrich, 1999; Young & Murphy-Ullrich, 2004). TSP-2 lacks the KRFK motif but this isoform has WXXW motifs, as does TSP-1, that have also been implicated in its binding to TGF-β1. However, it remains unclear whether this binding might increase or decrease the activation of latent TGF-β1 (Murphy-Ullrich & Poczatek, 2000).

The roles of TSP-1 and TSP-2 in modulating the response of specific tissues such as the pancreas to injury have not been examined in detail. Blocking the interaction of TSP-1 with TGFβ1 with specific peptides decreased TGFβ1 activation and diminished glomerular extracellular matrix accumulation in a rat model of glomerulonephritis (Daniel et al., 2004). The purpose of the studies described here was to determine whether pancreatic stellate cells might be source of TSP isoforms and whether the expression of the TSPs is increased by exposure to serum, a stimulus to serum-starved PSC that recapitulates in culture many of the changes in gene expression that correlate with activation of these cells and fibroblasts to a pro-fibrogenic smooth muscle phenotype (Chang et al., 2004; Reif et al., 2003).

2. Materials and methods

2.1. Animal treatment and pancreatic stellate cell isolation

Sustained pancreatic injury was induced by repetitive supraphysiological hormonal stimulation with cerulein (Neuschwander-Tetri, Bridle, Wells, Marcu, & Ramm, 2000). Cerulein (ICN Biomedicals, Costa Mesa, CA) was prepared as a 0.1 mM stock solution in 0.1 M $NaHCO_3$, pH 8.75, and diluted in sterile 0.15 M NaCl immediately before use. Fed female Swiss-Webster mice weighing 15–18 g received six hourly 0.1 ml intraperitoneal injections of cerulein (50 μg/kg/h) or an equal volume of saline to induce pancreatitis. The 6 hourly injections given in one day constitute one treatment. Animals were sacrificed by carbon dioxide inhalation followed by cervical dislocation and pancreatic RNA was isolated from 5 animals in each treatment group and pooled for expression analysis. Animal use was in accordance with current guidelines and was approved by the institutional animal care committee of Saint Louis University. Unless otherwise noted, reagents were obtained from Sigma Chemical Company (St. Louis, MO).

2.2. Isolation of pancreatic RNA

To prepare total RNA for real-time PCR, pancreatic tissue was immediately removed and homogenized in a phenol/guanidine/isothiocyanate reagent (Trizol, Life Technologies, Grand Island, NY) at 4 °C. The aqueous layer containing RNA was re-extracted once with phenol-chloroform-isoamyl alcohol (25:24:1) and the RNA was precipitated with isopropyl alcohol, washed with ethanol and resuspended in deionized formamide for storage at −80 °C, a technique necessary to facilitate stability (Sparmann, Jäschke, Löhr, Liebe, & Emmrich, 1997). The quality of RNA was confirmed by agarose gel electrophoresis. All samples were assayed in duplicate or triplicate and the means of the repeated assays were used as the assay results.

2.3. mRNA measurement

RNA abundance was measured by real-time PCR (ABI Prism 7700 Sequence Detector, Applied Biosystems, Foster City, CA). PCR primers were designed using Primer Express software (Applied Biosystems) according to the manufacturer's instructions and purchased from Gibco-BRL. PCR primer sequences used for these experiments were: