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Pancreatitis-Associated Protein Inhibits Human Pancreatic Stellate Cell MMP-1 and -2, TIMP-1 and -2 Secretion and RECK Expression

Ling Li[a, d] Max G. Bachem[a] Shaoxia Zhou[a] Zilin Sun[d] Jinfei Chen[d]
Marco Siech[b] Daniel Bimmler[c] Rolf Graf[c]

[a]Department of Clinical Chemistry, University Hospital Ulm, Ulm, and [b]Department of General and Vascular Surgery, Ostalb-Klinikum Aalen, Aalen, Germany; [c]Swiss HPB Center, Department of Visceral and Transplantation Surgery, University Hospital of Zurich, Zurich, Switzerland; [d]Department of Internal Medicine, Zhongda Hospital of Southeast University, Nanjing, China

Key Words
Pancreatitis-associated protein · Pancreatic stellate cell · Matrix metalloproteinase · Tissue inhibitors of matrix metalloproteinases

Abstract
Background/Aims: Pancreatic stellate cells (PSCs) play a key role in fibrogenesis associated with acute and chronic pancreatitis. Pancreatitis-associated protein (PAP), an acute-phase protein, is dramatically upregulated during acute and chronic pancreatitis. Assuming a protective role of PAP, we investigated its effects on human PSCs. ***Methods:*** PSCs were obtained by outgrowth from fibrotic human pancreas tissue. PAP was expressed in the yeast *Pichia pastoris*. PAP was added at 10 ng/ml to cultured PSCs. Cell proliferation was determined by bromodeoxyuridine incorporation. PSC migration was assessed by a wound healing assay. Collagen types I and III, fibronectin, matrix metalloproteinases (MMPs), tissue inhibitors of MMPs (TIMPs) and reversion-inducing cysteine-rich protein with Kazal motifs (RECK) were demonstrated on protein and mRNA level. ***Results:*** PAP had no significant effect on PSC proliferation and migration. Cell-associated fibrillar collagen types I and III and fibronectin increased after addition of PAP to PSCs. PAP diminished the expression of MMP-1 and -2 and TIMP-1 and -2 and their concentrations in PSC supernatants. RECK was detected on the surface of PSCs and its expression was reduced after PAP application. ***Conclusions:*** Our data offer new insights into the biological functions of PAP, which may play an important role in wound healing response and cell-matrix interactions.

Introduction

The identification and characterization of pancreatic stellate cells (PSCs) few years ago had a significant impact on research related to pancreatic fibrogenesis [1, 2]. Accumulating evidence from in vivo and in vitro studies indicates a central role for PSCs in fibrogenesis associated with acute and chronic pancreatitis [3]. In normal pancreas, PSCs are quiescent and can be identified by the presence of vitamin A-containing lipid droplets in the cytoplasm. In response to pancreatic injury or inflammation, PSCs change their phenotype from the fat-storing to the highly active myofibroblast-like cell type. They proliferate [1, 2], migrate [4], and produce significant amounts of extracellular matrix (ECM) [5], matrix metalloproteinases (MMPs) [6] and tissue inhibitors of metalloproteinases (TIMPs) [7, 8].

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PD Dr. Rolf Graf
Department of Visceral and Transplant Surgery, University Hospital of Zurich
Raemistrasse 100
CH–8091 Zurich (Switzerland)
Tel. +41 44 255 2071, Fax +41 44 255 4449, E-Mail rolf.graf@usz.ch

Isolated PSCs undergo spontaneous activation when cultured on plastic for more than 48 h [1, 2]. PSCs assume the myofibroblast-like phenotype and secrete increased amounts of ECM proteins including collagen types I and III and fibronectin. ECM accumulation and degradation are dynamic and regulated processes in physiological and pathological events [9]. The mutual interaction between the cells and ECM determines cell fate, tissue specificity, and homeostasis.

Myofibroblastic activated PSCs are not only the major source of the ECM but also produce MMPs which remodel these proteins. The MMP family is a group of proteases including collagenases, gelatinases, stromelysins, and membrane-type MMPs with different ECM substrate specificities [10, 11]. The activity of MMPs is potentially regulated at the levels of transcription, proenzyme activation, or inhibition of activated enzyme by TIMPs [12, 13].

TIMPs have been shown to be secreted glycoproteins that bind to and inhibit the active forms of MMPs on a 1:1 stoichiometric basis. Four TIMPs (TIMP-1 to -4) have been identified in vertebrates and they share similar functional motifs [14–17]. By inhibiting MMP activities, they participate in tissue remodeling of the ECM. The balance between MMP and TIMP activities is involved in both normal and pathological events such as wound healing, tissue remodeling, and angiogenesis.

Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) was recently discovered as an endogenous cell surface glycoprotein that inhibits the secretion and proteolytic activity of MMP-9 and the proteolytic activities of MMP-2 and MT1-MMP [18, 19]. RECK is widely expressed in human tissues, and it acts as a unique regulator of ECM turnover.

Pancreatitis-associated protein (PAP) is an acute-phase stress protein of the pancreas [20, 21]. It is constitutively synthesized by pancreatic acinar cells and secreted into pancreatic juice at low concentrations [22]. In experimental acute and chronic pancreatitis, PAP is dramatically increased [23, 24]. PAP levels significantly correlate with severity of the pancreatic injury and even survival [25]. Several approaches toward elucidation of PAP function have been pursued, and it has been shown to be antiapoptotic [26, 27], mitogenic [28], anti-inflammatory [29], and able to promote cell adhesion to the ECM [30]. More recently, the antiapoptotic and anti-inflammatory role of PAP was corroborated in a knockout mouse lacking HIP/PAP (regIIIβ) [31]. Nevertheless, the biological role of PAP, particularly during pancreatitis, needs to be further clarified.

The aim of this study was to investigate the effects of PAP on proliferation, migration, ECM synthesis, MMP secretion, TIMP secretion and RECK expression of activated human PSCs, to evaluate the role of PAP with regard to fibrogenesis during acute and chronic pancreatitis.

Material and Methods

Material

Reagents were purchased from the following sources: bisbenzimide from Hoechst (Frankfurt, Germany); Delfia Eu-labeled streptavidin and enhancement solution from PE-Life-Science-Wallac (Turku, Finland); mouse anti-human RECK from BD Biosciences (Heidelberg, Germany); goat anti-human RECK, mouse anti-human TIMP-2, mouse anti-human MMP-1 and -2, and biotinylated anti-human MMP-1 and -2 from R&D Systems Inc. (Minneapolis, Minn., USA); rabbit anti-human collagen type I and mouse anti-human TIMP-1 from Chemicon International (Temecula, Calif., USA); biotin-labeled rabbit anti-human collagen type III from Acris (Herford, Germany); rabbit anti-fibronectin from Dade-Behring (Marburg, Germany); rabbit anti-mouse IgG, anti-bromodeoxyuridine (BrdU), horseradish peroxidase (HRP) anti-mouse, HRP anti-rabbit, biotinylated swine anti-goat, and HRP-conjugated streptavidin from DAKO (Glostrup, Denmark); Alexa Fluor 568-conjugated streptavidin, Alexa Fluor 488-conjugated streptavidin, Alexa Fluor 488-conjugated rabbit anti-mouse IgG and propidium iodide from Molecular Probes (Eugene, Oreg., USA); High Pure RNA Isolation Kit and LightCycler DNA Master SYBR Green I from Roche (Mannheim, Germany); Superscript from GIBCO-BRL (Paisley, UK); QIAQuick Gel Extraction Kit from Qiagen (Hilden, Germany); LightCycler-Primer Set for human RECK, MMP-1, TIMP-1 and -2 from Search-LC (Heidelberg, Germany).

Cell Isolation and Culture

Human PSCs were isolated by outgrowth, using surgically resected pancreas as described previously [2]. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 (1:1 v/v) containing 10% fetal calf serum (FCS) and used from passage 3–8. Purity of the cells was assessed by morphology and cytofilament staining of vimentin (100%), α-smooth muscle actin (>90%) and desmin (20–40%).

Pancreatitis-Associated Protein

The cloning and production of human recombinant PAP followed the same strategy as that presented for the rat and mouse isoforms [22, 32]. PAP was expressed in the yeast *Pichia pastoris* and purified from medium supernatants. A sterile filtered stock solution (100 μg/ml) was stored in aliquots at –20°C and protected from light. Before use, the stock solution was diluted to the final concentration in DMEM, and added to cell cultures at 10 ng/ml.

Determination of Cell Proliferation

PSC proliferation was determined by BrdU incorporation as previously described [33]. BrdU-positive nuclei and total nuclei were counted. BrdU incorporation is presented as the percentage of positive nuclei to total cell number.