Uncoupling protein 2 deficiency reduces proliferative capacity of murine pancreatic stellate cells

Sarah Müller, Sandra Maria Klingbeil, Andreea Sandica and Robert Jaster

Rostock, Germany

BACKGROUND: Uncoupling protein 2 (UCP2) has been suggested to inhibit mitochondrial production of reactive oxygen species (ROS) by decreasing the mitochondrial membrane potential. Experimental acute pancreatitis is associated with increased UCP2 expression, whereas UCP2 deficiency retards regeneration of aged mice from acute pancreatitis. Here, we have addressed biological and molecular functions of UCP2 in pancreatic stellate cells (PSCs), which are involved in pancreatic wound repair and fibrogenesis.

METHODS: PSCs were isolated from 12 months old (aged) UCP2$^{-/-}$ mice and animals of the wild-type (WT) strain C57BL/6. Proliferation and cell death were assessed by employing trypan blue staining and a 5-bromo-2'-deoxyuridine incorporation assay. Intracellular fat droplets were visualized by oil red O staining. Levels of mRNA were determined by RT-PCR, while protein expression was analyzed by immunoblotting and immunofluorescence analysis. Intracellular ROS levels were measured with 2', 7'-dichlorofluorescin diacetate. Expression of senescence-associated β-galactosidase (SA β-Gal) was used as a surrogate marker of cellular senescence.

RESULTS: PSCs derived from UCP2$^{-/-}$ mice proliferated at a lower rate than cells from WT mice. In agreement with this observation, the UCP2 inhibitor genipin displayed dose-dependent inhibitory effects on WT PSC growth. Interestingly, ROS levels in PSCs did not differ between the two strains, and PSCs derived from UCP2$^{-/-}$ mice did not senesce faster than those from corresponding WT cells. PSCs from UCP2$^{-/-}$ mice and WT animals were also indistinguishable with respect to the activation-dependent loss of intracellular fat droplets, expression of the activation marker α-smooth muscle actin, type I collagen and the autocrine/paracrine mediators interleukin-6 and transforming growth factor-β1.

CONCLUSIONS: A reduced proliferative capacity of PSC from aged UCP2$^{-/-}$ mice may contribute to the retarded regeneration after acute pancreatitis. Apart from their slower growth, PSC of UCP2$^{-/-}$ mice displayed no functional abnormalities. The antifibrotic potential of UCP2 inhibitors deserves further attention.

(*Hepatobiliary Pancreat Dis Int 2016;15:647-654*)

KEY WORDS: pancreatitis; proliferation; stellate cell biology; uncoupling protein 2

Introduction

Regeneration of the exocrine pancreas after pancreatitis is a tightly regulated process that involves transient phases of inflammation, metaplasia, and redifferentiation. The regenerative process is driven by coordinated interactions between resident and non-resident cells with acinar cells, leukocytes and extracellular matrix (ECM)-producing cells as the key players.[1] Pancreatic stellate cells (PSCs) are the main source of ECM proteins in the diseased pancreas.[2, 3] Quiescent PSC (roughly 4% of all pancreatic cells[4]) store vitamin A in fat droplets[4, 5] and display properties of stem cells.[6] In response to pancreatic injury, the cells undergo a transition from a quiescent to an activated stage,[2, 3] which is characterized by an enhanced expression of the myofibroblastic marker α-smooth muscle actin (α-SMA).[4, 5] PSC activation involves cell proliferation, an enhanced capability to synthesize large amounts of collagen type I and other ECM proteins, as well as exhibition of a secretory phenotype that is characterized by the secretion of various profibrogenic and mitogenic mediators.[2, 3]

Author Affiliations: Department of Medicine II, Division of Gastroenterology, Rostock University Medical Center, E.-Heydemann-Str. 6, 18057 Rostock, Germany (Müller S, Klingbeil SM, Sandica A and Jaster R)

Corresponding Author: Robert Jaster, MD, Department of Medicine II, Division of Gastroenterology, Rostock University Medical Center, E.-Heydemann-Str. 6, 18057 Rostock, Germany (Tel: +49-381-4947349; Fax: +49-381-4947482; Email: jaster@med.uni-rostock.de)

doi: 10.1016/S1499-3872(16)60154-6
Published online November 4, 2016.

Potent triggers of ECM synthesis and PSC proliferation include transforming growth factor β1 (TGF-β1) and platelet-derived growth factor (PDGF), respectively.[7, 8] In addition, ethanol metabolites[9] and oxidative stress[10, 11] have been directly implicated in the activation process. Enhanced deposition of ECM is considered as an essential step of pancreatic wound healing and not pathological *per se*. Noteworthy, PSC activation has been shown to contribute to regeneration early after acute necrotizing pancreatitis in humans.[12] In the context of chronic pancreatitis and pancreatic cancer, however, PSC activation persists and results in pancreatic fibrosis, a characteristic feature and a putative progression factor of both diseases.[2, 3] An improved understanding of the underlying pathophysiological mechanisms may eventually lead to the development of novel therapies for chronic pancreatitis and pancreatic cancer, which are based on the inhibition of fibrosis. On the other hand, promoting the physiological wound healing function of activated PSC may be an effective approach to support regeneration after acute pancreatitis or episodes of chronic pancreatitis progression.

We have recently shown that mice which are lacking uncoupling protein 2 (UCP2), a carrier protein of the inner mitochondrial membrane,[13-15] are prone to a retarded tissue regeneration after acute pancreatitis: Compared with wild-type (WT) mice, a more severe pancreatic damage at late time points after the induction of cerulein pancreatitis (24 hours and 7 days, respectively) was observed.[16] Our findings are in line with previous investigations, which have shown an increased expression of UCP2 in two independent models of acute pancreatitis, cerulein-induced pancreatitis in mice and taurocholate-induced pancreatitis in rats (at two degrees of severity).[17] Interestingly, in our study a retarded regeneration was detected in aged mice (12 months old) only, and unrelated to acinar cell function.[16]

UCP2 has been suggested as a negative regulator of mitochondria-derived reactive oxygen species (ROS) production by decreasing the mitochondrial membrane potential,[18-20] and UCP2 expression in different tissues increases with age.[21] Enhanced UCP2 expression may therefore be important to attenuate ageing-associated oxidative stress burden, as also suggested by studies in $UCP2^{-/-}$ mice.[22, 23]

In this study, we addressed the question if functional defects or peculiarities of PSC from $UCP2^{-/-}$ mice may account for the longer persistence of the pancreatic damage in aged mice. Since oxidative stress has also been implicated in PSC activation,[10, 11] we hypothesized that putative malfunctions of $UCP2^{-/-}$ PSC might be related to the intracellular ROS level, which was therefore monitored experimentally.

Methods

Animals and reagents

$UCP2^{-/-}$ mice[23] were purchased from Charles River Laboratories (Sulzfeld, Germany) and kept on the C57BL/6 background (WT control strain). The mice had access to water and standard laboratory chow *ad libitum*. For cell isolation, balanced numbers of female and male mice at an age of 12 months were employed. Unless stated otherwise, all reagents were obtained from Sigma-Aldrich (Deisenhofen, Germany).

Cell culture

PSCs were isolated from mice pancreas by collagenase digestion followed by Nycodenz® (Nycomed, Oslo, Norway) density gradient centrifugation as described before for pancreata from rats.[24] Immediately after isolation, PSCs were resuspended in cryopreservation medium [fetal calf serum (FCS) supplemented with 10% dimethyl sulfoxide] and stored at -150 ℃ until required as previously described.[25] After thawing, the cells were cultured in Iscove's modified Dulbecco's medium supplemented with 17% FCS, 1% non-essential amino acids (dilution of a 100× stock solution), 10^5 U/L penicillin and 100 mg/L streptomycin (all reagents from Merck Millipore, Darmstadt, Germany). After approximately 7 days in primary culture, proliferating PSC reached subconfluency and were harvested by trypsination. Afterwards, the cells were recultured according to the experimental requirements. Unless stated otherwise, experiments were performed with cells passaged no more than one time. Trypan blue staining was used to distinguish live from dead cells and to determine absolute cell counts.

Quantification of DNA synthesis

Cell proliferation was assessed by quantifying incorporation of 5-bromo-2'-deoxyuridine (BrdU) into newly synthesized DNA, using the BrdU labelling and detection enzyme-linked immunosorbent assay kit (BrdU ELISA; Roche Diagnostics, Mannheim, Germany). Therefore, cells were plated in 96-well plates at equal seeding densities (2×10^3 cells per well) and allowed to adhere overnight. Subsequently, recombinant mouse PDGF, recombinant human TGF-β1 (both from R&D Systems, Minneapolis, MN, USA), genipin, or solvent only (controls) were applied as indicated. After 24 hours, BrdU labelling was initiated by adding labelling solution at a final concentration of 10 μmol/L. Another 24 hours later, labelling was stopped, and BrdU uptake was measured according to the manufacturer's instructions.

Measurement of ROS

For the detection of intracellular ROS levels, 2', 7'-di-