cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTER

Peroxiredoxin-I Sustains Inflammation During Pancreatitis

Acute pancreatitis is a transient and local inflammation of the pancreas characterized by immune cell infiltration, fibrosis, and edema.[1] It mainly affects acinar cells, causing acinar metaplasia, and thereby constitutes a favorable environment for the development of pancreatic cancer in human beings and mouse models.[2] Despite the significant involvement of redox-dependent mechanisms in pancreatitis (eg, mitogen-activated protein kinase signaling, autophagy, disulfide stress, calcium signaling), supplementation with generic antioxidants is therapeutically unsuccessful,[3] highlighting the need to identify specific targets amenable to pharmacologic therapy.

To identify redox targets relevant to pancreatitis, we first compared the transcriptional landscape of Fluorescence-activated cell sorting (FACS)-sorted acinar cells from control and cerulein-treated mice (cerulein is a pancreatitis-inducing compound). We identified an increased expression of activators of the peroxiredoxin pathway such as peroxiredoxin-1 (*Prdx1*), sulfiredoxin (*Srxn1*), and thioredoxin (*Txn1*) (Supplementary Figure 1*A*). Among the typical 2-cystein family members, mouse and human peroxiredoxin-1 protein (PRX-I), -II, -III, and -IV, only the expression of PRX-I was selectively induced in metaplastic acinar cells, at advanced stages of acute pancreatitis (Figure 1*A* and Supplementary Figure 1*B-G*). Accordingly, in primary human acinar cells cultured under conditions that mimic pancreatitis-induced metaplasia, we found substantially higher levels of PRX-I in metaplastic cells (days 3–4) compared with normal acini (day 0) (Figure 1*B* and Supplementary Figure 1*E* and *F*). PRX-I has been shown to interact with inflammatory factors, such as nuclear factor κB (NF-κB) and macrophage migration inhibitory factor, suggesting its involvement in the pathophysiology of pancreatitis.[4] To investigate the role of PRX-I in pancreatitis, we genetically ablated its expression using clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) (Supplementary Figure 2*A–D*). This constitutive inactivation recapitulates the clinical context in which a drug administrated to patients would inhibit its target in all cell types. Although a previous report showed that long-term constitutive PRX-I deletion causes anemia and a shortened lifespan,[5] we did not observe any pancreas-specific anomaly in *Prdx1*$^{-/-}$ mice (age, 3 mo). *Prdx1*$^{-/-}$ mice were born at the expected Mendelian frequency, showed normal postnatal development, and were fertile.

Next, we analyzed the histology of pancreata from *Prdx1*$^{+/+}$, *Prdx1*$^{+/-}$, and *Prdx1*$^{-/-}$ mice treated with cerulein in early and late acute settings (Supplementary Figure 2*E* and *F*). At early acute pancreatitis time points, *Prdx1*$^{+/+}$ and *Prdx1*$^{+/-}$ pancreata (considered together as controls [Ctrl]) showed a slight increase in PRX-I expression (Supplementary Figure 3*A* and *B*). The extent of edema and immune infiltration observed in Ctrl pancreata was not affected in *Prdx1*$^{-/-}$ mice; the low PRX-I expression, at early pancreatitis, probably explains the minimal effects observed after its genetic ablation (Supplementary Figure 3*C* and *D*). Interestingly, at late acute pancreatitis, PRX-I expression was strongly increased in metaplastic acini (Supplementary Figure 3*E* and *F*). At this time point, pancreata from *Prdx1*$^{-/-}$ mice showed a well-preserved architecture with a significantly 2-fold higher number of normal acini and a 3-fold reduction in metaplastic area compared with Ctrl (Figure 1*C* and *D* and Supplementary Figure 4*A* and *B*). CD45-positive immune cell infiltration and collagen deposit both were decreased significantly by 2-fold in *Prdx1*$^{-/-}$ compared with Ctrl mice (Figure 1*C* and *D*).

PRX-I usually is described as an antioxidant enzyme with high catalytic efficiency.[6] Interestingly, the content of protein carbonyls and 4-hydroxynonenal (4-HNE)-protein adducts was comparable in pancreata from Ctrl and *Prdx1*$^{-/-}$ mice (Figure 1*E*). This suggested that the antioxidant function of PRX-I is not playing a predominant role in pancreatitis, which prompted us to search for additional roles of PRX-I. Previous reports have shown that PRX-I can be secreted from cultured cells in response to inflammatory stimuli and can bind to Toll-like receptor 4 to activate NF-κB–mediated production of proinflammatory cytokines.[7–9] Accordingly, we detected PRX-I in the culture medium of primary mouse acinar cells undergoing metaplasia, highlighting their ability to release PRX-I (Figure 2*A*). Strikingly, primary mouse acinar cells treated with recombinant PRX-I protein released significantly more proinflammatory cytokines interleukin 6 and tumor necrosis factor-α compared with untreated cells (Figure 2*B*). In line with this result, *Prdx1*$^{-/-}$ pancreata showed a reduced expression of interleukin 6 and tumor necrosis factor-α, in the interstitial space between acinar cells, compared with their Ctrl counterparts (Figure 2*C* and *D*). Similarly, the expression and nuclear translocation of signal transducer and activator of transcription 3 and NF-κB (subunit p65), 2 transcriptional factors controlling the expression of proinflammatory cytokines, were decreased by 2- to 3-fold in *Prdx1*$^{-/-}$ pancreata (Figure 2*C* and *D* and Supplementary Figure 4*A*). Thus, our findings show

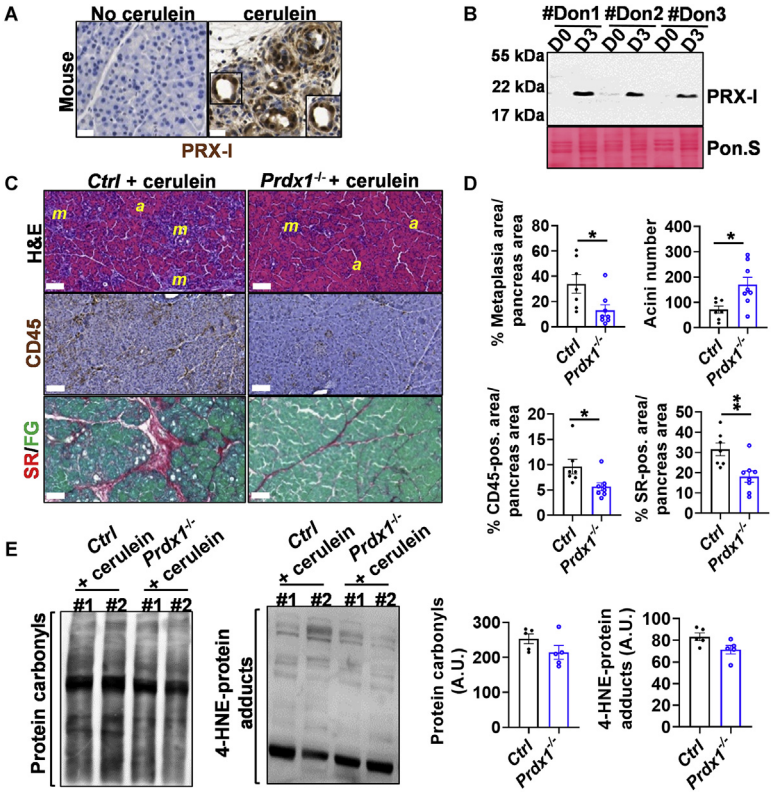

Figure 1. Ablation of PRX-I reduces the severity of late acute pancreatitis. (*A*) PRX-I staining on pancreas sections from mice treated or not with cerulein (n = 3). (*B*) Western blot for PRX-I performed on human pancreas culture lysates (n = 4). Day 0 (D0), normal acini; D3, metaplastic acini. Ponceau (Pon). S was used as loading control. (*C*) Histologic analysis on pancreas sections from cerulein-treated Ctrl(n = 7) and *Prdx1*$^{-/-}$ (n = 8) mice. *Scale bars*: 50 μm. (*D*) Whole-tissue quantification for data shown in panel *C*. (*E*) Similar levels of oxidative damages, protein carbonyls, and 4-hydroxynonenal (4-HNE)–protein adducts detected by Western blot on pancreatic lysates from cerulein-treated Ctrl and *Prdx1*$^{-/-}$ mice (n = 5). The corresponding densitometry quantifications also are available. Data are means ± SEM. Statistical significance was tested by the Student *t* test: **P* < .05; ***P* < .01.Ctrl*: Prdx1*$^{+/+}$ and/or *Prdx1*$^{+/-}$. a, normal acinar area; Don, donor; FG, Fast Green; m, metaplasia area; Pos, Positive; SR, Sirius Red.

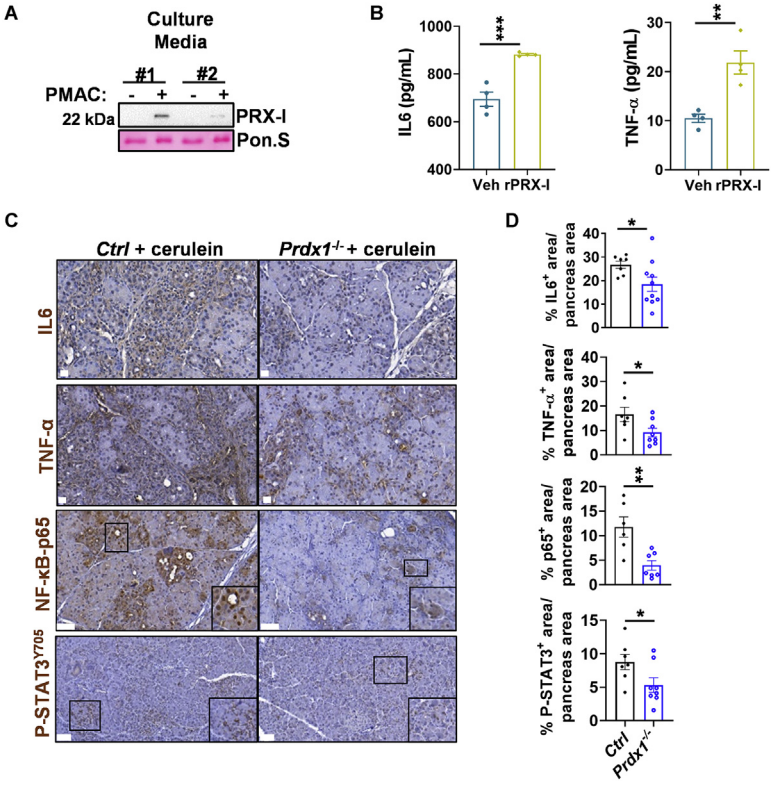

Figure 2. PRX-I promotes the production of proinflammatory cytokines. (*A*) Western blot analysis for released PRX-I in culture medium in the presence or absence of primary mouse acinar cells (PMACs) undergoing metaplasia. PMACs were cultured for 2 days (n = 3). Ponceau (Pon). S was used as loading control. (*B*) Enzyme-linked immunosorbent assay for interleukin (IL)6 and tumor necrosis factor-α (TNF-α) on the culture medium of metaplastic acinar cells treated with vehicle (Veh) (0.02 mol/L HEPES, pH 7) or recombinant PRX-I (rPRX-I) (100 nmol/L) for 1 day (n = 4). (*C*) IL6 and TNF-α (*scale bars*: 20 μm), as well asPhospho-signal transducer and activator of transcription 3 (P-STAT3^{Y705}) and NF-κB (p65) (*scale bars*: 50 μm) immunostaining on pancreata from cerulein-treated Ctrl (n = 6–7) and *Prdx1*$^{-/-}$ (n = 7–10) mice. (*D*) Whole-tissue quantification for data shown in panel *C* (+, positive). Data are means ± SEM. Statistical significance was tested by the Student *t* test: **P* < .05, ***P* < .01, and ****P* < .001. Ctrl: *Prdx1*$^{+/+}$ and/or *Prdx1*$^{+/-}$.

that a mechanism linking the secretion of PRX-I to the production of proinflammatory cytokines may operate in vivo.

In summary, we discovered that the ablation of PRX-I reduces the severity of inflammation and related acinar-to-ductal metaplasia (Supplementary Figure 4*C*). Our results support PRX-I as a potential therapeutic target to reduce pancreatic inflammation and related damage.

HORTENSE BUCKENS[1]
SOPHIE PIRENNE[1]
YOUNES ACHOURI[1,2]
JONATHAN BALDAN[3]
HAJAR DAHOU[1]
LUC BOUWENS[3]
FRÉDÉRIC P. LEMAIGRE[1]
PATRICK JACQUEMIN[1]
MOHAMAD ASSI[1]
[1]de Duve Institute, [2]Transgene Technology Platform, Université Catholique de Louvain, Brussels, Belgium
[3]Cell Differentiation Laboratory, Vrije Universiteit Brussel, Brussels, Belgium

Correspondence
Address correspondence to: Patrick Jacquemin, PhD and Mohamad Assi, PhD. de Duve Institute, 75 Avenue Hippocrate, 1200, Brussels, Belgium. e-mail: patrick.jacquemin@uclouvain.be and m.assi@uclouvain.be.

References

1. Habtezion A, et al. Gastroenterology 2019;156:1941–1950.
2. Guerra C, et al. Cancer Cell 2007; 11:291–302.
3. Perez S, et al. Redox Biol 2015; 5:1–14.
4. Bertoldi M. Protein Pept Lett 2016;23:69–77.
5. Neumann CA, et al. Nature 2003; 424:561–565.
6. Ogusucu R, et al. Free Radic Biol Med 2007;42:326–334.
7. Mullen L, et al. Mol Med 2015; 21:98–108.
8. Riddell JR, et al. J Immunol 2010; 184:1022–1030.
9. Liu DL, et al. Int Immunopharmacol 2016;41:82–89.

Abbreviations used in this letter: Ctrl, control; NF-κB, nuclear factor κB; PRX-I, mouse and human peroxiredoxin-1 protein

Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2021.03.013

Received December 21, 2020. Accepted March 24, 2021.

Acknowledgments
The authors thank Mourad El Kaddouri, Jean-Nicolas Lodewyckx, Freddy Abrassart, and Nicolas Dauguet for technical help.
Transcript profiling: GSE163254.

Conflicts of interest
The authors disclose no conflicts.

Funding
This work was supported by the National Fund for Scientific Research (FNRS), grant B.017.19F (M.A.) and the Foundation for Cancer Research and Télévie, grants 2016-089, 2018-078, 2018-076, and 7.8515.18 (F.P.L. and P.J.).