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HIF1-alpha Regulates Acinar Cell Function and Response to Injury in Mouse Pancreas

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Abstract

We investigated whether intra-pancreatic coagulation, with deposition of the fibrinogen-γ dimer (Fib-γD) and hypoxia, affect the severity of acute pancreatitis (AP) in mice. Pancreata of mice with AP induced by administration of cerulein or by L-arginine, or from patients with AP, had increased deposition of Fib-γD compared to control pancreata. Heparin administration protected mice from cerulein-induced AP and prevented Fib-γD formation. Cerulein administration resulted in activation and stabilization of HIF1-alpha in pancreata of ODD-luc HIF1-alpha reporter mice. Cerulein also led to induction of genes regulated by HIF1-alpha, including *VEGFA* and *ERO1A*, before evidence of Fib-γD deposition or histologic features of AP. Expression of tissue factor, which is regulated by VEGF, also increased following cerulein administration. Mice with acinar cell-specific disruption of *Hif1a* (*Hif1a*Ac−/−) developed spontaneous endoplasmic reticulum stress and less severe AP, but did not accumulate Fib-γD following administration of cerulein. Feeding mice increased pancreatic expression of HIF1-alpha, indicating a physiologic role in the exocrine pancreas. Therefore, HIF1-alpha has bifunctional roles, in exocrine pancreas homeostasis and progression of AP that is promoted by intra-pancreatic coagulation.

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Author contributions MJP, SI, JAW, YMS and MBO designed the experiments; MJP, SI, XX, JBC, DM and SG performed the experiments; MJP, SI, XX, JBC, SG, DM, SWP, JAW, DMS, YMS and MBO analyzed the data; MJP and MBO wrote the manuscript. All authors read and approved the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Keywords

mouse model; fibrinogen; blood clotting; factor VIII

Systemic alterations in coagulation are associated with complications from acute pancreatitis (AP), and are one of the reasons for the high mortality rate of AP[1,2]. Fibrinogen, a major coagulation protein, is composed of a dimer of three polypeptide chains (α,β,γ), of which γ-chains form protruded structures and contain sites allowing interaction with other factors such as clotting factors and cytokines, including vascular endothelial growth factor (VEGF) and fibroblast growth factor-2[3,4]. Notably, insoluble fibrinogen-γ dimers (Fib-γD) deposit in liver during acute liver injury in mice and humans and are an early marker of tissue damage[5], however, no other tissues were assessed and the underlying mechanism is poorly understood.

To investigate whether intra-parenchymal coagulation occurs during AP, pancreatitis was induced in mice by cerulein administration. As expected, histologic and serologic changes were noted including interstitial edema, intracellular vacuoles and inflammatory infiltration, and elevated serum amylase (Fig. S1A). Notably, Fib-γD was readily detectable in the insoluble protein fractions from the pancreata, and crosslinked fibrin was dramatically increased without changes in serum D-dimers, in parallel with severity of the AP (Fig. 1A,B; Fig. S1B). Examination of early time points after cerulein administration showed that Fib-γD begins to accumulate in the early stage of AP (Fig. 1C; Fig. S1C). Another AP mouse model, induced by L-arginine, also showed elevated Fib-γD and crosslinked fibrin (Fig. S2). The Fib-γD forms at the earliest stages when serum amylase is either normal or just beginning to increase in both AP models, before obvious histopathologic alterations. Importantly, Fib-γD was observed in human surgical pancreata samples from patients with pancreatitis (Fig. S3). Unlike the cerulein or L-arginine models, choline-deficient ethionine-supplemented (CDE) diet-induced AP did not lead to Fib-γD formation or fibrin crosslinking despite significant pancreatic injury (Fig. S4A–C). This is likely because the CDE-diet-induced injury also causes prominent liver damage with hemorrhage earlier than development of pancreatitis (Fig. S4D,E). Notably, administration of heparin as a potential therapy after initiation of cerulein-medicated injury alleviated the extent of pancreatic injury and prevented Fib-γD formation and fibrin crosslink-formation (Fig. 1D; Fig. S5), supporting a beneficial effect of heparin in improving the resolution of AP.

Coagulation is accomplished by activation of the intrinsic and extrinsic pathways. As an essential and terminal blood-clotting factor in the intrinsic coagulation pathway, the effect of factor VIII (FVIII) on Fib-γD formation was evaluated. During cerulein-induced AP, FVIII activity was elevated but FVIII-deficient mice had similar levels of Fib-γD deposition during AP (Fig. S6), indicating that Fib-γD formation is not directly related to the intrinsic coagulation pathway.

We hypothesized that the enhanced intra-pancreatic coagulation during AP causes hypoxia. Indeed, cerulein administration resulted in activation of hypoxia-inducible factor HIF1α in pancreata of ODD-luc HIF1α reporter mice, and promoted HIF1α stabilization with induction of HIF1α transcriptional targets such as *Vegfa* and *Ero1a* within 2h of cerulein

administration and before evidence of Fib-γD deposition or histologic AP (Fig. 1E–G; Fig. S7). Importantly, the HIF1α target, VEGF, is a well-known factor that binds to fibrinogen and regulates cell proliferation[6,7]. Consistent with this, mRNA and protein levels of the extrinsic initiator of coagulation, tissue factor (TF), increased (Fig. 1F,G), consistent with previously-known TF induction by VEGF[8,9]. These findings suggest a feed-forward cycle, in which the HIF1α-VEGF-TF cascade not only induces intra-pancreatic coagulation but this clotting, in turn, further enhances HIF1α signaling during AP.

The observation of early activation of HIF1α signaling, before Fib-γD formation, led us to hypothesize that HIF1α signaling contributes directly to Fib-γD formation rather than being an output of coagulation. Indeed, acinar cell-specific HIF1α deficiency (*Hif1a*$^{Ac-/-}$) prevented cerulein-induced Fib-γD accumulation and ameliorated the histopathologic abnormalities and amylase release (Fig. 2A,B; Fig. S8), thereby suggesting an upstream regulatory tissue hemostasis role of HIF1α during AP. Pancreatic HIF1α deficiency led to several pancreatic alterations including increased vacuolization, degranulation and ER dilation (Fig. 2B,C; Fig. S8; Fig. S9A), induction of ER stress proteins including GRP78 and CHOP, and alterations in autophagy-related proteins (p62,ATGs) (Fig. S9B). Pancreatic infiltration of leukocytes and cell death were also elevated in *Hif1a*$^{Ac-/-}$ mice without a significant change in fibrosis (Fig. S9C–F). Also, isolated acini from *Hif1a*$^{Ac-/-}$ mice were susceptible to cerulein despite the basally damaged pancreas (Fig. S9G,H), suggesting that HIF1α deficiency alleviates cerulein-induced severe AP at least in part through preventing coagulation, rather than basal damage preventing traditional pancreatitis responses. The decrease in amylase was observed in *Hif1a*$^{Ac-/-}$ pancreata without alterations in other pancreatic enzymes, while gene expressions of amylase, lipase and elastase were decreased implying potential direct or indirect regulation by HIF1α (Fig. 2D; Fig. S10). Additional evidence for the importance of HIF1α in normal pancreas function is the finding that re-feeding ODD-luc mice after fasting triggers marked upregulation of HIF1α, possibly through activation of Akt-mTOR signaling (Fig. 2E,F; Fig. S11).

Several prior findings lend support for our observations. For example, spontaneous pancreatitis and decreased tissue amylase have been reported in several acinar cell-specific ATGs null mice[10,11]. Similarly, Elastase-Cre mediated *Atg5*$^{-/-}$ mice showed normal morphology during basal conditions but protection from cerulein-induced injury[12]. In addition, the Human Protein Atlas database shows moderate expression of HIF1α in normal pancreas, particularly in exocrine cells[13], thereby suggesting a fundamental role of HIF1α during normal pancreatic exocrine function (Fig. 2G). Although a link between HIF1α and amylase regulation remains to be investigated, there is strong evidence for the involvement of HIF1α in insulin secretion in β-cells and the regulation of glucose metabolism genes including *Glut2, G6pi, aldoB* and *Hnf4a*, in a hypoxia-independent manner[14].

Coagulation abnormalities during AP lead to severe complications in some patients ranging from localized intravascular thrombosis to disseminated intravascular coagulation[15]. The findings herein demonstrate that Fib-γD formation is an early event during AP and is observed in mouse and human pancreata. Our findings show novel functions of HIF1α in promoting coagulation during AP through HIF1α-VEGF-TF cascade, and in the response of the exocrine pancreas to normal physiologic stimulation (Fig. 2G).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AP	acute pancreatitis
Fib-γD	fibrinogen-γ dimers
CDE	choline-deficient ethionine-supplemented
HIF1α	hypoxia-inducible factor-1α
VEGF	vascular endothelial growth factor
ODD	oxygen-dependent degradation domain
FVIII	factor VIII
TF	tissue factor
ATGs	autophagy-related proteins

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* Author names in bold designate shared co-first authorship.

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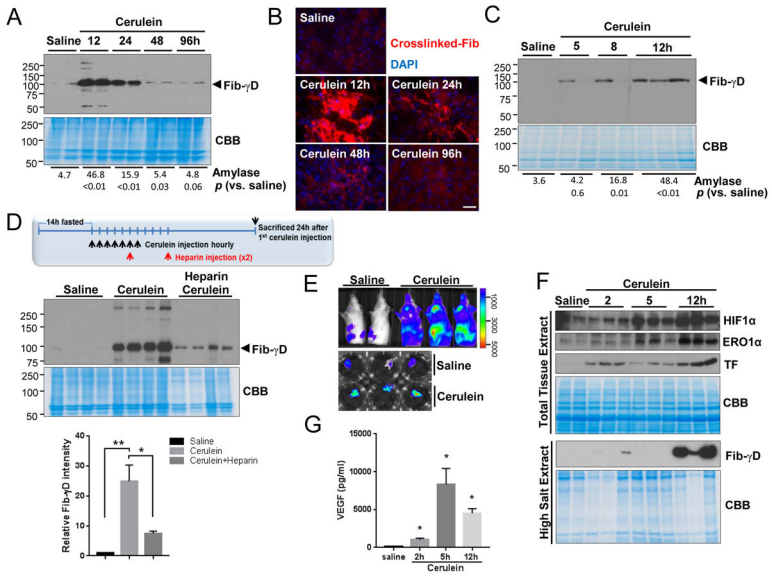

Fig. 1. Intra-pancreatic coagulation and HIF1α induction in pancreata of mice during pancreatitis

(A–C) Fibrinogen-γ immunoblotting of insoluble proteins isolated from pancreata of mice treated with saline or cerulean, and immunofluorescence (B) of crosslinked-fibrin (red) and DAPI-counterstaining (blue) in pancreata (bar=50μm). Serum amylase (U/ml, 3 mice/group) and *p*-values are included. **(D)** Heparin was administered 5h and 10h after the first cerulein injection as shown schematically. Insoluble proteins were blotted with antibodies to fibrinogen-γ. CBB, Coomassie stain to show protein loading (bottom panel: Fib-γD band intensity compared to CBB). **(E)** Bio-luminescence of ODD-luc mice (whole body and isolated pancreata) 12h after initial saline/cerulein injections. **(F)** Total lysates or insoluble fractions from pancreata of mice injected with saline/cerulein were blotted with antibodies to the indicated antigens. **(G)** VEGF levels in pancreata were measured using enzyme-linked immunosorbent assay. Mean±SEM (**p*<0.05).

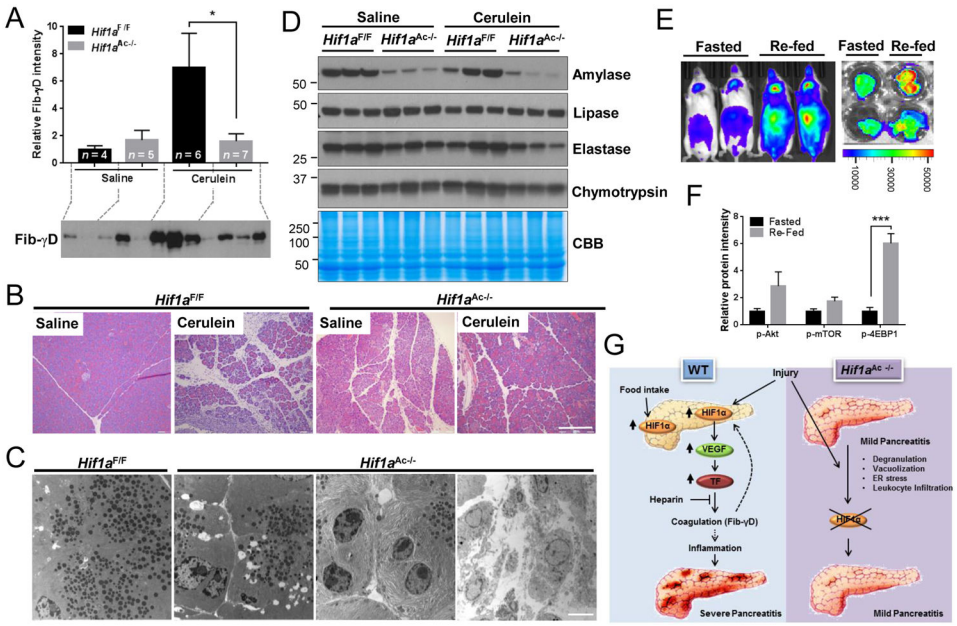

Fig. 2. HIF1α regulates pancreatic physiologic and pathophysiologic functions

(A) Fib-γD immunoblot (and relative intensity) of the insoluble protein fraction isolated from pancreata after saline or cerulein administration. **(B)** Histology (bar=200μm), and **(C)** Transmission electron microscopy (bar=5μm) images of pancreata from *Hif1a*$^{F/F}$ or *Hif1a*$^{Ac-/-}$ mice. **(D)** Total pancreas lysates were blotted with antibodies to the indicated antigens. **(E)** Bioluminescence of ODD-luc mice and pancreata after 14h-fasting or re-feeding. **(F)** Relative protein levels of immunoblots of pancreata lysates (see Fig. 11S for blots). **(G)** Summary of findings. HIF1α contributes to the post-prandial function of the pancreas. In addition, HIF1α is inducible by AP-causing triggers, followed by upregulation of downstream targets thereby promoting intra-pancreatic coagulation and tissue injury. In contrast, acinar cell-specific HIF1α deficiency prevents coagulation and provides protection from cerulein-induced pancreatitis.