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BRIEF REPORTS

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

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We investigated whether intrapancreatic 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 pancreatitis, had increased deposition of Fib-γD compared with control pancreata. Heparin administration protected mice from cerulein-induced AP and prevented Fib-γD formation. Cerulein administration resulted in activation and stabilization of hypoxia-inducible factor-1α (HIF1α) in pancreata of oxygen-dependent degradation domain–luciferase HIF1α reporter mice. Cerulein also led to induction of genes regulated by HIF1α, including *Vegfa* and *Ero1a*, before evidence of Fib-γD deposition or histologic features of AP. Expression of tissue factor, which is regulated by vascular endothelial growth factor, 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 HIF1α, indicating a physiologic role in the exocrine pancreas. Therefore, HIF1α has bifunctional roles, in exocrine pancreas homeostasis and progression of AP that is promoted by intrapancreatic coagulation.

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 3 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 intraparenchymal 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 (Supplementary Figure 1*A*). 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 (Figure 1*A* and *B*; Supplementary Figure 1*B*). Examination of early time points after cerulein administration showed that Fib-γD begins to accumulate in the early stage of AP (Figure 1*C*; Supplementary Figure 1*C*). Another AP mouse model, induced by L-arginine, also showed elevated Fib-γD and crosslinked fibrin (Supplementary Figure 2). 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 (Supplementary Figure 3). Unlike the cerulein or L-arginine models, choline-deficient ethionine-supplemented diet-induced AP did not lead to Fib-γD formation or fibrin crosslinking despite significant pancreatic injury (Supplementary Figure 4*A*–*C*). This is

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Abbreviations used in this paper: **AP, acute pancreatitis; Fib-γD, fibrinogen-γ dimers; FVIII, factor VIII; HIF1α, hypoxia-inducible factor-1α; TF, tissue factor; VEGF, vascular endothelial growth factor.**

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EDITOR'S NOTES

BACKGROUND AND CONTEXT

The role of intra-parenchymal coagulation, as assessed by fibrinogen dimer (Fib-γD) deposition, hypoxia, and hypoxia inducible factor-1α (HIF1α) in modulating the progression of pancreatitis is not known.

NEW FINDINGS

Intra-pancreatic coagulation is augmented during experimental pancreatitis and reversed by heparin, with upregulation of HIF1α early during pancreatitis. Feeding induces HIF1α, while its acinar cell-specific deficiency causes ER stress but protects from pancreatitis.

LIMITATIONS

The mechanism of feeding-associated HIF1α induction remains to be determined.

IMPACT

The study demonstrates novel associations of HIF1-alpha with normal pancreas function and with progression of acute pancreatitis. It also links HIF1α with intra-pancreatic coagulation/Fib-γD formation, and pancreatitis, with therapeutic implications.

likely because the choline-deficient ethionine-supplemented diet-induced injury also causes prominent liver damage with hemorrhage earlier than development of pancreatitis (Supplementary Figure 4*D* and *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 (Figure 1*D*; Supplementary Figure 5), 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 (Supplementary Figure 6), indicating that Fib-γD formation is not directly related to the intrinsic coagulation pathway.

We hypothesized that the enhanced intrapancreatic coagulation during AP causes hypoxia. Indeed, cerulein administration resulted in activation of hypoxia-inducible factor-alpha (HIF1α) in pancreata of oxygen-dependent degradation domain–luciferase HIF1α reporter mice, and promoted HIF1α stabilization with induction of HIF1α transcriptional targets such as *Vegfa* and *Ero1a* within 2 hours of cerulein administration and before evidence of Fib-γD deposition or histologic AP (Figure 1*E*–*G*; Supplementary Figure 7). 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 (Figure 1*F* and *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 intrapancreatic 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 (Figure 2*A* and *B*; Supplementary Figure 8), 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 endoplasmic reticulum dilation (Figure 2*B* and *C*; Supplementary Figure 8 and 9*A*), induction of endoplasmic reticulum stress proteins including GRP78 and CHOP, and alterations in autophagy-related proteins (p62, ATGs) (Supplementary Figure 9*B*). Pancreatic infiltration of leukocytes and cell death were elevated in $Hif1a^{Ac-/-}$ mice without a significant change in fibrosis (Supplementary Figure 9*C*–*F*). Also, isolated acini from $Hif1a^{Ac-/-}$ mice were susceptible to cerulein despite the basally damaged pancreas (Supplementary Figure 9*G* and *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, whereas gene expressions of amylase, lipase, and elastase were decreased, implying potential direct or indirect regulation by HIF1α (Figure 2*D*; Supplementary Figure 10). Additional evidence for the importance of HIF1α in normal pancreas function is the finding that refeeding oxygen-dependent degradation domain–luciferase mice after fasting triggers marked up-regulation of HIF1α, possibly through activation of Akt–mammalian target of rapamycin signaling (Figure 2*E* and *F*; Supplementary Figure 11).

Several prior findings lend support to our observations. For example, spontaneous pancreatitis and decreased tissue amylase have been reported in several acinar cell–specific autophagy-related proteins in 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 (Figure 2*G*).