Type I interferons mediate pancreatic toxicities of PERK inhibition

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The great preclinical promise of the pancreatic endoplasmic reticulum kinase (PERK) inhibitors in neurodegenerative disorders and cancers is marred by pancreatic injury and diabetic syndrome observed in PERK knockout mice and humans lacking PERK function and suffering from Wolcott-Rallison syndrome. PERK mediates many of the unfolded protein response (UPR)-induced events, including degradation of the type 1 interferon (IFN) receptor IFNAR1 in vitro. Here we report that whole-body or pancreas-specific *Perk* ablation in mice leads to an increase in IFNAR1 protein levels and signaling in pancreatic tissues. Concurrent IFNAR1 deletion attenuated the loss of PERK-deficient exocrine and endocrine pancreatic tissues and prevented the development of diabetes. Experiments using pancreas-specific *Perk* knockouts, bone marrow transplantation, and cultured pancreatic islets demonstrated that stabilization of IFNAR1 and the ensuing increased IFN signaling in pancreatic tissues represents a major driver of injury triggered by *Perk* loss. Neutralization of IFNAR1 prevented pancreatic toxicity of PERK inhibitor, indicating that blocking the IFN pathway can mitigate human genetic disorders associated with *PERK* deficiency and help the clinical use of PERK inhibitors.

PERK | pancreas | interferon | diabetes | Wolcott-Rallison syndrome

Tumor microenvironment-associated deficit in oxygen and nutrients activate numerous pathways that aid cancer and tumor stroma cells by increasing their ability to survive, withstand anticancer therapies, and ultimately select for more aggressive and viable clones capable of metastasizing (1). Activation of the unfolded protein response (UPR) plays a central role in these processes (2). Three branches of this response include stimulation of activating transcription factor-6 and activation of two kinases, inositol requiring enzyme 1α/β and the eukaryotic translation initiation factor 2-alpha kinase 3 [also termed double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase, or pancreatic endoplasmic reticulum kinase (PERK)]. The latter kinase contributes to phosphorylation of the eukaryotic translation initiation factor 2-alpha and controls the rate of global translation and noncanonical induction of specific proteins that help cope with stress (reviewed in ref. 2).

Among three main UPR pathways, signaling through PERK has received the most attention for its central role in cancer (3–6). Genetic studies have demonstrated that PERK is essential in supporting tumor growth and progression via diverse mechanisms, including stimulation of angiogenesis (7–12), potential effects on antitumor immunity (13, 14), and direct increase in cancer cell viability by altering its metabolic status (15), promoting survival autophagy (16–18), and induction of prosurvival microRNAs (19). Accordingly, development of novel, potent, and selective PERK inhibitors as a means to treat cancers has been proposed (20, 21). Several PERK inhibitors have shown promising results in various preclinical tumor models (22–24). Furthermore, some of these inhibitors can protect against the prion-mediated neurogenerative disorders (25).

Regrettably, PERK knockout and small-molecule inhibitors also showed serious toxic effects primarily affecting the pancreas (22, 25–27). Importantly, PERK has been indeed shown to play a key role in the maintenance of normal pancreatic exocrine, and especially endocrine, function (28–31). Failure of the insulin-producing pancreatic function is characteristic for Wolcott-Rallison syndrome, caused by inactivating mutations of *PERK* in humans (28). Pancreatic inflammation, loss of pancreatic tissue (including the β cells), and development of insulin-dependent diabetic syndrome was also described in mice either constitutively lacking *Perk* or undergoing inducible *Perk* ablation (32–38).

Intriguingly, we have recently identified an important role of PERK in the hypoxia- or virus replication-induced UPR-mediated ubiquitination and down-regulation of the IFNAR1 chain of type 1 IFN receptor (39–42). IFNs play important antiviral, antitumor, and immunomodulatory functions (43), yet can elicit and mediate pathologic scenarios (44). IFN has long been linked to pancreatic dysfunction in humans via elevated IFN expression in pancreatic tissues of patients with type 1 diabetes mellitus (45, 46) and induction of pancreatitis (47–49) and diabetogenic effects (50, 51) by pharmaceutical IFN used for treatment of tumors or viral infections. In addition, experiments in mouse models demonstrated that transgenic expression of IFN in β cells leads to

Significance

Inactivating pancreatic endoplasmic reticulum kinase (PERK) mutations cause pancreatic degeneration and diabetes in patients with Wolcott-Rallison syndrome. Pancreatic injury is also observed in mice upon PERK genetic ablation or treatment with PERK inhibitors. This toxicity (the mechanisms of which are poorly understood) impedes the clinical development of PERK inhibitors, which show promise against cancers and neurodegenerative diseases. Here we demonstrate that activation of type 1 interferon signaling occurs upon PERK ablation and is responsible for pancreatic injury and the loss of exocrine and endocrine tissues and functions. Neutralization of interferon signaling protects the pancreas from deleterious effects of PERK inhibitors. Temporally targeting the interferon pathway may help with the treatment of patients with Wolcott-Rallison syndrome and the use of PERK inhibitors against other diseases.

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diabetes (52), and that development of diabetes in the nonobese diabetic mice depends on production of IFN (53), as well as functional status of IFNAR1 (54).

Given that maintenance of threshold IFNAR1 levels is essential for the antiproliferative, proapoptotic, and immunopathological effects of IFN (55, 56), and that stabilization of IFNAR1 exacerbates acute and chronic inflammation in the pancreas (57), we proposed to test the role of IFN in the pancreatic toxicity of PERK inactivation. Work described here reveals that IFN is induced in the pancreas of mice lacking *Perk* ubiquitously or specifically in the pancreas. Knockout of IFNAR1 alleviated pancreatic tissue damage and endocrine dysfunction induced by *Perk* ablation. Conversely, an accelerated development of diabetic syndrome can be generated in mice lacking *Perk*, yet expressing the mutant *Ifnar1* allele, whose protein product is insensitive to all known inducers of ubiquitination and degradation. Furthermore, either knockout of *Ifnar1* or the use of neutralizing anti-IFNAR1 antibodies attenuated the pancreatic toxicities of PERK inhibitor in vitro and in vivo. These results indicate that IFN signaling plays a central role in mediating the pancreatic toxicity of PERK inactivation and suggests that modulating IFN responses may help treat the patients with Wolcott-Rallison and broaden the use of PERK inhibitors for therapeutic purposes.

Results

Activation of IFN Signaling upon PERK Inactivation Contributes to Apoptosis in Pancreatic Islets in Vitro. We previously demonstrated that acute excision of PERK in $Perk^{l/l}$;$Ubc9\text{-}Cre^{ERT}$ mice (ubiquitous *Perk* deletion, $uPerk^{\Delta/\Delta}$) resulted in a rapid decline in β-ell number and in development of a diabetic syndrome (32). Analysis of mRNA from purified islets from these mice revealed a significant induction of *Ifnb* and *Ifna4* and IFN-stimulated gene *Isg15* mRNA (Fig. S1*A*) associated with PERK excision. This induction may reflect stimulation of the IFN pathway in the pancreatic gland cells or/and infiltration of degenerating pancreas by immune cells that highly express these genes. To directly assess IFN signaling in these islet cells, we purified and cultured pancreatic islets from $Perk^{l/l}$;$Ubc9\text{-}Cre^{ERT}$ mice before 4-hydroxytamoxifen-mediated Cre excision in vitro. This treatment efficiently decreased levels of PERK in cultured islets (Fig. S1*B*). Analysis of mRNA from these islets demonstrated that ablation of *Perk* resulted in a moderate induction of UPR-stimulated binding immunoglobulin protein (*Bip*) and *Grp94* (Fig. 1*A*). Remarkably, *Perk* knockout robustly increased expression of IFN ligands (*Ifna4* and *Ifnb*) and IFN-stimulated gene interferon regulatory factor 7 (*Irf7*) (Fig. 1*A*). Furthermore, levels of IRF7 protein were increased in islet cells after 4-hydroxytamoxifen treatment (Fig. S1*C*). These results suggest that *Perk* ablation leads to activation of IFN signaling in the pancreatic islets in vitro.

In cultured fibroblasts and HeLa cells, UPR signaling accelerated ligand-independent ubiquitination and degradation of IFNAR1 (58) in a manner that required activities of PERK (39, 41, 42), p38 kinase (40), and casein kinase 1α (59). Accordingly, ablation of *Perk* in cultured islets in vitro led to a robust increase in the IFNAR1 levels (Fig. 1*B* and Fig. S1*D*). The specificity of this signal was ensured by comparison with a negative control represented by the islets from $Perk^{l/l}$ mice lacking *Ifnar1* and positive control [islets from $Perk^{l/l}$ mice that harbor the knocked-in $Ifnar1^{SA}$ alleles encoding the $IFNAR1^{S526A}$ mutant protein, which is insensitive to ubiquitination induced via PERK, as well as other PERK-independent pathways (57)]. Given the data from biochemical studies implicating PERK in the regulation of IFNAR1 ubiquitination and turnover (39, 42), and the fact that we did not observe a concurrent increase in *Ifnar1* mRNA levels (Fig. 1*A*), these data are indicative of posttranscriptional mechanisms that increase IFNAR1 protein (e.g., stabilization) in *Perk*-deficient pancreatic islets in vitro.

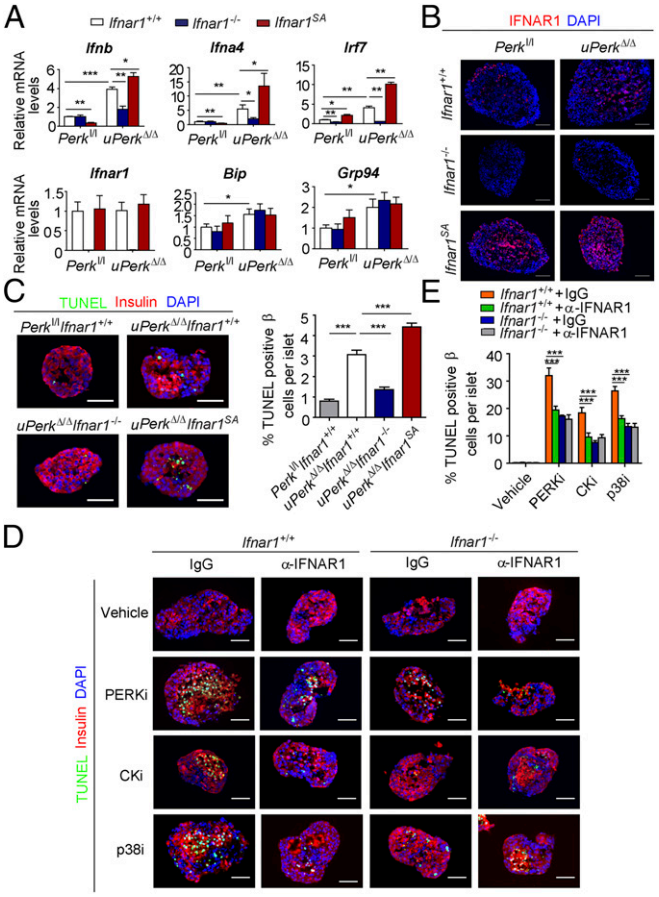

Fig. 1. Activation of IFN signaling upon PERK inactivation contributes to apoptosis in pancreatic islets in vitro. (*A*) Expression of mRNA of indicated genes from in vitro cultured pancreatic islets from indicated mice was assessed by quantitative PCR. Asterisks here and thereafter indicate statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (*B*) Immunofluorescent analysis of IFNAR1 protein levels in the cultured islets from indicated mice. (Scale bars, 100 μm.) (*C*) Analysis of cell death in the indicated cultured islets was carried out using the TUNEL labeling counterstained with antibody against insulin and DAPI. (*Right*) Quantitation of results from at least 10 islets observed in three independent experiments. (Scale bars, 50 μm.) (*D*) Analysis of cell death in the indicated islets cultured in presence of anti-IFNAR1 neutralizing or control antibody and treated with vehicle or inhibitors of CK1 (D4476, 50 μM), p38 kinase (SB203580, 10 μM), or PERK (GSK2606414, 1 μM) for 2 d. (Scale bars, 50 μm.) (*E*) Quantitation of results from *D*. Average data from at least 10 islets observed in three independent experiments are shown.

Importantly, ablation of both *Perk* and *Ifnar1* attenuated IRF7 mRNA and protein induction (Fig. 1*A* and Fig. S1*C*), consistent with reduced IFN-dependent signaling. Conversely, even a greater induction of IRF7 was seen in islets whose cells expressed an ubiquitination-deficient $IFNAR1^{SA}$ (Fig. S1 *A* and *C*). Collectively, these results suggest that ablation of *Perk* may induce IFN signaling by concurrent induction of IFN expression and partial stabilization of IFNAR1.

IFN is well-known for its proapoptotic and antiproliferative effects (43). Given that ablation of *Perk* triggers islets cell death (32, 38), we next sought to examine the role of IFN and IFNAR1 in the islet cells. The increase in TUNEL-positive cells in *Perk*-deficient cultures was attenuated after *Ifnar1* loss, yet further enlarged in $Perk^{\Delta/\Delta}$, $Ifnar1^{SA}$ islets (Fig. 1*C*). Furthermore, cell death induced by treatment of islets from wild-type mice (but not from mice lacking *Ifnar1*) with the PERK inhibitor, GSK2606414, could be attenuated by culturing the islets in the presence of an IFNAR1-blocking antibody (Fig. 1 *D* and *E*). Importantly, use of

this antibody or knockout of IFNAR1 also attenuated cell death induced by inhibitors of either p38 kinase (SB203580) or CK1 (D4476, Fig. 1 *D* and *E*), which are kinases known to function downstream of PERK in stimulating IFNAR1 ubiquitination and degradation (40–42, 59). Collectively, these results suggest that PERK inactivation triggers the induction of IFN and stabilization of IFNAR1, leading to the activation of the cell death pathways in pancreatic islets in vitro.

Perk Antagonizes IFN Signaling, Thereby Preventing Pancreatic Exocrine and Endocrine Tissue Injury and Dysfunction. We sought to determine the role of IFN signaling in pancreatic dysfunction caused by ubiquitous *Perk* inactivation ($uPerk^{\Delta/\Delta}$) in vivo. Consistent with previous work (32), tamoxifen treatment of $Perk^{l/l}$;$Ubc9\text{-}Cre^{ERT}$ mice led to efficient *Perk* ablation (Fig. S2 *A* and *B*) and signs of pancreatic injury, including increased β-cell death, loss in β-cell mass, reduced pancreatic mass, decreased insulin production, increased amylase levels, signs of acinar cells loss, and the development of a fully manifested diabetic syndrome within 5 wk (Fig. 2 and Fig. S3*A*). Analysis of pancreatic tissues from these $uPerk^{\Delta/\Delta}$ mice revealed a robust increase in the levels of IFNAR1 protein (Fig. S2*C*). Pancreatic tissues from wild-type, *Ifnar1*-deficient, and $Ifnar1^{SA}$ mice exhibited a similar efficacy of *Perk* excision (Fig. S2 *A* and *B*). Furthermore, IFNAR1 status did not affect the severity of UPR, judging by the similar induction of UPR-stimulated genes and proteins (e.g., BiP; Fig. S4 *B* and *C*) or similarly extended endoplasmic reticulum analyzed by electron microscopy (Fig. S4*D*). However, ablation of *Ifnar1* significantly decreased the expression of IFN (*Ifnb* and *Ifna4*) and practically abrogated the expression of IFN-stimulated genes (*Irf7* and *Isg15*), whereas the latter levels were superinduced in $Ifnar1^{SA}$ mice (Fig. S4*A*). Importantly, these changes were mirrored by changes in the frequency of pancreatic cell death after *Perk* excision. Concurrent ablation of *Ifnar1* attenuated cell death in the *Perk*-deficient pancreata, whereas a significantly greater level of cell death was seen in $Ifnar1^{SA}$ tissues (Fig. 2*A* and Fig. S5). These results collectively suggest that PERK functions to partially suppress the IFN signaling in the normal pancreas. Furthermore, these data further implicate IFN signaling in pancreatic cell death caused by *Perk* inactivation.

Loss of IFNAR1 ($Ifnar1^{-/-}$) attenuated, whereas stabilization of IFNAR1 ($Ifnar1^{SA}$) dramatically exacerbated, *Perk* excision-induced alterations in the islet structure, size, and β-cell numbers (Fig. 2 *B* and *E* and Fig. S2*D*); in overall size and weight of wet pancreata (Fig. 2 *C* and *D*); and in underlying histopathologic changes (Fig. 2*E* and Fig. S3*A*). These changes included atrophic alterations in the acinar cells whose death manifested itself in increased serum amylase levels attenuated in *Ifnar1*-null mice and aggravated in $Ifnar1^{SA}$ animals (Fig. 2*F*). Profound loss of islets (Fig. 2*E*) and a decrease in serum insulin levels (Fig. 2*F*) followed the same trend. Finally, stabilization of IFNAR1 in $Ifnar1^{SA}$ animals accelerated development of *Perk* deficiency-induced diabetes, which was dramatically delayed and moderated in the *Ifnar1* knockout animals (Fig. 2 *F*–*I*). In all, these data strongly suggest that effects of IFN are largely responsible for pancreatic toxicity that occurs upon inactivation of *Perk*.

Ubiquitous ablation of *Perk* in these studies could use IFN signaling to elicit detrimental changes in the pancreas directly or via *Perk*-dependent alterations in the immune cells. Indeed, the severity of IFNAR1-dependent changes in pancreatic injury and dysfunction (Fig. 2) paralleled the extent of pancreatic tissue infiltration with leukocytes (Fig. 3*A*), including myeloid cells (but not macrophages, Fig. S3 *B* and *C*) and T lymphocytes (Fig. S6). Thus, we have examined the role of IFN signaling under conditions in which Perk ablation occurs via inducing Cre^{ERT}, which is expressed under the *Pdx1* promoter specifically in the pancreas (60). In $Perk^{l/l}$, $Pdx1\text{-}Cre^{ERT}$; $Ifnar1^{+/+}$ mice, treatment with tamoxifen (resulting in $pPerk^{\Delta/\Delta}$ genotype) also triggered efficient *Perk* excision in the pancreas (Fig. S7*A*) and was associated

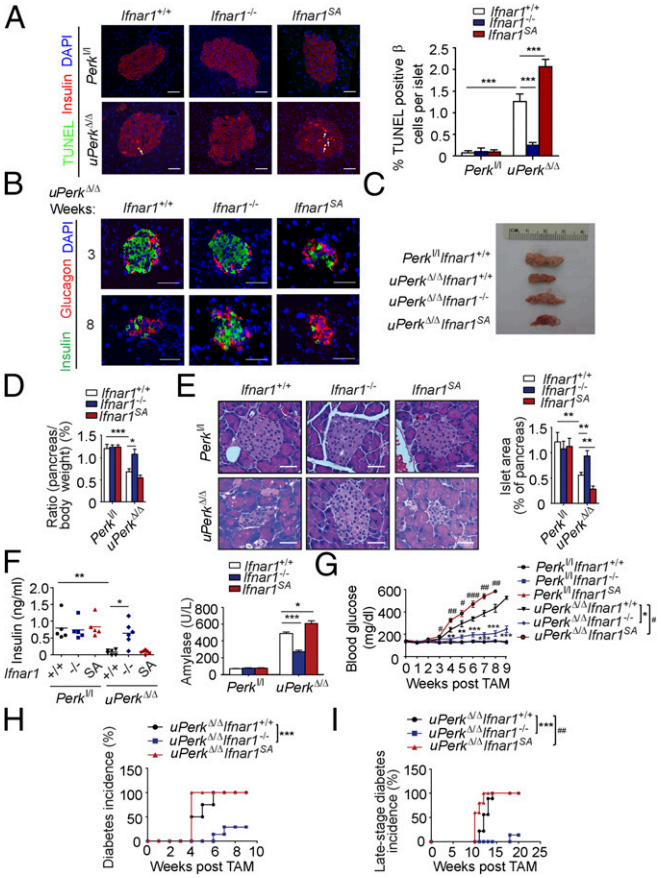

Fig. 2. Ubiquitous inactivation of PERK in vivo up-regulates IFNAR1 and its signaling, leading to pancreatic exocrine and endocrine tissue injury and dysfunction. (*A*) Analysis of TUNEL-positive β cells in pancreatic tissues from indicated mice was carried out 3 wk after tamoxifen treatment. Arrows indicate TUNEL (green), insulin (red), and DAPI triple-positive cells. (*Right*) Quantitation of results from 14 to 22 islets. (Scale bars, 50 μm.) (*B*) A representative islet immunostaining for insulin (green) and glucagon (red) was performed on pancreatic tissues from indicated mice killed at the indicated time after tamoxifen treatment. (Scale bars, 50 μm.) (*C*) Gross appearance of whole pancreata harvested from $uPerk^{\Delta/\Delta}$ and control mice 12 wk after tamoxifen. (*D*) Quantification of specific weight of pancreata from indicated mice (n = 3–4 for each group). (*E*) H&E staining of the islets of the indicated mice 12 wk after tamoxifen treatment. (*Right*) Quantification of the islet area from three to four mice. (Scale bars, 50 μm.) (*F*) Analysis of serum insulin (*left*) and amylase (*right*) levels from the indicated mice 12 wk after tamoxifen treatment. (*G*) Blood glucose was measured every week after tamoxifen treatment (n = 5–8 mice in each case; data shown as mean ± SEM). (*G–I*) $^{\#}P = uPerk^{\Delta/\Delta}Ifnar1^{SA}$ vs. $uPerk^{\Delta/\Delta}Ifnar1^{+/+}$. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$. $^{*}P = uPerk^{\Delta/\Delta}Ifnar1^{-/-}$ vs. $uPerk^{\Delta/\Delta}Ifnar1^{+/+}$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (*H*) Diabetes incidence (blood glucose >250 mg/dL for two consecutive measurements) was analyzed from the indicated mice in *F*. (*I*) Late-stage diabetes (blood glucose >600 mg/dL for three consecutive measurements) incidence was analyzed from the indicated mice in *F*.

with the β-cell loss and the development of a diabetic syndrome (ref. 32 and Fig. 3 *B*–*F*).

Intriguingly, pancreas-specific *Perk* ablation in $pPerk^{\Delta/\Delta}$ mice also increased levels of pancreatic IFNAR1 (Fig. S7*B*), indicating that this increase is unlikely to result from recruitment of *Perk*-deficient leukocytes that highly express IFNAR1, as might be argued in $uPerk^{\Delta/\Delta}$ mice wherein PERK was excised ubiquitously. Similar to the results obtained in the $uPerk^{\Delta/\Delta}$ mice (Fig. 2), pancreas-specific $pPerk^{\Delta/\Delta}$ mice exhibited elevated IFN signaling that reflected the extent of expression and stability of IFNAR1 (Fig. S7*C*); the latter did not markedly alter UPR signaling (Fig. S7*C*). Importantly,

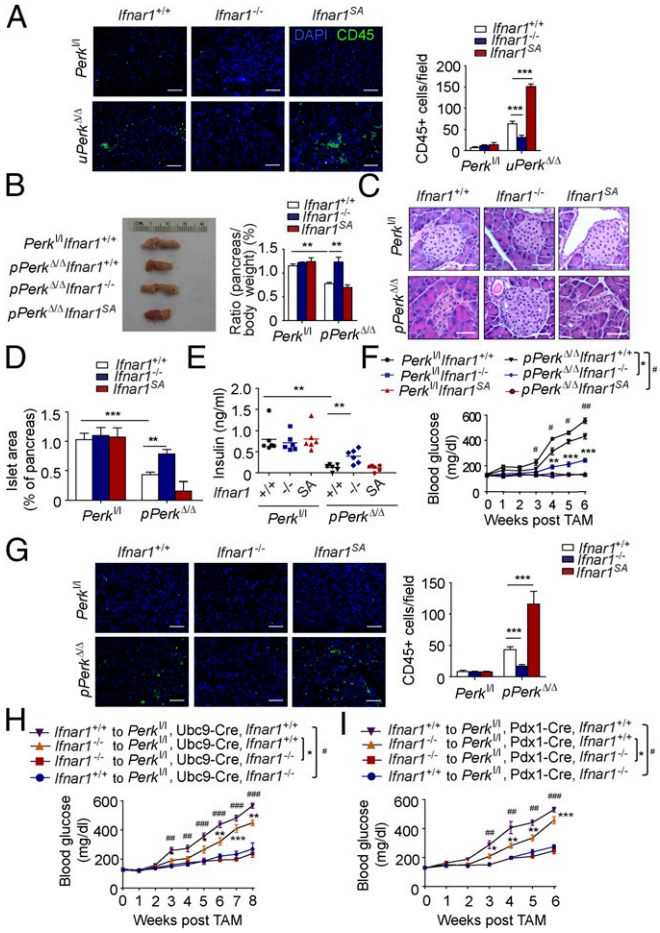

Fig. 3. Pancreatic PERK suppresses IFN signaling in the pancreas to prevent pancreatic tissue injury and dysfunction. (*A*) Immunofluorescent analysis of leukocyte infiltration into pancreata from *uPerk*$^{\Delta/\Delta}$ and control mice (harvested 3 wk after tamoxifen treatment) was carried out on frozen sections using anti-CD45 antibody. (*Right*) Quantitation of results from 10 fields observed in tissues from three mice. (Scale bars, 100 μm.) (*B*) The picture of whole pancreata harvested from p*Perk*$^{\Delta/\Delta}$ and control mice killed 9 wk after tamoxifen treatment. (*Right*) Quantification of results from three to four mice. (*C*) H&E staining of the islets of *pPerk*$^{\Delta/\Delta}$ and control mice 9 wk after tamoxifen treatment. (*D, Right*) Quantification of the islet area from three to four mice. (Scale bars, 50 μm.) (*E*) Analysis of serum levels of insulin in *pPerk*$^{\Delta/\Delta}$ and control mice 9 wk after tamoxifen treatment. (*F*) Blood glucose was measured every week after tamoxifen treatment (n = 5–8 mice in each case; data shown as mean ± SEM). $^{\#}P$ = *uPerk*$^{\Delta/\Delta}$*Ifnar1*^{SA} vs. *uPerk*$^{\Delta/\Delta}$*Ifnar1*$^{+/+}$. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$. $^{*}P$ = *uPerk*$^{\Delta/\Delta}$*Ifnar1*$^{-/-}$ vs. *uPerk*$^{\Delta/\Delta}$*Ifnar1*$^{+/+}$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (*G*) Immunofluorescent analysis of pancreata from *pPerk*$^{\Delta/\Delta}$ and control mice (harvested 3 wk after tamoxifen treatment) was carried out on frozen sections using anti-CD45 antibody. (*Right*) Quantification. (Scale bars, 100 μm.) (*H*) Weekly blood glucose levels in *Perk*$^{l/l}$*; Ubc9-Cre*^{ERT} mice (harboring indicated *Ifnar1* status) that received bone marrow from *Ifnar1*$^{-/-}$ or *Ifnar1*$^{+/+}$ mice, and 8 wk later being treated with tamoxifen (n = 5–8 mice in each case; data shown as mean ± SEM). (*I*) Weekly blood glucose levels in *Perk*$^{l/l}$*; Pdx1-Cre*^{ERT} mice (harboring indicated *Ifnar1* status) that received bone marrow from *Ifnar1*$^{-/-}$ or *Ifnar1*$^{+/+}$ mice, and 8 wk later being treated with tamoxifen (n = 5–8 mice in each case; data shown as mean ± SEM).

experiments using *Perk*$^{l/l}$*, Pdx1-Cre*^{ERT} *Ifnar1*-null and *Ifnar1*^{SA} mice clearly demonstrated that IFN signaling plays a key role in the *Perk* deficiency-induced loss of total pancreatic tissue (Fig. 3*B*), islets mass (Fig. 3 *C* and *D*), insulin levels (Fig. 3*E*), and development of diabetes (Fig. 3*F* and Fig. S8). These results in *pPerk*$^{\Delta/\Delta}$ mice support a model in which *Perk* functions to moderate IFN signaling in the pancreatic tissues to prevent IFN-dependent pancreatic injury and functional deficiency.

Notably, pancreas-specific deletion of Perk still elicited immune infiltration of pancreas that was modulated by the IFNAR1 status (Fig. 3*G* and Fig. S9). Given that null or SA alleles of *Ifnar1* are ubiquitous, it is plausible that *Perk* status in the pancreas signals to IFN pathway in the immune system, rather than in the pancreatic cells themselves. To test this possibility, we transferred bone marrow from *Ifnar1*$^{+/+}$ or *Ifnar1*$^{-/-}$ mice into lethally irradiated *Perk*$^{l/l}$*; Ubc9-Cre*^{ERT} or *Perk*$^{l/l}$*; Pdx1-Cre*^{ERT} recipients before administering tamoxifen to excise *Perk*. Although chimeras receiving bone marrow from *Ifnar1*$^{-/-}$ mice exhibited only a modest delay in diabetes development (most likely associated with an additional immune role of IFN), the status of IFNAR1 in peripheral tissues was the major determinant of pancreatic toxicity because the *Ifnar1*-deficient recipients lacking *Perk* in all peripheral tissues (Fig. 3*H*), or specifically in pancreas (Fig. 3*I*), displayed a dramatic suppression of diabetic phenotype. In all, these results suggest that *Perk* negatively regulates IFN signaling within the peripheral tissues (including pancreas) to prevent the injury and dysfunction of the pancreas.

Pharmacologic Inactivation of IFN Signaling Protects Pancreas from Toxic Effects of PERK Inhibitor. Given that IFNAR1 signaling contributes to apoptosis induced by GSK2606414 in cultured pancreatic islets (Fig. 1*D*), we aimed to determine whether knockout

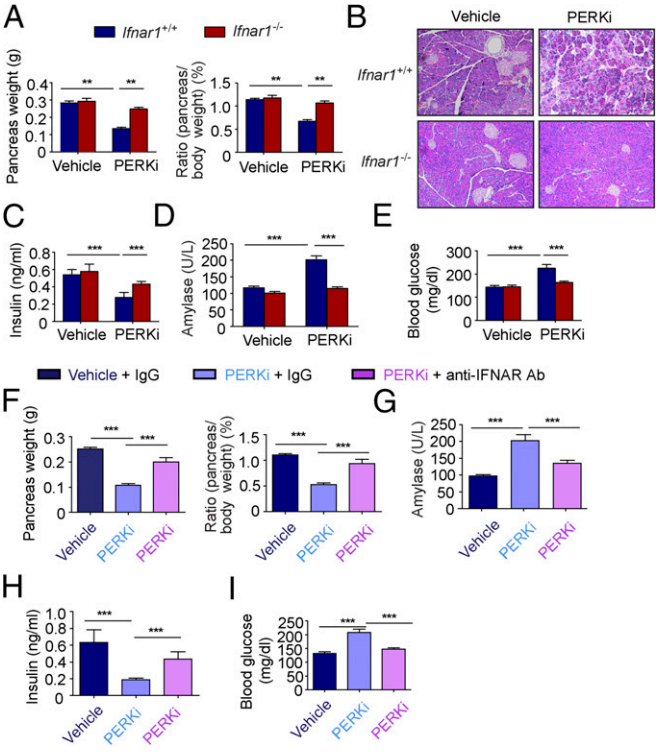

Fig. 4. Pharmacologic inactivation of IFN signaling protects pancreas from toxic effects of PERK inhibitor. (*A*) Analysis of absolute (*Left*) and specific (*Right*) weight of the pancreatic glands from wild-type or *Ifnar1*$^{-/-}$ mice treated with vehicle or PERK inhibitor GSK2606414 (150 mg/kg) for 14 d. (*B*) Representative H&E staining of the pancreas from the mice treated as in *A*. (Scale bars, 100 μm.) (*C*) Analysis of serum insulin levels from the mice treated as in *A*. (*D*) Analysis of serum amylase levels from the mice treated as in *A*. (*E*) Analysis of blood glucose levels from the mice treated as in *A*. (*F*) Analysis of absolute (*Left*) and specific (*Right*) weight of the pancreatic glands from wild-type mice treated with vehicle or PERK inhibitor GSK2606414 for 14 d combined with anti-IFNAR1 antibody or its isotype control treatment (every 5 d, 1 mg i.p. injection per mouse). (*G*) Analysis of serum amylase levels from the mice treated as in *F*. (*H*) Analysis of serum insulin levels from the mice treated as in *F*. (*I*) Analysis of blood glucose levels from the mice treated as in *F*. $^{**}P < 0.01$; $^{***}P < 0.001$.

or antibody-based blocking of IFNAR1 can protect animals from pancreatic toxicity generated after treatment with this PERK inhibitor in vivo. Consistent with previously published reports (22), extended treatment with PERK inhibitor markedly decreased pancreas weight in wild-type mice (Fig. 4*A*) and caused noticeable degenerative changes in pancreatic islets and acinar cells (Fig. 4*B*), as well as decreased insulin levels (Fig. 4*C*) and increase in serum levels of amylase (Fig. 4*D*) and glucose (Fig. 4*E*). In addition, upon glucose challenge, wild-type mice treated with PERK inhibitor exhibited defects in stimulated insulin secretion and glucose tolerance (Fig. S10). Importantly, all these detrimental changes in pancreatic morphology and function were alleviated by *Ifnar1* knockout (Fig. 4 *A–E* and Fig. S10).

Furthermore, administration of anti-IFNAR1 neutralizing antibody [previously shown to alleviate development of diabetes in NOD mice (54)] at least partially rescued GSK2606414-induced changes in pancreatic mass (Fig. 4*F*), amylase (Fig. 4*G*), insulin (Fig. 4*H*), and glucose levels and tolerance (Fig. 4*I*). These data indicate that IFN signaling plays an important role in pancreatic dysfunction caused by PERK inhibitors and provides a proof of principle for blocking the IFN pathway to reduce pancreatotoxic effects of PERK inactivation.

Discussion

Genetic (28–31) or pharmacologic (22, 25–27) inactivation of *Perk* in mice and humans contributes to degenerative changes in the pancreas and its ensuing exocrine and endocrine disorders, including development of diabetes mellitus. Here we demonstrate that either knockout of IFNAR1 or its blockade, using specific antibody, elicits a profound rescue effect on the viability and number of pancreatic exocrine and endocrine cells. The results provide strong support for a model in which *Perk* normally functions to restrict IFN signaling in the normal pancreas. Furthermore, pancreatic injury and dysfunction triggered by *Perk* inactivation reflect increased IFN signaling and are largely (but not exclusively) mediated by IFN effects.

Comparison of in vivo data from $uPerk^{\Delta/\Delta}$ mice (Fig. 2) with in vitro data obtained in cultured islets (Fig. 1) suggests that toxic effects of IFN are at least in part mediated by direct IFN action on the β cells. Importantly, IFN signaling is induced and can cause damage even if *Perk* is specifically inactivated in the pancreas itself ($pPerk^{\Delta/\Delta}$; Fig. 3). Additional experiments using bone marrow transplantation indicate that pancreatotoxicity is largely caused by the IFN action on the peripheral tissues. Although the role of IFN in eliciting the immunopathologic injury to the pancreas via additional changes in the immune system cannot be ruled out, our current data strongly suggest that *Perk*-deficient pancreatic parenchymal cells expressing IFNAR1 are directly sensitive to the toxic effects of IFN.

PERK was shown to mediate the UPR-induced IFNAR1 ubiquitination and degradation in vitro (42, 58, 59).The increase in IFNAR1 protein, but not mRNA levels after *Perk* ablation, has provided in vivo proof regarding the importance of PERK in regulating the IFNAR1 protein levels. Hyperactivation of the IFN pathway upon *Perk* deletion is likely a sum of IFN induction triggered by the UPR, as well as increased ability of this IFN to elicit the signaling via engaging highly expressed IFNAR1 (Figs. 1–3). Indeed, although complete IFNAR1 stabilization ($Ifnar1^{SA}$ mice) further exacerbated *Perk* deficiency-induced pancreatic toxicity, ablation of IFNAR1 alleviated detrimental effects to pancreatic cells and tissues in vitro and in vivo (Figs. 1–4).

The ability of anti-IFN therapy to antagonize pancreatic toxicity caused by *Perk* loss or inhibition is of significant practical importance. Current medical efforts are focused on the development of antibody-based drugs (sifalimumab, rontalizumab, etc.) for neutralizing IFNAR1 in patients with diverse inflammatory/autoimmune syndromes (61, 62). Our data suggest that a similar strategy could be potentially envisioned for relieving IFN-mediated pancreatotoxicity in patients with Wolcott-Rallison or patients who receive PERK inhibitors for treating tumors (22–24) and prion-mediated neurogenerative disorders (25).

Materials and Methods

Animals. All experiments with animals were carried out under the protocols 803995 and 804470 approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. All mice had water ad libitum and were fed regular chow. Ubc9-Cre^{ERT} (gift from E. Brown, University of Pennsylvania) and Pdx1-Cre^{ERT} mice (gift from X. Hua, University of Pennsylvania) were crossed with $Perk^{l/l}$ mice (kindly provided by D. Cavener, Penn State University) and either $Ifnar1^{-/-}$ mice (a kind gift of Dr. Dong-Er Zhang, University of California, San Diego) or $Ifnar1^{S526A/S526A}$ mice ($Ifnar1^{SA}$, described in ref. 57) to generate future $uPerk^{\Delta/\Delta}$ or $pPerk^{\Delta/\Delta}$ littermates and their controls. Genotyping of mice using tail DNA or islet DNA was performed by PCR. Only male mice were used for the experiments. The method to induce *Perk* deletion is described in *SI Materials and Methods*.

For induction of pancreatic toxicity in mice by GSK2606414 treatment, IFNAR1 neutralizing antibody (63) treatment in mice, bone marrow transplantation assay, glucose tolerance test, and measurement of blood glucose, insulin, and amylase, see *SI Materials and Methods*.

Islet Culture and Treatments. Islet isolation was described previously (32). For details regarding islet isolation and treatment, and TUNEL assay to assess β-cell death, see *SI Materials and Methods*.

Histopathology, Immunological, and Other Techniques. For the immunostaining of frozen sections, H&E staining, the immunostaining of paraffin sections, TUNEL assay, and immunoblotting, see *SI Materials and Methods*. Detailed imaging protocols, Fuji software (64) and Illustrator image processing, data analyzing, and statistics were included in *SI Materials and Methods*. For details about electron microscopy, methods for RNA isolation, cDNA synthesis, quantitative PCR, and the sequences of the primers, see *SI Materials and Methods*.

Statistics. Every shown quantified result is an average of at least three independent experiments carried out in either triplicate or quadruplicate and calculated as means ± SE. The *P* values were calculated using the two-tailed Student *t* test. Diabetes incidence was compared by using the log-rank test.

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