Epidermal growth factor receptor regulates pancreatic fibrosis

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Blaine SA, Ray KC, Branch KM, Robinson PS, Whitehead RH, Means AL. Epidermal growth factor receptor regulates pancreatic fibrosis. Am J Physiol Gastrointest Liver Physiol 297: G434–G441, 2009. First published July 16, 2009; doi:10.1152/ajpgi.00152.2009.—The development of pancreatic fibrosis has been shown to be a major component in several diseases of the pancreas including pancreatic cancer, chronic pancreatitis, and type 2 diabetes mellitus, but its actual role in the progression of these disorders is still unknown. This fibrosis is characterized by stromal expansion and the excessive deposition of extracellular matrix (ECM) that replaces pancreatic tissue. This eventually leads to dysregulation of ECM turnover, production of cytokines, restriction of blood flow, and often exocrine and endocrine insufficiencies. Activated pancreatic stellate cells (PSCs) have been identified as key mediators in the progression of pancreatic fibrosis, serving as the predominant source of excess ECM proteins. Previously, we found that overexpression of the growth factor heparin-binding epidermal growth factor-like growth factor (HB-EGF) in pancreatic islets led to intraislet fibrosis. HB-EGF binds to and activates two receptors, epidermal growth factor receptor (EGFR) and ErbB4, as well as heparin moieties and CD9/DRAP27. To understand the mechanism underlying the induction of fibrogenesis by HB-EGF, we utilized a hypomorphic allele of Egfr, the Waved-2 allele, to demonstrate that EGFR signaling regulates fibrogenesis in vivo. Using an in vitro cell migration assay, we show that HB-EGF regulates both chemoattraction and stimulation of proliferation of PSCs via EGFR activation.

The development of pancreatic fibrosis is a major element in both chronic pancreatitis (26) and pancreatic cancer (3). Although it likely plays a role in the progression of both diseases as excessive deposition of extracellular matrix (ECM) replaces pancreatic tissue, its actual role in these processes is still unknown. Fibrosis may contribute to disease progression by the dysregulation of ECM turnover, production of cytokines, and restriction of blood flow, resulting in exocrine and endocrine insufficiencies (4). Although fibrosis in both cases predominately involves exocrine pancreatic tissue, there is evidence from both animal models and human patients suggesting that intraislet fibrosis also occurs and is a component in the progression of type 2 diabetes mellitus (6, 13). Islet dysfunction in the form of impaired insulin secretion is a predominant factor in nonobese patients with type 2 diabetes (18), and this may be induced by insufficient β-cell mass. Early loss of β-cells with increased fibrosis and eventual destruction of the pancreatic islets is seen in both human patients and animal models as the disease progresses, and a recent study suggests that fibrotic islet destruction mediated by activated pancreatic stellate cells (PSCs) may be one cause for this loss of β-cell mass (13).

PSCs have been identified as key mediators in the progression of pancreatic fibrosis (1, 2, 5, 8). These normally quiescent cells are found in low abundance throughout the pancreas and are characterized by their star-shaped morphology, the presence of intracellular vitamin A-storing fat droplets, and the expression of intermediate filament proteins such as desmin, glial fibrillary acidic protein (GFAP), and nestin, distinguishing them from fibroblasts and other stromal components (11, 22). PSCs reside in the periacinar regions of the pancreas where their long cytoplasmic processes encircle the base of the acinus. Upon activation by tissue injury, oxidative stress, growth factors, or cytokines, they are transformed to a myofibroblast-like phenotype that can isolate and/or repair damaged tissue. PSCs contribute to the fibrotic response in multiple ways, including ECM remodeling and tissue contraction. In these cases, fibrosis arises and resolves as the damage is contained. However, chronic fibrosis can occur when damage is not repaired or when abnormal signaling events perpetuate the activated state of PSCs. Although a number of growth factors have been found to induce or enhance PSC activation, little is known about the regulation of its maintenance or resolution.

During PSC activation, cells undergo enlargement of the nucleus, the loss of vitamin A fat droplets, increased proliferation, increased migration, expression of α-smooth muscle actin (α-SMA), and increased synthesis and secretion of ECM molecules like collagen type I (2, 23). Two of the strongest mediators of PSC activation, transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF), have been shown to produce different effects, with PDGF being a mitogen and chemotactic agent, whereas TGF-β increases α-SMA expression and the synthesis of ECM proteins (16). Profibrogenic mediators of PSC activation can be secreted by neighboring pancreatic parenchymal or inflammatory cells or from activated PSCs themselves in an autocrine loop that allows for perpetuation of activation. Both PDGF and TGF-β are thought to be important modulators of a persistent activated state.

The role of the epidermal growth factor receptor (EGFR) in PSC activation has not been explored. However, overexpression of EGFR ligands resulted in extensive pancreatic fibrosis in vivo. Overexpression of TGF-α in the exocrine pancreas (25) led to progressive interstitial fibrosis in the exocrine parenchyma with little to no fibrosis within the islets. On the other hand, overexpression of another ligand, heparin-binding epidermal growth factor-like growth factor (HB-EGF), in the endocrine islets led to fibrosis within the islets but rarely within the exocrine pancreatic tissue (19). These studies suggest that EGFR ligands act locally, inducing fibrosis around the expressing tissue. In vitro coculture of islets from transgenic HB-EGF-overexpressing mice with normal stellate cells demonstrated
that HB-EGF expressed in the islets could induce fibrogenic responses within the islets, but recombinant HB-EGF added to the culture medium could not. These data suggest that additional factors found within the islet microenvironment contributed to HB-EGF-mediated fibrosis (19). Signaling through HB-EGF is complicated by its many binding partners. HB-EGF can activate both the EGFR and ErbB4 receptors, as well as bind to heparin-containing proteins (9) and CD9/DRAP27 (21). We demonstrate here that attenuating EGFR activity via a point mutation in the Egfr tyrosine kinase domain, the Waved-2 mutation, reduces the incidence of pancreatic fibrosis in vivo. We further show that HB-EGF acts as a chemottractant and mitogen to PSCs, thus uncovering two roles for this growth factor pathway.

MATERIALS AND METHODS

Animals. Mice were isogenic except for the presence or absence of a transgene and for a point mutation in the Egfr gene. Mice carrying the Pdx1-Hbegf transgene (19) were bred for at least 10 generations into the C57BL/6J background. Pdx1-Hbegf transgenic mice were then crossed to mice carrying the Waved-2 mutation (also in the C57BL/6J background) to generate Pdx1-Hbegf, Egfr<sup>+/W2</sup> and Pdx1-Hbegf, Egfr<sup>W2/W2</sup> mice. Mice were euthanized at 5 mo of age, 2 mo after the age of onset of fibrogenesis previously determined for Pdx1-Hbegf mice (19). All experiments were done with approval of the Vanderbilt Institutional Animal Care and Use Committee.

Histology. Pancreatic samples were fixed for 4 h in 4% paraformaldehyde, washed, and dehydrated through a series of ethanol and xylene (Sigma), 100 U/ml penicillin (Invitrogen, Carlsbad, CA), 100 μg/ml streptomycin (Invitrogen), and 5 U/ml interferon-γ (Peprotech, Rocky Hill, NJ) at 33°C and 5% CO₂.

Immunoblotting. Whole cell extracts were prepared simultaneously from three separate dishes each of IPS-1 and IPS-Wa2 cells by lysing cells in RIPA buffer (0.1% SDS, 50 mM Tris·HCl, 150 mM NaCl, 1% NP-40, and 0.5% Na deoxycholate) plus protease inhibitor cocktail (Sigma). Fifty micrograms of protein for each sample were denatured in NuPage sample buffer plus reducing agent (Invitrogen), electrophoretically separated on a 10% NuPage Bis-Tris gel, and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After being blocked with 5% nonfat dry milk in Tris-buffered saline plus 0.1% Tween-20, blots were probed with a mouse monoclonal antibody against α-tubulin (Sigma) followed by incubation with horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) and detection with SuperSignal West Femto Max Sensitivity Substrate (Pierce, Rockford, IL). Blots were sequentially stripped with Restore Western Blot Stripping Buffer, blocked with 5% nonfat dry milk, and reprobed with either a mouse monoclonal antibody against GFAP (clone GA-5, Sigma), a mouse monoclonal antibody against α-SMA (clone 1A4, Sigma), a rabbit polyclonal antibody against type I collagen (Rockland, Gilbertsville, PA), or a rabbit polyclonal antibody against fibronectin (F3648, Sigma). Band intensities were quantified using densitometry and NIH ImageJ software.

Immunofluorescence. Cells were grown on glass coverslips coated with 100 μg/ml poly-d-lysine (Chemicon, Billerica, MA) and fixed with 4% paraformaldehyde for 10 min at room temperature. After being permeabilized with 0.5% Triton X-100 in PBS for 10 min at room temperature, coverslips were blocked in 5% donkey serum and 1% BSA in phosphate-buffered saline for 1 h at room temperature and incubated with mouse monoclonal antibodies against GFAP and α-SMA (diluted 1:400 in 1% BSA in PBS) overnight at 4°C. Coverslips were incubated with Cy3-conjugated donkey anti-mouse IgG (Molecular Probes, Invitrogen, Carlsbad, CA) for 1 h at room temperature and counterstained with anti-GFAP mouse monoclonal antibody (Pierce) before being dried and mounted with Prolong Gold antifade reagent (Molecular Probes). Images were captured with a Spot Color Digital camera (Diagnostics Instruments, Sterling Heights, MI) using a Zeiss Axiohot (Oberchon, Germany) microscope with a ×20 objective.

Migration assays. Cells were serum starved overnight in nonpermissive conditions at 37°C with PSC medium containing only 0.5% FBS and without interferon-γ. Assays were performed using 8.0-μm pore size cell culture inserts (BD Biosciences, San Diego, CA) placed in 24-well culture plates. The upper surface of the membrane was coated with a thin layer of type I collagen, and IPS-1 cells were seeded at 30,000 cells/upper chamber in starvation medium containing only 0.5% FBS and without interferon-γ before placing the insert into the lower chamber containing starvation media with and without 25 ng/ml HB-EGF (R&D Systems, Minneapolis, MN). For some samples 25 ng/ml HB-EGF was added to the cell suspension in the upper chamber with starvation media in the lower chamber. After incubation for 24 h at 37°C, culture medium was removed and cells adhering to the membrane were fixed with 4% paraformaldehyde for 15 min at 37°C, followed by staining with 1 μg/ml DAPI. Membranes were allowed to air dry, cut from the inserts, and mounted on glass slides with Prolong Gold antifade reagent (Molecular Probes). For each membrane DAPI staining on both the upper surface and underside in 11 different fields was captured using an Olympus FX-100 confocal microscope using the ×10 objective. The number of cells on each side of the membrane was counted with NIH Image J software and the rate of migration expressed as the fold change in migration toward HB-EGF-containing medium compared with control medium as measured by the number of cells on the underside/total number of cells. Each condition was...
repeated in triplicate in four separate experiments. For some assays, cells were suspended in a three-dimensional collagen matrix in the insert at 30,000 cells/upper chamber for IPS-1 cells and 60,000 cells/upper chamber for IPS-Wa2 cells before placing the inserts in the lower chambers containing starvation medium with and without either 25 ng/ml HB-EGF or 20 ng/ml PDGF-BB (Sigma). Cells were incubated for 72 h at 37°C, refreshing the culture media daily, and cells both attached to the membrane and suspended in collagen matrix were quantified as above. For each membrane DAPI staining in 15 different fields was captured using a Spot CCD Camera and a Zeiss Axioiophot microscope using the ×10 objective. Each condition was repeated in duplicate in three separate experiments. Significance was determined by two-tailed t-test.

RESULTS

Reducing EGFR activity decreases the incidence of pancreatic fibrosis. We have previously shown that overexpression of HB-EGF in pancreatic islets leads to disrupted islet architecture and intraislet fibrosis by 3 mo of age and that this stromal expansion is a fully penetrant phenotype (19). To further investigate the role of EGFR in HB-EGF-induced fibrogenesis in vivo, we have crossed our HB-EGF-overexpressing line, Pdx1-Hbegf, with mice carrying the Waved-2 mutation (Egfrwa2/wa2). Waved-2 is a point mutation that occurs in the EGFR tyrosine kinase domain, resulting in a 80–95% attenuation in EGFR activity (7, 17). Egfrwa2/wa2 mice are fully viable although they exhibit hair follicle, eye, and mammary gland defects. A single copy of the wild-type Egr allele has been shown to be sufficient for normal development and function (20, 27), and thus Egfrwa2/wa2 mice serve as a control for comparing normal EGFR activity with Waved-2-reduced EGFR activity. We analyzed Pdx1-Hbegf; Egfrwa2/wa2 mice and Pdx1-Hbegf; Egfrwa2/wa2 at 5 mo of age, 2 mo after the age of onset of fibrosis in Pdx1-Hbegf mice (19), to assure that we were not merely observing a delay in fibrogenesis. Sections throughout each pancreas were analyzed at 200-μm intervals to control for any possible regional bias. Fibrosis was quantified by staining sections with Masson trichrome for the accumulation of fibrillar collagen, a prominent feature of pancreatic fibrosis. Pdx1-Hbegf; Egfrwa2/wa2 mice had a level of fibrosis (Fig. 1A) comparable to that observed in Pdx1-Hbegf; Egfrwa2/wa2 mice alone and thus were treated as wild-type for Egfr in further analysis (data not shown). However, Pdx1-Hbegf; Egfrwa2/wa2 mice showed a significant reduction in the incidence of pancreatic fibrosis (Fig. 1B). Only 51.7 ± 0.08% of islets from Pdx1-Hbegf; Egfrwa2/wa2 mice showed signs of fibrosis compared with 81.6 ± 0.05% of islets from Pdx1-Hbegf; Egfrwa2/wa2 mice (P = 0.005) (Fig. 1C). These data clearly show that reducing EGFR activity significantly decreased the incidence of pancreatic fibrosis in vivo. Interestingly, despite identical genetic backgrounds, not all Pdx1-Hbegf; Egfrwa2/wa2 mice developed the same level of fibrosis. Rather, 38% of the mice showed a dramatic decrease in intraislet fibrosis, indistinguishable from that seen in wild-type mice, indicating a complete rescue of HB-EGF-induced fibrosis. On the other hand, 46% had a similar percentage of fibrotic islets as their Pdx1-Hbegf; Egfrwa2/wa2 counterparts, implying that reduction of EGFR signaling had little to no effect in these mice. Because all mice were in the C57BL/6J background, these differences suggest a stochastic difference in response to lowered EGFR signaling. This response determines whether or not fibrosis reaches detectable levels, rather than influencing the rate of fibrotic progression.

HB-EGF increases the migration of PSCs. In previous work, we found that, although recombinant HB-EGF added to the culture medium was not sufficient to induce fibrogenic changes...
in cultured PSCs, overexpression of HB-EGF from islets cocultured with PSCs in vitro induced PSCs to assume an activated morphology (19). One key difference between these two assays was localized vs. wide-spread release of HB-EGF, which may have affected PSC migration. To investigate the mechanism for the HB-EGF-mediated intraislet fibrosis observed in our Pdx1-Hbegf mice and to determine whether HB-EGF acted directly as a chemoattractant to draw activated PSCs into islets, we isolated conditionally immortalized PSCs both from mice carrying wild-type Egfr alleles and mice homozygous for the Waved-2 Egfr allele. These cell lines are referred to as IPS-1 and IPS-Wa2, respectively. Conditionally immortalized PSCs were used in place of primary PSCs to ensure stable and identical PSC populations across all experiments. Both cell populations were characterized as activated stellate cells by the expression of both GFAP and α-SMA using immunofluorescence (Fig. 2, A–D) and by absence of cytoplasmic lipid droplets by oil red O staining (data not shown). It has been our experience that PSCs are rapidly activated by in vitro culture conditions after isolation, an observation that has been reported by others (1, 5). To further characterize and verify that both cell populations were equivalent, we compared expression levels of the PSC markers GFAP, α-SMA, collagen type I, and fibronectin in whole cell lysates by immunoblotting (Fig. 3A). Expression of α-tubulin was used as a loading control. Both IPS-1 and IPS-Wa2 cells expressed comparable amounts of all PSC markers tested, indicating that they both consisted of activated PSCs (P > 0.14 in all cases, n = 3) (Fig. 3B). IPS cells expressed very high levels of α-SMA and collagen type I that were not significantly increased by TGF-β treatment (data not shown), again indicating that both PSC cell populations were already activated. Interestingly, EGFR expression was decreased by approximately threefold in IPS-Wa2 cells compared with IPS-1 cells (P = 0.0346, n = 3). The IPS-Wa2 cells, therefore, had lowered EGFR activity attributable both to decreased kinase activity and lower protein level.

IPS-1 cells were initially examined for their ability to respond to recombinant HB-EGF in an in vitro migration assay. Cells were cultured in modified Boyden chambers for 24 h in media containing very low serum with or without the addition of 25 ng/ml recombinant HB-EGF in the lower chamber. To control for possible growth effects of HB-EGF, migration rates were calculated as the percentage of migrating cells to total cells on both sides of the membrane. The presence of HB-EGF in the lower chamber increased IPS-1 migration by 2.3-fold over control medium alone (70 ± 1.3% vs. 31 ± 5.4% for control medium alone, P = 0.0004, n = 4) (Fig. 4). To determine whether this increase in migration was the result of increased chemotaxis or increased chemokinesis, we added recombinant HB-EGF to the upper chamber and control medium alone to the lower chamber and found no difference in IPS-1 migration (20 ± 9.0% vs. 31 ± 5.4% for control medium alone, P = 0.37, n = 4). These data suggest that HB-EGF acted as a potent chemoattractant for PSCs.

**HB-EGF-stimulated migration of PSCs requires EGFR signaling.** Because HB-EGF binds both EGFR and the related ErbB4 receptor, we examined whether signaling through EGFR was required for increased migration. We compared migration of IPS-1 and IPS-Wa2 PSCs in a three-dimensional matrix to more closely simulate migration in vivo. IPS-1 or IPS-Wa2 cells were embedded in a collagen matrix and cultured in modified Boyden chambers for 72 h in media containing very low serum with or without the addition of 25 ng/ml recombinant HB-EGF or 20 ng/ml recombinant PDGF in the lower chamber. As described above, migration rates were calculated as the percentage of migrating cells to total cells on the membrane and remaining in the collagen matrix to control for differences in proliferation. In three-dimensional matrix, overall migration was lower than through a thin layer of matrix. However, in accord with the data in Fig. 4, HB-EGF still stimulated a twofold increase in chemotaxis of IPS-1 cells over control medium alone (8.94 ± 0.75% migrating cells vs. 4.54 ± 0.30% for control medium alone, P = 0.0016, n = 4)
On the other hand, IPS-Wa2 cells did not migrate significantly toward a source of HB-EGF (4.50 ± 0.35% vs. 4.48 ± 0.43% for control medium alone, \( P = 0.972, n = 7 \)). However, chemotaxis of IPS-Wa2 cells was increased twofold in the presence of PDGF (8.72 ± 0.97% vs. 4.48 ± 0.43% for control medium, \( P = 0.0012, n = 4 \)), a known chemoattractant for PSCs, suggesting that there is no impairment in the general migration machinery (Fig. 5A). Thus we have shown that HB-EGF acting through EGFR can stimulate the migration of PSCs, thus identifying one mechanism by which EGFR signaling may mediate pancreatic fibrosis.

HB-EGF stimulates proliferation of PSCs via EGFR activation. Observations from our migration assays suggested that HB-EGF also had an effect on proliferation of PSC cells. To verify this observation, total cell numbers in each sample were calculated after treatment with 25 ng/ml rHB-EGF for 72 h. Total cell counts from rHB-EGF-treated cells vs. cells given control medium alone were compared for both IPS-1 and IPS-Wa2 cell samples. Exogenous HB-EGF induced the proliferation of IPS-1 cells by approximately twofold (\( P = 0.012, n = 4 \)) over control medium alone, whereas the presence of HB-EGF had no effect on the growth of IPS-Wa2 cells (\( P = 0.535, n = 7 \)) (Fig. 5B). Therefore, we have demonstrated here that HB-EGF-mediated activation of EGFR signaling can stimulate both PSC migration and proliferation.

**DISCUSSION**

We have found that EGFR signaling plays a role in pancreatic fibrosis, a pathology that is known to impact multiple diseases, including type 2 diabetes, chronic pancreatitis, and pancreatic cancer. Although it has been shown that overexpression of either TGF-\( \alpha \) or HB-EGF in the mouse pancreas results in extensive pancreatic fibrosis in vivo (19, 25), the molecular mechanisms leading to its development are not understood. In addition, the role that their common receptor, EGFR, plays in this process has not been investigated. On the other hand, the fact that overexpression of amphiregulin, another EGFR-specific ligand, did not induce a strong fibrogenic response in the...
suggests that there is a stochastic reason for this difference, these mice were all on a pure C57BL/6J background, which is distinguishable from that in mice expressing wild-type allele(s). We found that all Waved-2 mice exhibited similar coat defects. However, eye defects were incompletely penetrant with no eye, small eye, or normal eye developing seemingly at random (data not shown).

The bipartite response to Egfr mutation suggests that EGFR signaling acts as a checkpoint rather than just as an enhancer of fibrotic expansion. Only two of the thirteen Pdx1-Hbegf; Egfr<sup>Wa2/Wa2</sup> mice had an intermediate level of fibrosis that would be expected if EGFR signaling acted only by influencing proliferation of activated PSCs. Rather, Egfr mutation either completely prevented or had no effect on intraislet fibrogenesis. Although EGFR has never been implicated in the activation of quiescent PSCs, it may act at a subsequent step leading to maintenance of the fibrotic state. By its nature, the pancreas is an injury-prone organ; dysfunction or damage of one acinar cell can lead to the aberrant release of digestive enzymes that can damage surrounding tissue. Therefore, fibrogenic mechanisms may routinely be employed to minimize pancreatic dysfunction or damage that under normal circumstances would quickly be resolved. However, in the presence of HB-EGF, activated PSCs may be drawn to the source of growth factor production, where they expand and lead to chronic fibrosis. When EGFR activity is lowered in PSCs, HB-EGF may be less likely to overcome the signals that normally resolve this expansion. The inability of the Waved-2 mutation to affect fibrosis in some mice may indicate that HB-EGF is capable of acting through a low level of EGFR activity that is sufficient to bypass antifibrotic signals, particularly if PSCs become activated near islets where they receive high levels of HB-EGF protein. The signals responsible for resolving the fibrotic response are not known and are clearly of clinical importance in treatment of fibrotic diseases. Results presented here suggest that EGFR signaling should be investigated for its role in this process.

The Waved-2 mutation also produced an incompletely penetrant phenotype in a mouse model of intestinal cancer. Using the Apc<sup>min</sup> mouse model of familial adenomatous polyposis on the Waved-2 background, Roberts et al. (24) found that, although attenuated EGFR activity drastically decreased the number of intestinal tumors found in adult mice, it did not affect the size, proliferation, or morphology of any polyps that did form. Microadenomas formed with normal frequency in the Waved-2 background, demonstrating that EGFR activity was not required for the onset of tumorigenesis. Rather, inhibition of EGFR activity decreased the number of lesions progressing from microadenoma to macroadenoma. However, in the lesions that were able to bypass this requirement for EGFR-induced progression, the tumors grew unchecked. In other words, activation of EGFR affected only an early stage of intestinal tumor development and had little effect on the subsequent expansion of established tumors.

Although we speculated from our in vivo data that EGFR acts at an early stage in the fibrogenic process, its exact role in fibrogenesis was not known. One critical early step in the development of pancreatic fibrosis is the attraction of activated PSCs to the appropriate area(s) within the tissue. We investigated whether EGFR and one of its ligands, HB-EGF, may possibly attributable to low EGFR activity occasionally overcoming the threshold necessary for fibrosis to proceed. In the C57BL/6J background, we have found that the Waved-2 mutation results in both fully penetrant and incompletely penetrant phenotypes in other organs. We found that all Waved-2 mice exhibited similar coat defects. However, eye defects were incompletely penetrant with no eye, small eye, or normal eye developing seemingly at random (data not shown).
play a role in this early step. We found that HB-EGF acting through EGFR serves as a chemoattractant for activated PSCs in vitro and thus may function in vivo by drawing PSCs toward cells expressing a high level of this growth factor. HB-EGF and other EGFR ligands are known to be highly expressed in early stages of pancreatic cancer (14, 15, 31) where they may have a function in the extensive fibrosis that characterizes this devastating disease. The presence of activated PSCs has been demonstrated in the stromal reaction in tumor samples from patients with pancreatic cancer (3) where they are thought to play a role in creating a permissive microenvironment that promotes the growth and survival of the tumor cells (10, 28).

Our results suggest that the overexpression of EGFR ligands, HB-EGF in particular, may function in chemoattraction of activated PSCs to the tumor microenvironment as well as in maintenance of their activated state.

Although the etiology underlying type 2 diabetes in nonobese individuals is often not clear, it has been suggested that pancreatic fibrosis may play an important role (13). Reports suggest that a loss of β-cell mass replaced by fibrosis and leading to impaired insulin secretion and islet dysfunction may be an early pathology in the progression of the disease in both human patients and animal models. Kim, et al. (13) demonstrated that high levels of glucose stimulated both proliferation and the activation of PSCs as well as deposition of extracellular matrix proteins in the Otsuka Long Evans Tokushima Fatty rat model of diabetes. These rats also showed severe islet destruction attributable to fibrosis as the disease progressed and this was accompanied by increased presence of activated PSCs, especially surrounding the destroyed islets. This same group found increased intrasitial α-SMA staining in pancreatic tissue sections from human diabetic patients (13). These studies suggest that intrasitial fibrosis mediated by activated PSCs plays a role in the progression of type 2 diabetes, and this intrasitial fibrosis may serve as a potential target in treatment of the disease. It remains to be seen in these models whether HB-EGF or other EGFR ligands are upregulated in pancreatic islets. However, we have previously shown that overexpression of HB-EGF in pancreatic islets both affected islet architecture and contributed to the onset of late stage diabetes in our transgenic mouse model (19).

In conclusion, we have shown that reducing EGFR activity significantly decreases the incidence of pancreatic fibrosis in vivo and propose that the role of EGFR signaling occurs during establishment or maintenance rather than just in proliferation of activated PSCs. The data reported here suggest that this role may be to draw activated stellate cells into areas expressing high levels of ligand where they can then facilitate fibrosis through proliferation and the secretion of ECM. The findings presented here may have clinical ramifications by identifying HB-EGF and EGFR signaling as possible targets in the treatment of several pancreatic diseases in which fibrosis has been shown to be an important component.

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