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EDITORIAL

Pancreatic stellate cells: small cells with a big role in tissue homeostasis

Anna L Means

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In the current issue, exciting new evidence indicates a role for pancreatic stellate cells (PaSCs) in normal pancreas function, not just in response to damage and disease. PaSCs are present in low abundance in the healthy adult pancreas where they are considered 'quiescent' but expand into 'activated' cells that are perhaps the most abundant cell type of the damaged pancreas.[1] Owing to this dramatic and critical response to pancreatic damage, PaSCs have been extensively studied for their role in pancreatitis and cancer. Their role in normal pancreas function has been overlooked until now.

In healthy tissue, stromal cells often have support roles such as supplying blood flow and providing scaffolding for epithelial integrity. A well-studied example is the intestinal tract, which comprises a folded, single layer of specialized epithelial cells for nutrient absorption and barrier function. Underlying the epithelium is the stromal lamina propria, supplying architectural support, blood flow, and inflammatory cells[2] (Figure 1a).

The pancreas is organized completely differently. The exocrine pancreas, composed of acini that produce digestive enzymes and ducts that transport those enzymes, is a highly convoluted layer of epithelium that is densely packed and has no underlying stromal layer comparable to the intestinal lamina propria (Figure 1b). Rather, vasculature travels along major ducts and in spaces between acini where small numbers of inflammatory and mesenchymal cells are also found. The major mesenchymal cell in the pancreas is the PaSC (reviewed in Omary *et al*[1]). PaSCs in the healthy pancreas are considered quiescent, proliferating rarely and expressing few cell-specific markers. However, when the pancreas is damaged, PaSCs quickly become activated. They proliferate rapidly and undergo substantial changes in gene expression, triggering fibrosis (Figure 2). Through extracellular matrix (ECM) production and expression of the intermediate filament protein α-smooth muscle actin, activated PaSCs can wall off and constrict blood flow to damaged regions. After the damage is repaired, this fibrosis resolves through unknown mechanisms, and PaSCs are again a small, quiescent population of cells. Owing to this damage response, it is likely that a primary role of quiescent PaSCs is that of damage surveillance.

In the current manuscript by Riopel *et al*[3], a new role for quiescent PaSCs is presented. Even though PaSCs are normally a minor pancreatic component, compared with lamina propria in the intestine, these researchers demonstrate a function for PaSCs in epithelial integrity via maintenance of the basement membrane. The basement membrane is a specialized extracellular matrix that lies immediately subjacent to most epithelial tissues and provides a scaffold for epithelial architecture (reviewed in LeBleu *et al*[4]). Basement membranes typically contain long, fibrillar proteins, such as collagens and laminins, and proteins such as nidogen and perlecan that crosslink and anchor these fibers. Connection to the basement membrane via integrin receptors allows epithelium to maintain cytoskeletal architecture and tissue cohesion.

While basement membranes function to support the architecture of epithelial cells, the components of basement membranes are often produced by surrounding mesenchymal cells. For example, in the developing intestine and mammary gland, collagen IV and laminin are produced by

Department of Surgery, Vanderbilt University Medical Center, Nashville, TN, USA
Correspondence should be addressed to: anna.means@vanderbilt.edu

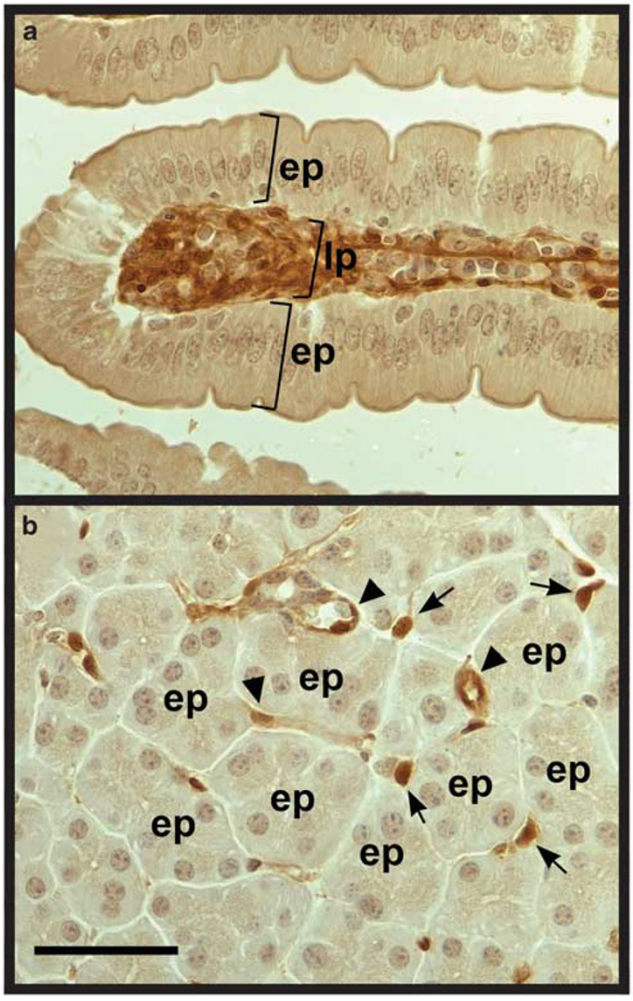

Figure 1 Labeling of mesodermally derived cells identifies mesenchymal cells in adult mouse intestine (**a**) and pancreas (**b**). Nkx3.2$^{Cre/+}$; R26R$^{EYFP/+}$ mice were immunolabeled for EYFP protein as a marker for cells derived from the lateral plate mesoderm.[11,12] These cells make up the bulk of the lamina propria in the duodenum where an abundance of mesenchymal cells are present underlying epithelium. In the pancreas, there is no apparent stromal layer similar to the intestinal lamina propria. Rather, endothelial (arrowheads) and mesenchymal (arrows) EYFP-positive cells are sporadically located between acinar clusters. Ep, epithelium; lp, lamina propria. Size bar, 50 μm.

mesenchymal cells apposed to the epithelium.[5–8] However, in other organs, such as the developing stomach and kidney, both epithelium and surrounding mesenchyme synthesize collagen IV and laminin.[6,8,9] Collagen IV and laminin are also a major component of the acinar basement membrane (Figure 3) but with no obvious underlying lamina propria structure for its synthesis. Owing to the production of RNases by acinar cells, it is not known which cells in the pancreas express which basement membrane proteins nor how the basement membrane links different cellular components of the healthy pancreas.

Riopel *et al*[3] have begun to address this issue of basement membrane maintenance and function by eliminating integrin β1 expression from PaSCs. Integrin β1 is one of the primary receptors linking cells to basement membrane proteins. Loss of integrin β1 did not just affect attachment of PaSCs to the extracellular matrix, but triggered a cascade effect on basement membrane and acinar function. Integrin β1 expression was removed from PaSCs of the adult mouse pancreas via loxP-mediated recombination and the Col1a2-CreERT transgene and tamoxifen administration. Following integrin β1 loss in PaSCs, ECM proteins

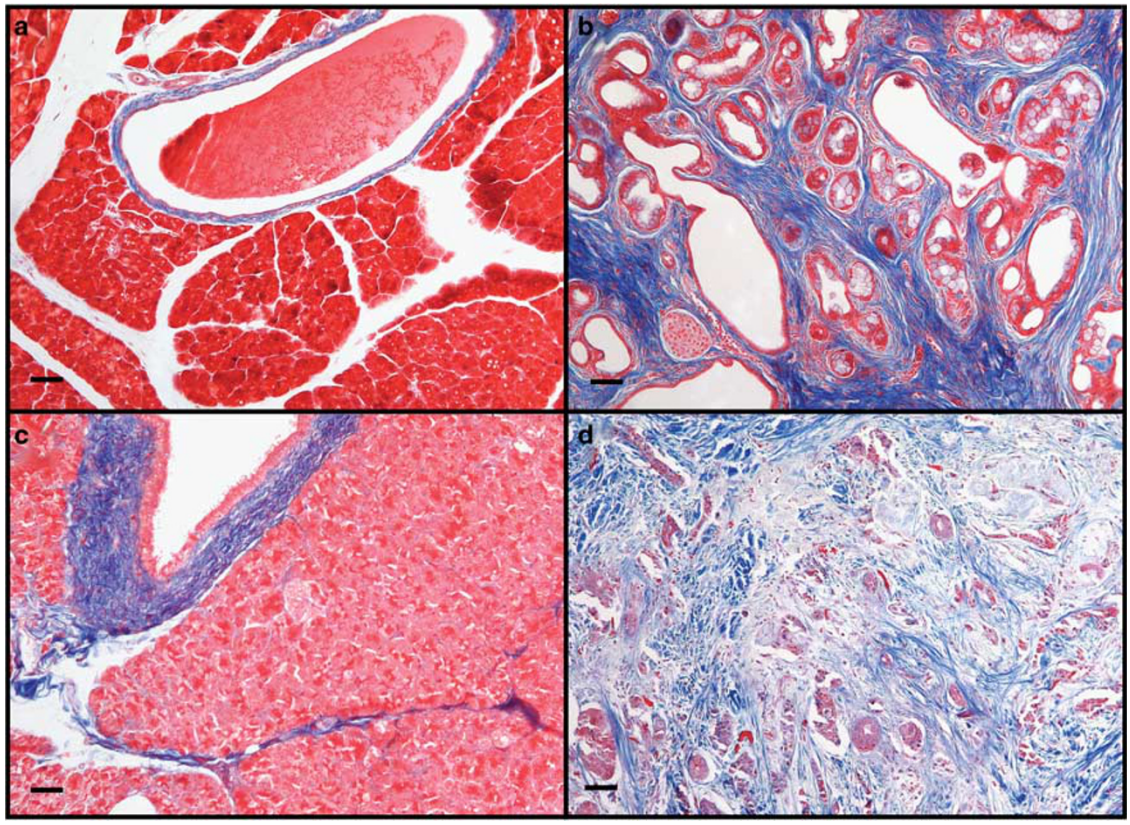

Figure 2 Collagen I is produced primarily around large ducts and blood vessels in normal pancreas in which PaSCs are quiescent (**a**, **c**) but is produced throughout the pancreas by activated PaSCs in diseased pancreas (**b**, **d**). Mouse (**a**, **b**) or human (**c**, **d**) pancreas was stained with Gomori trichrome to label fibrilar collagen I in blue and cells red. Normal mouse pancreas (**a**) has little collagen I accumulation confined mainly to large ducts and blood vessels. When $Kras^{G12D}$ expression[13] is introduced into the mouse pancreas (**b**), cancer precursors arise in the epithelium and extensive collagen deposition indicates rampant fibrosis mediated by activated PaSCs present as individual red cells within the collagen matrix. In normal human pancreas (**c**), collagen deposition is seen mostly around large ducts and blood vessels as in the mouse, but also in septal areas. In human pancreatic cancer (**d**), collagen deposition is extreme, and the collagen-producing mesenchymal cells, such as the activated PaSCs, outnumber the epithelial tumor cells. Size bars, 50 μm.

were rapidly reduced followed by loss of integrin β1 in acinar cells. With loss of basement membrane and integrin receptors, acinar cells had decreased zymogen production and decreased survival. Thus, affecting the interaction of PaSCs with basement membrane led to impaired acinar–basement membrane interaction and loss of acinar function. These studies indicate that quiescent PaSCs regulate pancreas function via maintenance of basement membrane.

So, do quiescent PaSCs produce the ECM proteins that are lost when integrin β1 is lost? This remains an open question owing to the difficulty of studying these small, sparse cells. They are certainly capable of producing large amounts of ECM proteins when activated. Unfortunately, the process of isolating and culturing these cells *ex vivo* is sufficient to induce activation and thus increased ECM production. Saotome *et al*[10] reported that human PSCs cultured *ex vivo* for three passages produced collagen protein. However, by two passages *ex vivo*, α-smooth muscle actin, a widely accepted marker of PaSC activation,[1] was also expressed in these PaSCs, indicating that they had become activated. These results strongly support a role of activated PaSCs in ECM production but leave open the question of how much of a role quiescent PaSCs have in basement membrane production under homeostatic conditions. The work by Riopel *et al*[3] begins to address this difficult question, showing a clear role for PaSCs in maintenance of the acinar basement membrane.

DISCLOSURE/CONFLICT OF INTEREST

The author declares no conflict of interest.

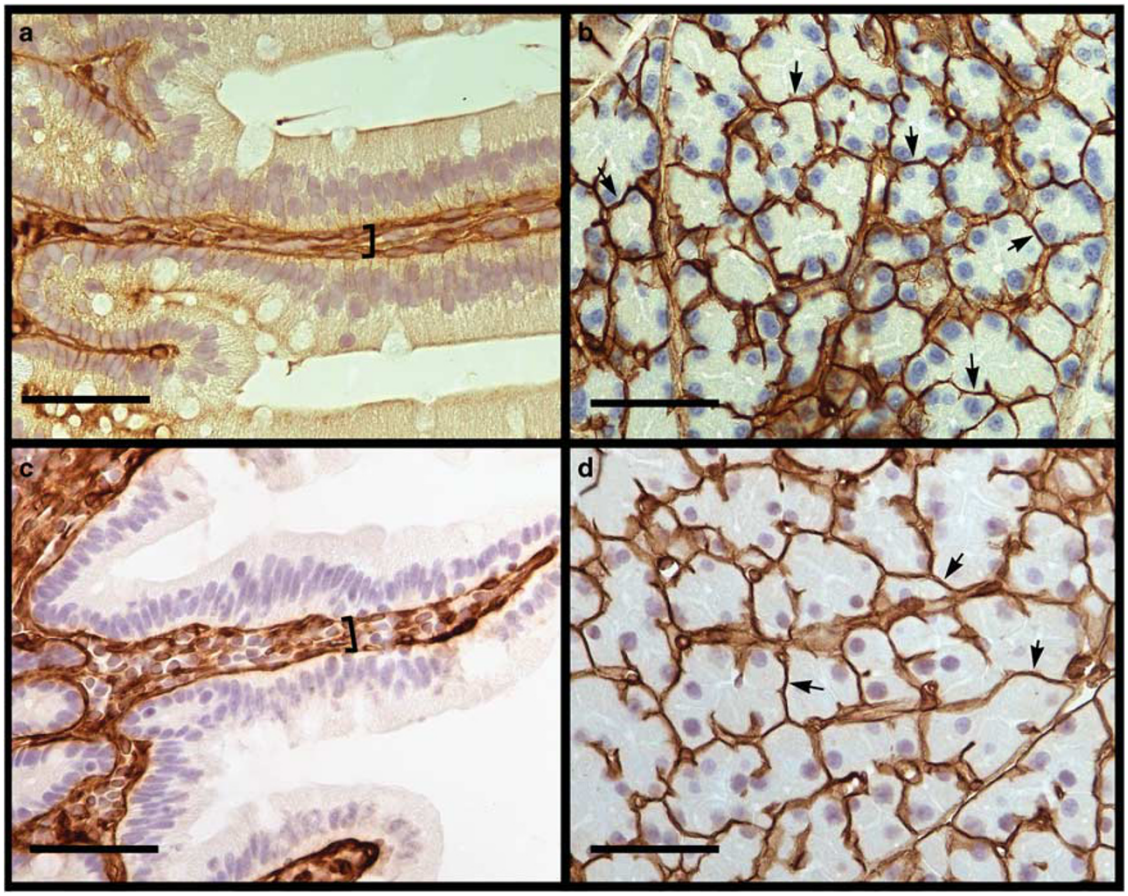

Figure 3 Basement membrane separates epithelium from lamina propria in intestine (**a**, **c**) while separating adjacent epithelial acinar clusters as well as the few stromal cells present in the pancreas (**b**, **d**). Collagen IV (**a**,**b**) and laminin (**c**, **d**) are labeled in brown, and nuclei counterstained in blue. Basement membrane proteins delineate epithelial sheets from subjacent lamina propria in intestine (brackets in **a** and **c**). Basement membrane proteins in pancreas (**b**, **d**) delineate closely apposed epithelial acinar clusters, not just epithelial–mesenchymal boundaries. Arrows in **b** and **d** indicate areas where basement membrane separates acinar epithelium with no apparent intervening stromal layer. However, it cannot be ruled out that very thin mesenchymal cell processes extend into these spaces between juxtaposed acini. Size bars, 50 μm.

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