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Brain meets pancreas:

netrin, an axon guidance molecule, controls epithelial cell migration

Matthias Hebrok and Louis F. Reichardt

Diabetes Center and Departments of Medicine and Physiology, University of California, San Francisco, CA 94143, USA

Abstract

A recent publication shows that two integrins, $\alpha 6 \beta 4$ and $\alpha 3 \beta 1$, interact with netrin-1, a protein best known for its role in axon guidance. Consistent with a role in pancreatic development *in vivo*, netrin is expressed together with these integrins in fetal pancreatic epithelium. The paper presents strong evidence that pancreatic epithelial cells adhere, spread and migrate on netrin-coated substrata through $\alpha 6 \beta 4$. Data also indicate that $\alpha 3 \beta 1$ mediates HGF-stimulated cell migration on netrin-coated substrata. These results indicate that netrins might have much broader functions than previously suspected.

Organogenesis is characterized by intricate interactions between adjacent tissues. During pancreas development (Figure 1), an epithelial sheet of endodermal cells invades the surrounding mesenchyme to form an elaborately branched structure [1]. Most of the highly specialized cell types of the mature pancreas, including exocrine cells and endocrine cells, are derived from this branched epithelium [2]. While much has been learned about the transcriptional control of differentiation within the distinct endocrine and exocrine cell lineages [3], less is known about how epithelial cells interact with the surrounding mesenchyme, how the elaborate branching structure is formed, and how specialized endocrine cells delaminate and migrate away from the epithelium to cluster in the islets of Langerhans. Because of the importance of cell migration in pancreatic development, the discovery that netrin-1, an axon guidance molecule, is expressed in the developing pancreas is particularly provocative [4-6]. Here, we discuss work from Yebra *et al.* that demonstrates a novel role for netrin-1 in cell adhesion and cell migration at the developmental stages when the pancreatic epithelium branches into the surrounding mesenchyme [6].

Netrin was first identified genetically in *Caenorhabditis elegans* as the gene product of *uncoordinated-6 (unc-6)* and purified biochemically from chick brain as a protein that diffuses from its sources to establish gradients that regulate axon guidance [7,8]. In previous work, netrin family members were shown to activate transmembrane receptors in the DCC (deleted in colorectal cancer) and Unc5 families to regulate attractive and repulsive interactions of cell and growth cone migration. In addition, recent work has suggested that adhesive interactions between netrin and the DCC-related receptor neogenin help stabilize cell layers during organogenesis of the mammary gland [9]. It has long been clear that integrins are also essential for organogenesis [10]. Integrins now appear to be a third family of receptors for netrins, distinct from the DCC and Unc5 receptor families.

Integrins are a large family of heterodimeric receptors, consisting of noncovalently associated α and β subunits, that associate with both extracellular and cytoplasmic proteins [10]. While the majority of integrin ligands are constituents of the extracellular matrix, integrins also bind to cell-adhesion molecules, such as VCAM and E-cadherin, proteases, such as matrix metalloproteases 1 and 2, and regulators of TGF β signaling including chordin and TGF β latency-associated polypeptide [11,12]. Integrins also bind to and mediate signaling by several heparin-binding multimodular proteins of the CCN family [13].

Netrin-integrin interactions in early pancreatic development

A key observation of Yebra *et al.* indicating that the role of netrin-1 at the early stages of pancreas formation is different from its function in neuronal cell migration is that DCC and neogenin, the usual receptors, are not involved in epithelial cell adherence [6]. In addition, a discrete area marked by high levels of netrin-1 expression is not found in the developing pancreas, suggesting that pancreatic epithelial cells do not migrate towards a localized source of netrin-producing cells. Rather, the broad distribution of netrin observed in the basement membrane that separates the pancreatic epithelial cells from surrounding mesenchyme suggested that netrin-1 might bind to components of the extracellular matrix (ECM), as suggested from earlier studies [14]. In biochemical studies, Yebra *et al.* show that netrin-1 binds to both the ECM protein collagen IV and the integrins $\alpha 6 \beta 4$ and $\alpha 3 \beta 1$ that are expressed on epithelial cells [6]. The binding appears specific for a subset of integrins as others, including $\alpha 2 \beta 1$ and $\alpha V \beta 8$, do not bind to netrin-1. Inhibition of netrin-integrin interactions affects cell adherence as well as migration of pancreatic epithelial cells. However, the data provided by Yebra *et al.* do not directly address whether netrin-1 function is crucial for the delamination of endocrine pre-cursors from the pancreatic epithelium and their subsequent migration during islet formation *in vivo*. While netrin-1 elicits the migration of putative epithelial progenitor cells (cytokeratin-19⁺/Pdx-1⁺), previous studies had suggested that different integrins, $\alpha v \beta 3$ and $\alpha v \beta 5$, promote endocrine cell migration and islet formation [15]. Inhibition of $\alpha v \beta 5$ activity does not reduce epithelial cell migration, indicating that netrin-mediated cell migration is organized through a distinct subset of integrins. Although additional studies will have to be performed, the current data would suggest that netrin-1-integrin interactions mediate migration of pancreatic epithelial cells at early stages of development, whereas migration of differentiated endocrine cells is dependent upon classical integrin-ECM interactions. Other groups have noted that other members of the netrin family are expressed in pancreatic tissue [5,16], so it will be interesting to determine whether those also interact with integrins or DCC/neogenin receptors to regulate, possibly redundantly, migration of differentiated endocrine cells during islet formation.

Netrin-integrin signaling

Netrin-integrin interactions might have more general importance in cell migration and adhesion, extending beyond their roles in pancreatic organogenesis. Integrins have unique properties that could potentially be important in regulation of netrin-mediated signaling. 'Inside-out' signaling regulates the ability of integrins to bind to extracellular ligands [10]. Thus intracellular signaling pathways might control cellular responsiveness to netrin through regulation of integrin conformation. In addition, cAMP and cGMP have been shown to control whether growth cone responses to netrin mediated by DCC are attractive or repulsive [17, 18]. Interactions of retinal ganglion cell growth cones with laminin, presumably mediated through integrins, can convert the response to netrin from attractive to repulsive through regulation of cAMP levels [19]. Thus, netrin association with integrins might control the output of netrin interactions with DCC and Unc5 family members through integrin-regulated signaling pathways.

Ligand engagement of integrins also activates signaling pathways through phosphoinositide 3-kinase, ras and rho family GTPases that control cell survival, proliferation, motility, cytoskeletal organization and gene transcription [10,20]. The data of Yebra *et al.* [6] suggest that netrin might activate each of these signaling pathways in the pancreas. In addition, similar to integrin engagement, netrin signaling through DCC activates Rho GTPases [21,22], so in cells that express both integrins and DCC, integrin engagement of netrin might reinforce the guidance role of DCC.

The integrins $\alpha 6\beta 4$ and $\alpha 3\beta 1$ are unusual integrins with novel properties and signaling mechanisms that could potentially confer specificity on netrin signaling [20]. In epithelia, both integrins bind to LN-5, a major epithelial laminin isoform. In addition, $\alpha 6\beta 4$ is specifically associated with the hepatocyte growth factor (HGF) receptor c-met and functions as an essential adaptor in its signaling pathway [23]. $\alpha 6\beta 4$ has a large cytoplasmic domain that is phosphorylated on tyrosines following either c-met activation or binding to laminin-5. This results in recruitment of Shc and activation of phosphoinositide 3-kinase, Rac and Ras signaling pathways [20]. Thus, in epithelial cells, binding of $\alpha 6\beta 4$ to laminin or netrin is expected to activate pathways that promote cell proliferation, survival, differentiation and migration. In neurons and other cells that express both $\alpha 6\beta 4$ and DCC receptors, stimulation of $\alpha 6\beta 4$ by netrin might synergize with Rac activation through DCC engagement. In addition to binding to laminin, $\alpha 3\beta 1$ associates tightly *in cis* with the tetraspan protein CD151, which stimulates signaling through PtdIns 4-kinase and protein kinase C (PKC), and with the GPI-linked urokinase receptor (uPAR), which initiates a protease cascade [24]. uPAR association with $\alpha 3\beta 1$ is promoted by binding of urokinase to uPAR as well as by integrin activation. uPAR not only controls cell behavior through urokinase but directly promotes integrin signaling through activation of Src. In epithelial cells, this results in stimulation of slug expression, downregulation of E-cadherin and stimulation of cell migration. Ligand engagement of $\alpha 3\beta 1$ by netrin might thus stimulate signaling through both uPAR and CD151. It seems possible that the inhibitory effect of antibodies against $\alpha 3\beta 1$ on cell migration over netrin-coated substrata is caused by perturbations of uPAR-integrin, instead of netrin-integrin, interactions [6].

Remaining issues

While the results described above are exciting, some issues remain unsettled. First, it will be important to determine the functional importance *in vivo* of the reported interactions. While netrin and the integrins partially colocalize *in vivo*, evidence of direct physical association is missing. In previous work on integrins, stronger evidence for physical association has been provided through co-immunoprecipitation. Second, the integrin-binding site in the C-terminal domain of netrin was identified solely through use of synthetic peptides. In the future, it will be important to examine the function of this region in its native conformation, using deletions and mutations within netrin. Of potential concern, netrin is probably localized *in vivo* through interactions that might make this C-terminal domain inaccessible to integrins. Heparin and heparan sulfate bind with nanomolar affinities to the same C-terminal basic domain in netrin [25]. By contrast, interactions of netrin with DCC are not inhibited by the presence of heparin [26]. Thus, experiments are needed to assess the ability of integrins to associate with netrin in physiological environments. Finally, data in the paper suggest that netrin binds to collagen IV, fibronectin and possibly laminin [6], but the affinity and specificity of these interactions were not well characterized. In the future, it will be important to characterize interactions of netrin with ECM constituents and proteoglycans more completely and to identify those interactions that are most relevant *in vivo*.

Concluding remarks

The study by Yebra *et al.* [6] describing a role for netrin-integrin interactions in pancreatic development and a complementary study by Srinivasan *et al.* [9] on the role of netrin-neogenin interactions in mammary gland development indicate that members of the netrin family might have hitherto unsuspected roles in embryonic development. Overlapping distributions of members of this family might have prevented identification of these functions through analysis of mice lacking individual family members. The concept that axon guidance molecules are 'reused' for additional purposes is particularly exciting and complements recent papers demonstrating that Hedgehog, Wnt and Bmp family members that are essential for early patterning of the embryo are 'reused' at later stages as axon guidance molecules [27-29]. While significant data on the role of netrin signaling during development have been presented, future studies might profitably address the participation of these proteins in tissue regeneration and adult organ maintenance.

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Figure 1.

Pancreas morphology and netrin-1 protein distribution. (a) At early stages of pancreas organogenesis, the endodermal epithelium buds into the surrounding mesenchyme. (b) During later stages, the epithelium undergoes elaborate branching morphogenesis and the mature cell types of the exocrine and endocrine lineage start to emerge. The exocrine component comprises acinar cells that produce and secrete digestive enzymes into an elaborate system of interconnected channels formed by the other main exocrine cell type, the duct cell. In addition to providing digestive enzymes, the pancreas also contains an endocrine portion located in islets of Langerhans, clusters of hormone-producing cells interspersed within the exocrine matrix. (b,c) Netrin-1 protein (shown in red) is distributed in a subset of epithelial as well as differentiated endocrine and acinar cells. In addition, expression is localized to the basement membrane that separates the epithelial and mesenchymal layers.