**Sorting out fate determination**

Mostafa Bakhti1-3, Aimée Bastidas-Ponce1-4 and Heiko Lickert1-4

*1Institute of Diabetes and Regeneration Research, Helmholtz Zentrum München, Neuherberg, Germany*

*2Institute of Stem Cell Research, Helmholtz Zentrum München, Neuherberg, Germany*

*3German Center for Diabetes Research (DZD),* *Neuherberg, Germany*

*4Technical University of Munich, Medical Faculty, Munich, Germany*

Corresponding author: [heiko.lickert@helmholtz-muenchen.de](mailto:heiko.lickert@helmholtz-muenchen.de)

**How organ morphogenesis specifies cell fate and whether organ progenitors are predetermined or specified via niche signals are critical developmental biology questions. In this issue of *Developmental Cell*, Nyeng et al. (2019) modulate cell-cell adhesion in the pancreas and provide evidence that progenitors are plastic and instructed by niche signals.**

The developmental mechanisms linking morphogenesis and cell fate decisions are not well understood. One example is pancreas organogenesis, which starts with formation of an epithelial primordium that progressively gets patterned and segregates into central trunk and peripheral tip domains. Further development of these domains results in the differentiation of acinar cells in the tip and ductal and endocrine cells from the trunk. During the establishment of the tree-like tubular network, endocrine progenitors (EPs) arise from the bipotent trunk epithelial cells. Increased levels of Neurogenin3 (Neurog3) expression specifies endocrine cells that delaminate to form hormone-producing cells in the islets of Langerhans (Bakhti et al., 2019). One of the key questions that remained to be addressed in this organ system is how lineage segregation occurs during pancreas development. Are the three main pancreatic lineages (acinar, ductal and endocrine) derived from intrinsic predetermined progenitors? Or does progenitor differentiation depend on extrinsic signal from the surrounding niche? Although the common notion is that pancreas architecture defines lineage decision, this idea is challenged by recent studies reporting that predetermined unipotent progenitors exist before organ patterning (Larsen et al., 2017; Sznurkowska et al., 2018). Now, in this issue of *Developmental Cell*, Nyeng et al. (2019) reconciles apparently opposing models of progenitor predetermination and niche instruction by showing that differential cell-cell surface tension (dictated by a combination of adhesion and cortical tension) in pancreatic progenitors is important for cell sorting and pattern formation before environmental signals in the trunk and tip niche further leads to cell-fate determination.

For their study, the authors established several mosaic mutant and/or reporter mouse lines and performed long-term live imaging on organotypic pancreatic cultures over 2 days. They found that during early stages of pancreas development, the cells in the center actively move towards the periphery over a remarkable long distance. This raised the question of whether the progenitors were predetermined before their movement or instructed by the niche that they moved into. A first hint came from the careful analysis of immune localization studies of the protein p120-catenin. Before tip-trunk patterning, pancreatic progenitors express heterogeneous levels of p120-catenin (p120ctnlow and p120ctnhigh) and are randomly intermingled. However, the authors observed that during pattern formation, p120ctnlow cells move towards the periphery and p120ctnhigh cells remain in the center (Fig. 1A). This suggested that differential cell-cell surface tension of progenitors determines cell sorting, pattern formation, and potentially through niche signals, define trunk (duct/endocrine) and tip (acinar) fates. To test this idea directly, the authors generated mosaic p120ctn or E-cadherin mutant mice to change cell-cell surface tension in a mosaic fashion. This led to the movement and enrichment of p120ctn-KO cells in the peripheral tips. Notably, the p120ctn-low or -KO cells induced an acinar cell program after relocating toward the peripheral tip, which implies that the cells were not predetermined before movement, but instead acquired the acinar fate in the tip domain. Thus, increased acinar differentiation is not a direct consequence of p120ctn reduction or loss but is a secondary result of cell repositioning, indicating that differential cell-cell surface tension eventually predetermines cellular fate through cell sorting.

The authors further found a remarkable reduction in p120ctn protein levels in insulin-expressing cells, as compared to bipotent ductal and Neurog3+ EPs in mouse and human (Fig. 1A). This finding indicates that after tip-trunk patterning, transient downregulation of cell-cell adhesion further segregates the endocrine lineage from the ductal epithelium. In addition, mosaic loss of p120ctn increased delamination of Neurog3+ KO cells, which resulted in decreased numbers of β-cells and increased numbers of α-cells. This suggests that the duration of EP residency within the epithelium impacts endocrine lineage allocation. Accordingly, -cells are formed at early stages during development when cell polarity and cell-cell adhesion are not yet well established in the trunk epithelium, and these cells leave the niche faster. On the other hand, β-cells are determined at later stages within the well polarized and p120ctnhigh trunk epithelium. They stay longer in the niche due to higher cell-cell adhesion, which is downregulated upon delamination. Along the same line, the Semb laboratory has previously shown the requirement of established apical cell polarity for β-cell, but not α-cell formation (Löf-Öhlin et al., 2017). Thus, the current study further supports the notion that cell polarity and adhesion-mediated progenitor residency within the epithelium define endocrine subtype specification. Additionally, the majority of β-cells were apically polarized and attached to the epithelial lumen upon differentiation. The authors observed that after β-cell birth, the cells lost polarity, formed membrane protrusions and exhibited motile features before they delaminated from the epithelium and formed clusters (Fig. 1A). These data challenges the current idea that β-cells form after delamination, instead demonstrating that β-cells are already specified and born in the trunk epithelium.

Over 60 years ago differential cell-cell adhesion was shown to sort out the primary germ layers during gastrulation, but the molecular basis was unknown at that time (Townes and Holtfreter, 1955). The work of Nyeng et al. (2019) now further show that differential cell-cell surface tension also directs cell sorting, tissue patterning, and cell-fate decisions in the pancreas. The authors have shown that differential expression of p120ctn first pattern pancreatic epithelium into tip and trunk and then further segregates endocrine and ductal lineages. These findings increase our understanding of pancreas patterning that drives cell differentiation. They also help to establish model systems for pancreas-related diseases (including pancreatic cancer, pancreatitis and diabetes), develop a way to generate compact 3D pancreatic spheres for pancreatic progenitor expansion, and provide a means to induce α- and β-cell fate by modulating timing of intraepithelial progenitor residency or delamination.

Future work should address how p120ctn and cell-cell surface tension heterogeneity is regulated and drives patterning and cell-fate determination. Moreover, how differential expression of p120ctn and other adhesion junction molecules in pancreatic progenitors is regulated on transcriptional and post-transcriptional level deserves further attention. This is particularly important in case of cancer metastasis that is highly connected to changes in cell-cell adhesion. For instance, targeting of p120ctn by miR-197 was shown to increase epithelial-mesenchymal transition (EMT) in pancreatic cancer cells (Hamada et al., 2013) (Fig. 1A). Modulating cell-cell adhesion in metastatic tumor cells might move them into a different niche and change the cellular phenotype, such as the initiating step of acinar-to-ductal metaplasia during pancreatic cancer initiation. Furthermore, the molecular signature of heterogenous progenitors based on their cell-cell adhesion levels needs to be studied using tools such as single-cell transcriptomics combined with measuring cell-surface adhesion proteins. This will contribute to a better understanding of how tissue morphogenesis, pattering and differentiation is linked. Finally, the evidence that human endocrine cells also reduce levels of p120ctn upon differentiation, suggests that modulating cell-cell adhesion in a temporal controlled manner might allow for the efficient generation of endocrine cell subtypes from human pluripotent stem cells (hPSCs) (Fig. 1B).

**Figure 1.** **Differential expression of p120ctn couples pancreas patterning and lineage segregation** (A) Heterogeneous population of pancreatic progenitors segregate into tip or trunk domains based on their p120ctn protein levels where they are specified by niche signals. During endocrinogenesis, differentiating endocrine cells reduce the levels of p120ctn as compared to nearby epithelial cells. p120ctn reduction by miR-197 results in EMT in pancreatic cancer cells. (B) Targeting of p120ctn in hPSCs may allow endocrine differentiation to be directed *in vitro*.

Bakhti, M., Böttcher, A., and Lickert, H. (2019). Modelling the endocrine pancreas in health and disease. Nat. Rev. Endocrinol. *15*, 155–171.

Hamada, S., Satoh, K., Miura, S., Hirota, M., Kanno, A., Masamune, A., Kikuta, K., Kume, K., Unno, J., Egawa, S., et al. (2013). miR-197 induces epithelial-mesenchymal transition in pancreatic cancer cells by targeting p120 catenin. J. Cell. Physiol. *228*, 1255–1263.

Larsen, H.L., Martín-Coll, L., Nielsen, A.V., Wright, C.V.E., Trusina, A., Kim, Y.H., and Grapin-Botton, A. (2017). Stochastic priming and spatial cues orchestrate heterogeneous clonal contribution to mouse pancreas organogenesis. Nat. Commun. *8*, 605.

Löf-Öhlin, Z.M., Nyeng, P., Bechard, M.E., Hess, K., Bankaitis, E., Greiner, T.U., Ameri, J., Wright, C. V., and Semb, H. (2017). EGFR signalling controls cellular fate and pancreatic organogenesis by regulating apicobasal polarity. Nat. Cell Biol. *19*, 1313–1325.

Nyeng, P., Heilmann, S., Löf-Öhlin, Z.M., Pettersson, N.F., Hermann, F.M., Reynolds, A.B., and Semb, H. (2019). p120ctn-Mediated Organ Patterning Precedes and Determines Pancreatic Progenitor Fate. Dev. Cell, this issue.

Sznurkowska, M.K., Hannezo, E., Azzarelli, R., Rulands, S., Nestorowa, S., Hindley, C.J., Nichols, J., Göttgens, B., Huch, M., Philpott, A., et al. (2018). Defining Lineage Potential and Fate Behavior of Precursors during Pancreas Development. Dev. Cell *46*, 360–375.e5.

Townes, P.L., and Holtfreter, J. (1955). Directed movements and selective adhesion of embryonic amphibian cells. J. Exp. Zool. *128*, 53–120.