Published in final edited form as:
Cell Metab. 2010 January ; 11(1): 2–3. doi:10.1016/j.cmet.2009.12.005.

Ductal origin hypothesis of pancreatic regeneration under attack

Jake A. Kushner[1,*], **Gordon C. Weir**[2,*], and **Susan Bonner-Weir**[2,*]

[1]Division of Endocrinology and Diabetes, Children's Hospital of Philadelphia, Institute of Diabetes Obesity and Metabolism, Institute for Regenerative Medicine, Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104, USA

[2]Section of Islet Transplantation and Cell Biology, Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts, USA, 02215

Although pancreatic beta cells are known to expand by self-renewal in postnatal life, contribution by ductal progenitors remains vigorously debated. In a recent issue of Developmental Cell Jorge Ferrer and colleagues report lineage-tracing studies that challenge the ductal origin hypothesis (Solar et al, 2010).

Reduced beta-cell mass is fundamental to diabetes mellitus, which has led to considerable interest in beta-cell regeneration. Despite decades of investigation, this regeneration process remains incompletely understood. Self-renewal by beta cells is the dominant mechanism of beta cell mass expansion postnatally, following stimuli such as pregnancy, or toxigene mediated beta cell ablation (Brennand et al., 2007; Dor et al., 2004; Nir et al., 2007; Teta et al., 2007). What remains debated is whether beta cells are formed postnatally from cells other than preexisting beta cells, and if so, from what cell of origin? Proposed mechanisms have included ductal progenitors, acinar transdifferentiation, circulating progenitors, and putative pancreatic stem cells (Granger and Kushner, 2009; Pittenger et al., 2009). The potential of ductal progenitors has been of interest for years, and recently Bonner-Weir and colleagues used carbonic anhydrase II (*CAII*) promoter activity to perform lineage tracing of ductal epithelium (Inada et al., 2008). From birth until age 4 weeks substantial numbers of acinar and endocrine cells were traced from *CAII*-expressing cells. Additionally, pancreatic duct ligation (PDL) mediated pancreatic injury resulted in a quarter of the beta cells being marked (Inada et al., 2008). Additional studies by Heimberg and colleagues showed that beta cell mass doubled in the ligated portion a week after PDL (Xu, 2008). PDL activated cells expressing neurogenin 3, a critical transcription factor for islet development. However, they were not able to trace the lineage of ductal cells to beta cells *in vivo*, leaving open the possibility that other cells could contribute to beta cell mass expansion.

Enter Ferrer and colleagues, who report in Developmental Cell lineage-tracing studies that test the contribution of ductal epithelium in beta cell mass expansion and regeneration (Solar et al, 2010). The authors observe that HNF1β expression is restricted to ductal epithelium in embryonic pancreas and in the adults is expressed throughout the ductal tree. They derived *Hnf1bCreER* mice, which express Cre recombinase fused to a tamoxifen-binding domain of the estrogen receptor driven by the *Hnf1b* promoter. *Hnf1bCreER* mice were crossed with Rosa26 reporter mice to perform lineage tracing of *Hnf1b* promoter activity. After tamoxifen treatment from E11.5–13.5, labeled duct, beta, alpha and acinar cells were found. These observations support the notion that *Hnf1b* is expressed in multipotent embryonic progenitors. Lineage tracing studies were then performed with late embryonic and adult

Correspondence: kushnerj@mail.med.upenn.edu (J.A.K.), gordon.weir@joslin.harvard.edu (G.C.W), susan.bonner-weir@joslin.harvard.edu (S.B-W).

mice and tracing was observed throughout the ductal epithelium including centro-acinar cells. But, there was no contribution by *Hnf1b*-expressing cells to acinar cells, beta cells or other endocrine cells. Thus, Ferrer and colleagues came to a surprising and especially provocative conclusion: pancreatic ductal epithelium does not contribute to endocrine nor acinar cells postnatally.

Ferrer and colleagues further challenged the hypothesized ductal origin of postnatal pancreatic regeneration with PDL. This intervention provoked massive pancreatic injury, causing reorganization of pancreatic tissue into whorls of epithelial ductal structures labeled by *Hnf1b* expression. As expected, beta cell mass was doubled within a week of PDL, but no beta cells were marked. The authors conclude that new beta cells must have originated from a source other than ductal epithelium. Additionally the authors performed beta cell ablation studies with alloxan followed by epidermal growth factor and gastrin, previously reported to stimulate islet neogenesis from duct cells (Rooman and Bouwens, 2004). However, Ferrer and colleagues again observed the lack of contribution from *Hnf1b*-derived progenitors, even though the beta cell mass increased 9 fold. The authors conclude that pancreatic ductal epithelium does not contribute to beta cell growth during acute regeneration.

The results of Ferrer and colleagues contradict earlier studies supporting a ductal origin of beta cells in postnatal life, but there are important caveats. *Hnf1bCreER* mice would ideally allow promoter lineage tracing of ductal epithelial cells in a sensitive and specific manner. But, *Hnf1bCreER* mice are only capable of extremely low labeling efficiency during embryonic development (1–2%). While the *Hnf1bCreER* mice label a much larger portion (~40%) of adult ductal epithelial cells, most duct cells are still unlabeled. Given the rapid PDL-induced beta cell mass expansion, it is possible that some ductal progenitors could have lower expression of *Hnf1b* that was inadequate for excision. Although their efforts to uncover *Hnf1b* expression heterogeneity in the ductal epithelium were unsuccessful, variability cannot be definitively ruled out. The cells that become marked could represent a population of duct cells with a higher activity of *Hnf1b* transcription or greater accessibility to Cre recombinase.

Data supporting a duct origin for islet cells postnatally in mice, rats and humans exist, but the ductal origin hypothesis remains controversial and in need of definitive experiments. Growth of the exocrine pancreas from duct cells during branching morphogenesis remains an attractive hypothesis, which, surprisingly, is unsupported by this paper. If duct cells are not the origin of this increase in beta cell number after PDL, what other candidates might produce so many beta cells this quickly? The demonstrated increase in beta cell replication after PDL must account for some of increased beta cell mass, with these replicating cells being from either newly formed or pre-existing islets. If there are non-duct precursor cells, they must exist in reasonable numbers, but then why are they not easier to identify?

Assuming that the above caveats turn out to be groundless, the studies by Ferrer and colleagues considerably advance our understanding of pancreas development. *Hnf1bCreER* mice can be viewed as a novel tool to identify components of pancreas development. It can be concluded that *Hnf1b* floxed duct cells do not substantially contribute to endocrine nor acinar pancreas. In the future we imagine that *Hnf1bCreER* mice can be applied to exclude pancreatic lineages when searching for progenitors of beta cell expansion. Ultimately, it will be important to confirm and expand the findings of Ferrer and colleagues with lineage tracing studies using additional markers that label either ducts or other candidate precursor cells.

In the end the experiments by Ferrer and colleagues challenge the duct origin hypothesis, but the above caveats must be addressed. If future similarly rigorous experiments succeed in disproving the duct cell origin, where do we look next to explain the impressive regenerative potential of the beta cells? We anticipate that the next few years will have many surprises in this exciting field.

REFERENCES

Brennand K, Huangfu D, Melton D. All beta cells contribute equally to islet growth and maintenance. PLoS Biol. 2007; 5:e163. [PubMed: 17535113]

Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature. 2004; 429:41–46. [PubMed: 15129273]

Granger A, Kushner JA. Cellular origins of beta-cell regeneration: a legacy view of historical controversies. J Intern Med. 2009; 266:325–338. [PubMed: 19765178]

Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A, Bonner-Weir S. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proc Natl Acad Sci U S A. 2008; 105:19915–19919. [PubMed: 19052237]

Nir T, Melton DA, Dor Y. Recovery from diabetes in mice by beta cell regeneration. J Clin Invest. 2007; 117:2553–2561. [PubMed: 17786244]

Pittenger GL, Taylor-Fishwick D, Vinik AI. A role for islet neogenesis in curing diabetes. Diabetologia. 2009; 52:735–738. [PubMed: 19271208]

Rooman I, Bouwens L. Combined gastrin and epidermal growth factor treatment induces islet regeneration and restores normoglycaemia in C57Bl6/J mice treated with alloxan. Diabetologia. 2004; 47:259–265. [PubMed: 14666367]

Solar M, Cardalda C, Houbracken I, Martin M, Maestro MA, De Medts N, Xu X, Grau V, Heimberg H, Bouwens L, Ferrer J. Pancreatic exocrine duct cells give rise to insulin-producing β-cells during embryogenesis but not after birth. Dev Cell. 2010

Teta M, Rankin MM, Long SY, Stein GM, Kushner JA. Growth and regeneration of adult beta cells does not involve specialized progenitors. Dev Cell. 2007; 12:817–826. [PubMed: 17488631]

Xu X, D'Hoker J, Stange G, Bonne S, De Lu N, Xiao X, De Casteele MV, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H. Beta cells can be regenerated from endogenous progenitors in adult mouse pancreas. Cell. 2008; 132:197–207. [PubMed: 18243096]