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Barrier function of the coelomic epithelium in the developing pancreas

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ABSTRACT

Tight spatial regulation of extracellular morphogen signaling within the close confines of a developing embryo is critical for proper organogenesis. Given the complexity of extracellular signaling in developing organs, together with the proximity of adjacent organs that use disparate signaling pathways, we postulated that a physical barrier to signaling may exist between organs in the embryo. Here we describe a previously unrecognized role for the embryonic coelomic epithelium in providing a physical barrier to contain morphogenic signaling in the developing mouse pancreas. This layer of cells appears to function both to contain key factors required for pancreatic epithelial differentiation, and to prevent fusion of adjacent organs during critical developmental windows. During early foregut development, this barrier appears to play a role in preventing splenic anlage-derived activin signaling from inducing intestinalization of the pancreas-specified epithelium.

1. Introduction

Tight spatial regulation of extracellular morphogen signaling within the close confines of an embryo would seem to be crucial for proper organogenesis. Morphogen signaling is thought to be contained and regulated through numerous mechanisms, such as cytoneme signaling and ligand/receptor specificity (Cadigan and Nusse, 1997; Chuang and McMahon, 1999; Gupta and Defranco, 2003; Parr and McMahon, 1994; Ramirez-Weber and Kornberg, 1999; Williams et al., 2004; Zeng et al., 2001). Physical barriers that contain such signaling have not been well-delineated. Here we describe a previously unrecognized function of the embryonic coelomic epithelial layer in the regulation of organogenesis signals in the developing mouse pancreas. This layer of cells appears to function as a barrier that prevents diffusion out of key factors required for pancreatic epithelial differentiation, and prevents diffusion in of non-pancreatic signals (specifically in the form of splenic anlage-derived activin signals), and lastly prevents inappropriate fusion with adjacent organs.

Compartmentalized morphogen signaling would seem to be necessary during organogenesis to avoid potentially chaotic cross-talk of signals between different developing organs. The very early embryo is known to have morphogen gradients that can signal across different embryonic structures or organs, but as organogenesis proceeds, we hypothesized that compartmentalization of signaling to individual organs may occur. In

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mature animals, such compartmentalization of signaling is achieved through the development of specialized cellular layers. Examples of cell layers that serve to compartmentalize distinct regions within the body include the peritoneal and pleural lining, which both derive from the embryonic coelomic epithelium. Coelomic epithelium in the embryo is derived from the lateral plate mesoderm and, in addition to peritoneum and pleura, has been shown to contribute to the formation of structures such as the Müllerian duct (Hashimoto, 2003), the developing gonad (Tanimura and Iwasawa, 1989), and endothelial and hematopoietic cell progenitors (Munoz-Chapuli et al., 1999).

We describe here that during early endoderm organogenesis the coelomic epithelium serves as a barrier to prevent the loss of morphogens required for proper development of the embryonic mouse pancreas by diffusion out. In addition, the coelomic epithelium may serve to prevent the inappropriate fusion of closely compacted embryonic viscera.

2. Results/discussion

2.1. *Ontogeny and characterization of embryonic coelomic epithelium overlying the pancreas*

We initially observed that the early embryonic mouse dorsal pancreas epithelium and mesenchyme are encased within an outer layer of cells that is continuous with the coelomic epithelium that surrounds the intestines of embryonic mice. At embryonic day 9–10 in the mouse, the coelomic epithelium is readily visible and appears thickened around the lateral aspects of the developing pancreatic epithelium (Fig. 1A,B). As the dorsal pancreatic epithelium buds out from the foregut endoderm and rotates laterally, we saw that the coelomic epithelium grows and intercedes between the dorsal aspect of the pancreatic bud and the dorsal aortae and notochord. Histological sections through the dorsal pancreas at embryonic day 10 show how the coelomic epithelial layer lies over the pancreatic mesenchyme and epithelium, and could potentially serve as a barrier to external influences (Fig. 1C). Ultrastructural examination using electron microscopy reveals tight junctions between cells of the coelomic epithelium, supporting such a barrier function (Fig. 1D). Further evidence for an epithelial barrier-type role for this layer is provided by immunohistochemical staining for laminin-1, which suggests that mature basement membrane underlies the coelomic epithelium (Fig. 1E). Ontogeny studies of the coelomic epithelium and the underlying pancreas show that the coelomic epithelium begins to cover the dorsal aspect of the pancreas coincident with the separation of the paired dorsal aortae away from the dorsal pancreas (data not shown) (Lammert et al., 2001), possibly indicating that dorsal pancreatic development is no longer dependent on signaling from the dorsal aortae. The coelomic epithelium may represent a transition from extra-pancreatic influences on pancreatic development (e.g. aortae) to intrapancreatic influences only (e.g. pancreatic mesenchyme). As the pancreas matures, the coelomic epithelium covering the pancreas begins to attenuate (Fig. 1F-H) as it develops into the mature mesothelial peritoneal lining that covers the adult pancreas (Fig. 1I). (Fig. 1H, I). Immunohistochemistry for laminin-1 and vimentin, in

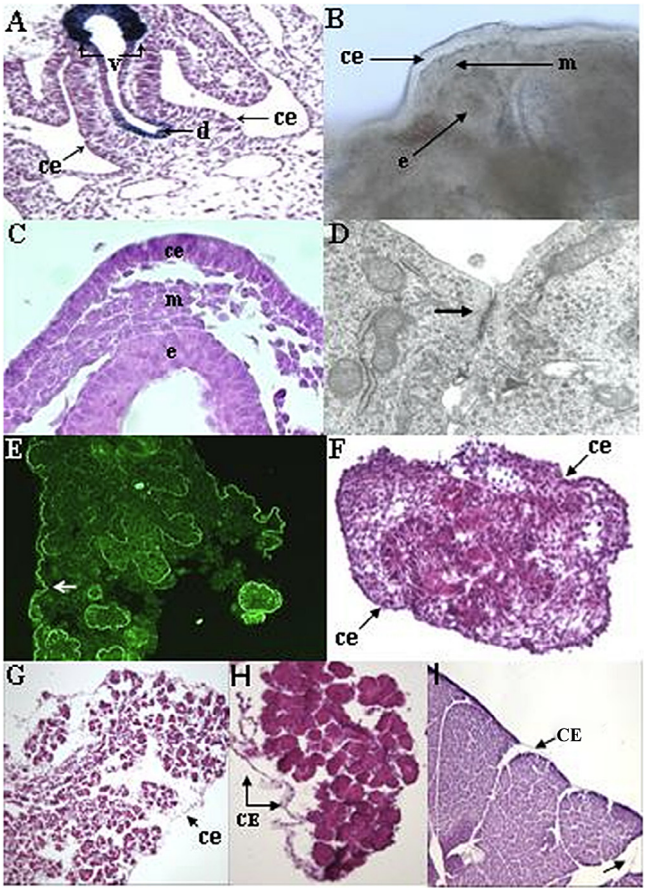

Fig. 1 – Characterization and ontogeny of coelomic epithelium. (A) e9 *Pdx-1-LacZ* transgenic embryo stained for beta-gal activity, (d) dorsal and (v) ventral regions of Pdx1 positive areas (the coelomic epithelium is indicated by ce). The right and left dorso-medial aspects of the ceolomic epithelium will later invaginate toward each other, which will lead to a walling off of the dorsal bud by day 10.5 (not shown). (B) Inverted microscope image of an e10 embryo in the region of the foregut. Pancreatic epithelium (e) has budded from the gut tube and is embedded in pancreatic mesenchyme (m) within the overlying coelomic epithelium (ce). The coelomic epithelium appears as a thickened transparent layer immediately dorsal to the developing pancreas. (C) H&E staining of a transverse section of an e10 pancreas showing the anatomic relationships of the pancreatic epithelium (e), mesenchyme (m), and coelomic epithelium (ce). (D) Electron microscopy of the e10 coelomic epithelium showing tight junctions between two cells (arrow). (E) Immunohistochemistry for laminin-1 on e12 mouse pancreas. Basement membrane is present beneath the coelomic epithelial layer (arrow), as well as at the interface between pancreatic epithelium and mesenchyme. (F) H&E of e12 mouse pancreas showing a thinning of the coelomic epithelium compared with e10 (arrows). H&E of e15 (G), e18 (H), and adult (I) mouse pancreas showing further thinning of the CE, with eventual maturation of the CE to become the peritoneal lining around the adult pancreas. Arrows on panels F–I indicate the positioning of the coelomic epithelium (or peritoneal mesothelial lining in the adult in I) at these developmental time points.