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Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues

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Abstract

The location and movement of mammalian gut tissue progenitors, prior to the expression of tissue-specific genes, has been unknown, but this knowledge is essential to identify transitions that lead to cell type specification. To address this, we used vital dyes to label exposed anterior endoderm cells of early somite stage mouse embryos, cultured the embryos into the tissue bud phase of development, and determined the tissue fate of the dye labeled cells. This approach was performed at three embryonic stages that are prior to, or coincident with, foregut tissue patterning (1–3 somites, 4–6 somites, and 7–10 somites). Short-term labeling experiments tracked the movement of tissue progenitor cells during foregut closure. Surprisingly, we found that two distinct types of endoderm-progenitor cells, lateral and medial, arising from three spatially separated embryonic domains, converge to generate the epithelial cells of the liver bud. Whereas the lateral endoderm-progenitors give rise to descendants that are constrained in tissue fate and position along the anterior–posterior axis of the gut, the medial gut endoderm-progenitors give rise to descendants that stream along the anterior–posterior axis at the ventral midline and contribute to multiple gut tissues. The fate map reveals extensive morphogenetic movement of progenitors prior to tissue specification, it permits a detailed analysis of endoderm tissue patterning, and it illustrates that diverse progenitor domains can give rise to individual tissue cell types.

Keywords: Endoderm; Progenitor cells; Fate map; Liver; Morphogenesis; Organogenesis

Introduction

The embryonic endoderm gives rise to the liver, pancreas, lungs, thyroid, and gastrointestinal tract. The ability to predictably induce endoderm derivatives from stem and progenitor cell populations is anticipated to provide cell therapies, facilitate drug development, and enhance basic science. However, all such cell differentiation that is not from the normal embryonic endoderm is sporadic (Wagers and Weissman, 2004). By contrast, a tissue bud that arises from the endoderm during development contains an entire field of cells that initiates a new tissue program. Definitive endoderm cells exist transiently in the post-gastrulation embryo and, unlike stem cells in culture, they neither proliferate (Jung et al., 1999) nor maintain an undifferentiated state (Deutsch et al., 2001). Tissue explant and transplant studies with both chick and mouse embryos show that broad domains of the gut endoderm are multipotent, and that specific domains gain tissue identities by local interactions with mesoderm (Beddington and Lawson, 1990; Bossard and Zaret, 2000; Gualdi et al., 1996; Kumar et al., 2003; Le Douarin, 1964; Serls et al., 2005; Wells and Melton, 2000). In order to identify and track undifferentiated endoderm cells in embryos, and thereby better understand the normal origins of tissue progenitors, we created a partial fate map of the mammalian foregut endoderm prior to the expression of tissue-specific genes and determined where the cells move before and during tissue patterning.

Endoderm fate maps have been made for non-mammalian model organisms, but since tissue patterning occurs

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during gut tube morphogenesis and such morphogenesis differs dramatically between organisms, it is difficult to compare progenitor cell locations between model systems. For example, cell labeling with vital dyes showed that in zebrafish, a sparse field of endoderm cells moves to the midline to generate a rod of gut cells (Horne-Badovinac et al., 2001; Warga and Nusslein-Volhard, 1999), but it is not yet clear if the liver and other tissues initially bud from the rod (Field et al., 2003) or form independently (Korzh et al., 2001; Wallace and Pack, 2003). Similar approaches showed that the *Xenopus* gut tube also forms from a rod of endoderm cells (Chalmers and Slack, 2000). By contrast, the chick and mouse gut develops from an epithelial sheet of endoderm cells that form anterior and posterior pockets to create the foregut and hindgut, with liver and other tissues being specified in the sheet prior to and during gut tube closure (Wells and Melton, 1999).

Kirby et al. (2003) used dye labeling and fate mapping to show that in the chick, growth of the foregut is facilitated by caudal extension of the ventral foregut "lip," at the anterior intestinal portal (AIP), apparently led by ventral midline endoderm cells derived from the rostral portion of the prechordal plate. AIP lip cells labeled lateral to the ventral midline moved toward the midline, but not across it, and caudally, leaving a trail of descendants that streamed along the anterior–posterior axis into the foregut. Fate mapping studies of the posterior chick gut, caudal to the liver and ventral pancreas, showed that lateral domains fold ventrally to close off the gut (Matsushita, 1996). In summary, lateral domains move ventrally toward the midline and the foregut contains a ventral midline population that separates the lateral descendants. However, the extent to which this morphogenetic mechanism applies to mammals is unknown and the early foregut endoderm fate mapping studies in the chick did not extend into the period of organogenesis.

Lawson and Pedersen (1987) performed extensive fate mapping studies of gastrulating mouse embryos by labeling individual endoderm cells, culturing the whole embryos, and determining descendant cell position. They found that axial (near midline) endoderm initially contributes to the anterior dorsal endoderm, while later axial endoderm contributes to successively more posterior dorsal endoderm (Lawson et al., 1986). The role of lateral endoderm cells was not addressed and the embryos did not grow sufficiently in vitro to locate the positions or movement of organ progenitors. However, a recent lineage mapping study of mouse embryos has shown that by the end of gastrulation, cell progenitors of lateral endoderm are already distinct from cells that will become axial (anterior foregut and dorsal) endoderm (Tam et al., 2004). However, how the lateral and axial cell populations contribute to organ bud fates was not addressed.

Limited fate mapping studies of 2–4 somite pair (2–4 S) chick embryos identified a paired lateral endoderm domain, adjacent or posterior to the third somite, that contains liver progenitors (Rosenquist, 1971). Paired lateral domains of endoderm also contribute to the liver in the zebrafish and frog (Chalmers and Slack, 2000; Warga and Nusslein-Volhard, 1999). Foregut organogenic fate mapping in the mouse has not been reported, and genetic methods to map gut tissue lineages have been limited to tissue-specific promoters that are activated after tissues are specified (Gu et al., 2002; Kawaguchi et al., 2002). Tissue explant studies show that the mouse liver is not specified prior to ~7 S (Gualdi et al., 1996; Jung et al., 1999) and the earliest known in situ marker for liver appears in the left and right lateral endoderm at E8.5 (9 S) (Watt et al., 2001). Given that the liver bud is at the ventral midline by 15 S (E9.0), it is not clear whether midline cells, in addition to lateral cells, also contribute to the mammalian liver, nor how differences in gut morphogenesis allow the fate maps of other model systems to apply to the mammal.

In summary, the origin, location, and movement of mammalian gut organ progenitors, including the liver, has been unknown, but this information is crucial for understanding the mechanisms of organogenesis.

Materials and methods

Endoderm fate map construction

To provide a common framework on which to present a foregut endoderm fate map, we created a grid that subdivides the left and right sides of the exposed endoderm into thirds along the rostral/caudal and medial/lateral axes (Fig. 1). We also divided the exposed ventral endoderm lip at the AIP into three segments. The size of each grid segment was 2- to 4-fold greater than the area we could readily resolve during dye labeling. We divided our experiments into three categories based on somite number: 1–3 S, 4–6 S, and 7–10 S. In each category, the rostral end of the fate map was the exposed endoderm on the AIP lip, and the caudal end of each fate map was the endoderm rostral to the first visible somite. The area of the fate map is smaller over developmental time due to the progression of the AIP towards the first visible somite.

Mouse embryos: collection, manipulation, and in vitro culture

C3H mouse embryos were harvested at E8.0–8.25 (1–10 S). Embryos were dissected free of decidual tissue and Reichart's membrane in warm dissection medium (DMEM, 7.5% FBS; (Lawson and Pedersen, 1987; Lawson et al., 1986) and maintained at 37°C, 5% CO_2 in culture medium [DMEM, 50% Rat Serum (Valley Biomedical, AS3061); (Lawson and Pedersen, 1987; Lawson et al., 1986)] until manipulation. The parietal endoderm was carefully dissected away, and only embryos with an intact yolk sac and ectoplacental cone were used (Tam, 1998). Embryos were placed in warmed dissection medium and each was