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Expression analysis of the Islet-1 gene in the developing and adult gastrointestinal tract

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ABSTRACT

LIM-homeodomain genes encode a family of proteins defined by the cysteine-rich protein/protein interacting (Lin-11, Isl-1, and Mec-3) LIM domain and a highly conserved DNA-binding domain. Studies in several organisms have shown that these transcriptional regulators control multiple aspects of embryonic development and are responsible for the pathogenesis of several human diseases. Here we report the expression of Islet-1 (Isl-1) in the gastrointestinal epithelium in developing and adult mice. At embryonic day (E) 9.5–10.5, Isl-1 expression was first detected in the ventral gastric mesenchyme, and expression in the dorsal mesenchyme initiated a few days later. Isl-1 expression was first observed in the gastric epithelium at E13.5 and at E14.5 was restricted to the posterior half of the stomach. In the mature stomach, Isl-1 expression was detected only in subsets of enteroendocrine cells. Furthermore, Isl-1 expression in the intestinal epithelium was first detected at E15.5 and was restricted to subpopulations of enteroendocrine cells in adult mice. These expression analyses suggest that Isl-1 might have an early broad role in stomach and intestinal cells and a secondary role in terminal differentiation and/or maintenance of mature enteroendocrine subtypes in the gastrointestinal epithelium.

1. Results and discussion

In mammals, the LIM–HD proteins form a family of 13 members of transcriptional regulators that control numerous developmental processes in the developing tissues and organs (Hunter and Rhodes, 2005). Some of these LIM–HD proteins have been associated with human diseases including leukemia, pituitary hormone deficiency, diabetes mellitus and nail-patella syndrome (Hunter and Rhodes, 2005), implicating this protein family in regulating key processes during embryonic and adult lives. Islet-1 (Isl-1), a LIM–HD family member, is required for cell specification and differentiation events in cardiac mesoderm, motor neuron and endocrine pancreas (Ahlgren et al., 1997; Cai et al., 2003; Du et al., 2009; Pfaff et al., 1996).

In the pancreas, Isl-1 is expressed in hormone expressing endocrine cells and has been shown to regulate their differentiation (Ahlgren et al., 1997; Du et al., 2009). Outside of the pancreas, endocrine cells are also found throughout the gastrointestinal (GI) mucosa (referred to as enteroendocrine cells). These cells play critical roles in regulating digestion, gut motility, appetite, and lipid absorption (May and Kaestner, 2010; Mellitzer et al., 2010). In contrast to endocrine cells of the pancreas, which cluster to form islets, enteroendocrine cells in the gastrointestinal tract are scattered as individual cells throughout the epithelium. Interestingly, while endocrine cells of the pancreas, stomach and intestine arise from different regions of the definite endoderm, they express a common set of genes and are also influenced by similar signaling pathways (May and Kaestner, 2010).

Differentiation of the mammalian gastric epithelium is a coordinated process involving morphogenesis, tissue interactions and cell differentiation. Initial specification of the gastric epithelium is dependent on the presence of the gastric mesenchyme (Koike and Yasugi, 1999); a similar dependence exists between the dorsal pancreatic bud and the pancreatic mesenchyme (Ahlgren et al., 1997). In mice, formation of the stomach begins as a bulge around E10.0, with subsequent extensive remodeling of the epithelium throughout development (Nyeng et al., 2007). While differentiation of cell lineages and invagination of the gastric epithelium occur at E15.5–E16.5, maturation of these cells continues into postnatal life (Nyeng et al., 2007). The mature stomach is divided into two main regions: the proximal stomach (also known as the

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forestomach) comprises keratinized squamous epithelium, while the posterior stomach (also known as glandular stomach) comprises the corpus and antrum and is made up of stratified epithelium. The glandular stomach is characterized by numerous tubular invaginations, also referred to as gastric units. Within each gastric unit, endocrine cells are found scattered at the bases surrounded by other cell types, including pepsinogen-secreting chief cells, gastric-acid producing parietal cells and mucus-producing pit cells (May and Kaestner, 2010).

Although formation of the gut tube occurs early during embryogenesis, remodeling of the pseudostratified intestinal epithelium to a monolayer does not begin until E14.0. Between E15.5 and E19.0, villus-like structures are present from duodenum to colon (Potten, 1995; Stappenbeck et al., 1998; Yang et al., 2001). The underlying mesenchyme differentiates into smooth muscle and connective stromal tissue, whereas the epithelium differentiates into enterocyte, goblet, paneth and endocrine cells (May and Kaestner, 2010). As in the stomach, maturation of these intestinal cell types continues from birth until weaning (Henning and Guerin, 1981).

In the adult, enteroendocrine cells are the least abundant cell types in the gastrointestinal epithelium and encompass at least 15 different subtypes classified based on their main hormonal products (i.e. somatostatin, gastrin, serotonin, cholecystokinin, and ghrelin), ultrastructure of their secretory granules, and marker gene expression (May and Kaestner, 2010; Rindi et al., 2004). Most, but not all, enteroendocrine cells are positive for chromogranin A, a pan-endocrine cell marker, staining (Cetin et al., 1989). Although chromogranin A-positive enteroendocrine cells are detected at high levels around E16.5 (Nyeng et al., 2007), other markers of enteroendocrine cell differentiation (i.e. Pdx1, Ngn3 and Pax6) can be detected earlier (May and Kaestner, 2010). Due to the large number of enteroendocrine subtypes and the low number of each specific subpopulation, many studies have focused only on a subset of endocrine subpopulations (May and Kaestner, 2010).

Many transcription factors that are essential for endocrine pancreas differentiation also play important roles in the development of enteroendocrine cells (May and Kaestner, 2010; Oliver-Krasinski and Stoffers, 2008). Isl-1 has been shown to regulate the differentiation of glucagon, insulin, somatostatin and pancreatic polypeptide-expressing cells in the endocrine pancreas (Ahlgren et al., 1997; Du et al., 2009). In the stomach, Isl-1 expression has been characterized in rat, where it is first expressed in G/D-cell precursors and a subpopulation of somatostatin-producing D-cells, but not gastrin-producing G-cells (Larsson et al., 1995). In the intestine, Isl-1 expression is completely uncharacterized. To investigate the possibility that Isl-1 regulates enteroendocrine cell differentiation in the gastrointestinal epithelium, we characterized Isl-1 expression during various stages of development and in adult mice.

1.1. Isl-1 expression is detected in the entire gastrointestinal tract

We first examined the presence of Isl-1 mRNA in different regions of the adult gastrointestinal tract by real-time PCR analysis. The expression of *hypoxanthine–guanine phosphoribosyltransferase* (*HPRT*) was used as an internal control. Highest levels of Isl-1 transcripts were observed in the corpus of the stomach, while squamous stomach and rectum had the least (Fig. 1A). The gastric antrum and the duodenum showed modest levels of Isl-1 transcripts, with lower amounts in the jejunum, ileum and colon (Fig. 1A).

To further characterize Isl-1 expression in the adult gastrointestinal tract, we performed immunohistochemistry (IHC) analysis using an Isl-1 antibody on different regions of the adult GI tract. Scattered Isl-1-positive cells were detected in the epithelium of the stomach, duodenum, jejunum, ileum and colon (Fig. 1C–G). Within the stomach, Isl-1 positive cells were more numerous in the corpus than the antrum region, and no Isl-1$^+$ cells were observed in the squamous stomach, consistent with the mRNA quantitation (Fig. 1A and B).

1.2. Isl-1 in enteroendocrine subpopulations in the adult stomach

The distribution and frequency of Isl-1$^+$ cells in the GI tract were suggestive of enteroendocrine cells. To confirm this, we carried out IHC analysis of Isl-1 with chromogranin A and various gastrointestinal hormones. Not all chromogranin A$^+$ cells were positive for Isl-1, indicating that Isl-1 is differentially expressed in endocrine subtypes (Fig. 2A–C). Most of the somatostatin, serotonin and ghrelin producing cells were positive for Isl-1 expression (Fig. 2D–L), while there were fewer number of Isl-1$^+$/gastrin$^+$ cells (Fig. 2M–O). Isl-1$^+$ cells co-expressing somatostatin and ghrelin were also detected in the adult stomach (Fig. 2P–R).

Our observations are in agreement with Larsson and colleagues, who demonstrated that rat Isl-1 is first expressed in the gastrin and somatostatin coexpressing G/D precursors then later is restricted to mature somatostatin expressing D cells (Larsson et al., 1995). Based on these observations and our IHC analyses, Isl-1 is likely to play an important role in the asymmetric division of the G/D precursors and the differentiation/maturation of the D cells (Larsson et al., 1995). In addition, Isl-1 has been shown to regulate somatostatin expression in pancreatic cell lines (Leonard et al., 1992), and *Isl-1* deficient mice lack somatostatin-expressing cells in the endocrine pancreas (Ahlgren et al., 1997; Du et al., 2009). By analogy, Isl-1 may regulate the differentiation of D cells and/or somatostatin gene expression in the gastric epithelium.

Isl-1 was also detected in cells expressing serotonin or ghrelin, peptides that are responsible for feeding behavior and modulating gastric peristalsis, sensation, and secretion (Arvat et al., 2001; Englander et al., 2004; Gershon and Tack, 2007). Interestingly, Isl-1 was also detected in the ghrelin/somatostatin co-expressing cells, suggesting that these two enteroendocrine subtypes may share a common precursor. Collectively, these observations suggest that Isl-1 alone or in combination with other transcriptional regulators may be involved in the differentiation of somatostatin, gastrin, ghrelin and serotonin producing cells in the gastric epithelium.

1.3. Isl-1 in somatostatin-expressing cells of the adult intestine

We next performed immunostaining to characterize Isl-1 expression with somatostatin and ghrelin in the adult intestine since these two enteroendocrine subtypes express Isl-1 abundantly in the stomach. IHC analyses demonstrated that while there were very few Isl-1$^+$ cells detected in the adult intestinal epithelium from duodenum to colon, most of the Isl-1$^+$ cells were positive for somatostatin or ghrelin expression (Fig. 3A–O). These results indicate that the function of Isl-1 in somatostatin- and ghrelin-expressing cells is likely to be conserved throughout the GI tract.

1.4. Isl-1 in the stomach and intestine during embryogenesis

Since the differentiation of enteroendocrine cells occurs during embryogenesis and Isl-1 showed highest levels of gene expression in the glandular stomach, we focused our embryonic/perinatal expression analysis on stomach at various developmental stages by real-time PCR (Fig. 4A). Expression levels at these stages were compared to that of the adult glandular stomach. Isl-1 expression was high at the onset of gastric epithelial differentiation and decreased as development progressed. At birth, Isl-1 expression reached a nadir and then began to increase (Fig. 4A).

To localize the developmental expression of Isl-1, IHC analyses were performed on transverse sections prepared from mouse embryos (E9.5–E17.5) and tissue sections from postnatal day (P)14.