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Expression of Isl1 during mouse development

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Abstract

The LIM-homeodomain transcription factor Isl1 plays essential roles in cell proliferation, differentiation and survival during embryogenesis. To better visualize Isl1 expression and provide insight into the role of Isl1 during development, we generated an Isl1 nuclear LacZ (nLacZ) knockin mouse line and analyzed Isl1nLacZ expression during development by Xgal staining and compared expression of Isl1nLacZ with endogenous Isl1 by coimmunostaining with antibodies to Isl1 and β -galactosidase. Results demonstrated that during development Isl1 nuclear LacZ is expressed in a pattern that recapitulates its endogenous protein expression. Consistent with previous in situ and immunohistochemistry data, we observed Isl1nLacZ expression in multiple tissues and cell types, including the central and peripheral nervous system, neural retina, inner ear, pharyngeal mesoderm and endoderm and their derivatives (craniofacial structures, thymus, thyroid gland and trachea), cardiovascular system (cardiac outflow tract, carotid arteries, umbilical vessels, sinoatrial node and atrial septum), gastrointestinal system (oral epithelium, stomach, pancreas, mesentery) and hindlimb. In some cases, Isl1nLacZ appears to be more readily detectable than Isl1 protein when expression level is low, and in others, Isl1nLacZ appears to act as a lineage tracer, likely owing to perdurance of the nuclear localized beta-galactosidase.

Keywords

Isl1; LIM-homeodomain; transgenic; gene expression; development

1. Results and discussion

The LIM-homeodomain (HD) proteins are evolutionarily conserved superfamily of transcription factors that play fundamental roles in cell differentiation, cell fate determination, and generation of cell diversity and segmental pattern during embryogenesis

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Disclosures

The authors confirm that there are no conflicts of interest.

(Gill, 2003; Lee and Pfaff, 2003; Hunter and Rhodes, 2005). Isl1 is a LIM-HD transcription factor and its gene contains six exons, located on chromosome 13 in mouse and chromosome 5 in human (Karlsson et al., 1990). Isl1 was originally identified as a protein that can bind to an insulin gene enhancer and regulate its expression (Karlsson et al., 1990). Isl1 regulates gene expression by binding of its homeodomain to AT-rich consensus element containing a core TAAT motif (YTAATGR) (Karlsson et al., 1990; Leonard et al., 1992; Wang and Drucker, 1995; Lien et al., 1999; Dodou et al., 2004; Takeuchi et al., 2005; Liu et al., 2011; Golzio et al., 2012).

In situ hybridization and immunohistochemistry studies have revealed expression of Isl1 in multiple tissues and cell types, including pancreas, the central and peripheral nervous system, progenitors of the second heart field, craniofacial structures, neural retina, inner ear, thyroid gland and pituitary gland (Karlsson et al., 1990; Pfaff et al., 1996; Ahlgren et al., 1997; Yuan and Schoenwolf, 2000; Cai et al., 2003; Radde-Gallwitz et al., 2004; Elshatory et al., 2007; Elshatory and Gan, 2008; Nathan et al., 2008; Sun et al., 2008; Westerlund et al., 2008). Genetic studies demonstrate that Isl1 plays essential roles in cell specification, differentiation and survival during development. In pancreas, Isl1 is expressed in beta-cells and mesenchymal cells surrounding the dorsal lobe, and plays an important role in pancreas development (Ahlgren et al., 1997). Isl1 is required for spinal MN survival and fate determination (Pfaff et al., 1996; Song et al., 2009; Liang et al., 2011). Lineage studies with Isl1-Cre have revealed that Isl1 marks progenitors of the second heart field that contribute to outflow tract (OFT), right ventricle and some atrial myocardium (Cai et al., 2003). However, when these progenitors migrate into the heart and differentiate, Isl1 expression is turned off. Mice null for Isl1 die at E9.5–10.5 with missing cardiac segments derived from Isl1 progenitors, including the OFT and right ventricle, and atrial tissues were greatly reduced (Cai et al., 2003). Recent studies have revealed that Isl1 expression persists in subdomains of the heart, including the OFT, sinoatrial node (SAN) and cardiac progenitors within the heart (Sun et al., 2007; Sizarov et al., 2011). Lineage studies have revealed contribution of Isl1Cre lineage to cardiac and non-cardiac vasculature, including aortic and cardinal vein endothelial cells and entire umbilical cord (Milgrom-Hoffman et al., 2011; Keenan et al., 2012). However roles of Isl1 in these cardiac cells within the heart remain unknown. Isl1 is required for neuronal survival of Drg nociceptive neurons and cranial ganglion neurons (Sun et al., 2008; Liang et al., 2011). Isl1 is required for development of a subset of basal forebrain cholinergic neurons (Elshatory and Gan, 2008). In addition, Isl1 is required for the development of the retina and thyroid gland (Elshatory et al., 2007; Pan et al., 2008; Westerlund et al., 2008). Lineage study has revealed that Isl1 progenitors contribute to a majority of hindlimb cells, but Isl1 expression is downregulated as Isl1+ progenitors migrate into the hindlimb. Isl1 is required for hindlimb development (Yang et al., 2006; Narkis et al., 2012). Despite these insights, our understanding of the role of Isl1 is still fragmental. To better visualize Isl1 expression and provide further insight into its potential roles during development, we generated an Isl1nlacZ mouse line, and initial analyses of Isl1nlacZ expression at early developmental stages have revealed that Isl1nlacZ expression recapitulates its endogenous expression (Sun et al., 2007; Liang et al., 2011).

1.1. Expression of *Isl1nlacZ* during development revealed by Xgal staining

Expression of *Isl1nlacZ* during development was analyzed by wholemount and section Xgal staining. In previous studies, we have reported expression of *Isl1nlacZ* at early developmental stages in cranial ganglia, spinal motor neurons, pharyngeal and cardiac regions (Sun et al., 2007; Liang et al., 2011). Consistent with these data and previous *in situ* data (Cai et al., 2003), we observed expression of *Isl1nlacZ* at E8.5 and E9.5 in pharyngeal mesoderm dorsal to the heart tube (the second heart field), pharyngeal and foregut endoderm, umbilical cord and surrounding mesenchyme. *Isl1nlacZ* expression was not observed within the heart, except the outflow tract (Fig 1A, B). At E10.5 and E11.5, expression of *Isl1nlacZ* was observed in multiple tissues (Fig 1C, D). Besides aforementioned structures, *Isl1nlacZ* was expressed in subregions of midbrain and basal forebrain, spinal motor neurons, cranial ganglia, dorsal root ganglia, the sympathetic chain and sinus venosus (Fig 1C, D). *Isl1nlacZ* expression was also observed in the posterior hindlimb (Fig 1D) at E11.5 and then it was downregulated at E12.5, but re-expressed at E15.5 in the posterior hindlimb (Fig 1D–F). Expression of *Isl1nlacZ* was observed in the outflow tract (OFT) at these stages and up to early postnatal life (Fig 1B–D, I, not shown). At E15.5, *Isl1nlacZ* expression remained on in all aforementioned structures (basal forebrain, midbrain, diencephalon, motor columns of the hindbrain and spinal cord, the cranial ganglia, dorsal root ganglia) (Fig 1F, G, G'), and it was also expressed in cranial skeletal structures and the muscle of the body wall (Fig 1F, arrowhead), autonomic nervous system (superior cervical ganglia, stellate ganglia, cardiac ganglia and prevertebral sympathetic chain) and medulla of adrenal gland, thymus and sinoatrial node (Fig 1H–J). Consistent with previous *in situ* data (Yuan and Schoenwolf, 2000; Davis et al., 2008), expression of *Isl1nlacZ* was observed in the upper gastrointestinal tract, including esophagus, the pyloric end of the stomach and duodenum, and pancreas (Fig 1K).

At E9.5, Section Xgal staining revealed expression of *Isl1nlacZ* in subdomains of the outer mantle layer of midbrain, ventral hindbrain, Rathke's pouch, trigeminal ganglia (Fig 2A, B), mesodermal core of pharyngeal arches, pharyngeal pouch and foregut endoderm (Fig 2C, D). *Isl1nlacZ* expression was also observed in thyroid primordium, outflow tract, sinus venosus, primordial lung bud (lb) and tracheal epithelium (Fig 2D, E). At E11.5, *Isl1nLaZ* expression was observed in mantle layer of midbrain and diencephalon (Fig 2F–I), trigeminal motor nucleus of midbrain (Fig 2H), mantle layer of basal forebrain, hindbrain and spinal motor neurons, cranial ganglia (v, vii/viii, xi), Rathke's pouch, and the ventral and dorsal pole of otic vesicle (Fig 2I). Within the heart, *Isl1nlacZ* continued to be expressed in outflow tract, sinus venosus, sinoatrial node and atrial septum (Fig 2J, K). In addition, *Isl1nlacZ* expression was observed in the arytenoid swelling, tracheal epithelium and mesenchyme ventral to the trachea (Fig 2J, K). *Isl1nlacZ* was also expressed in the sympathetic chain, dorsal root ganglia, the distal-middle part of the stomach, pancreas and the surrounding mesenchyme, superior mesenteric vein and umbilical artery (Fig 2L, M).

At E13.5, *Isl1-nLacZ* continued to be expressed in cranial ganglia (v, vii/viii, ix/x, xi), the basal forebrain (caudate nucleus), diencephalon and inner ear/otic vesicle (Fig 3A–C), and it was also expressed in the retina (Fig 3C). *Isl1nlacZ* was expressed in the thymus, ventral tracheal epithelium and surrounding mesenchyme/arytenoid swelling, ultimobranchial

bodies, and thyroid glands (Fig 3D). In addition, Isl1nlacZ was expressed in the carotid artery (Fig 3D), aortic arch, right ventricular outlet, sinoatrial node, atrial septum and cardiac ganglia (Fig 3E–G). Isl1nlacZ was also expressed in the mesenchyme lateral to the left and right main bronchi (Fig 3F, G). Isl1nlacZ was expressed in the adrenal medulla, the middle part of the stomach, lesser omentum, pancreatic islets and the dorsal esophageal-mesentery, umbilical artery and surrounding mesenchyme (Fig 3H). In addition, Isl1nlacZ expression remained on in hindbrain and spinal motor neurons, dorsal root ganglia, sympathetic chain (Fig 3A–H).

1.2. Isl1nlacZ recapitulates endogenous Isl1 expression revealed by co-immunostaining with Isl1 and β -galactosidase antibodies

To examine the extent to which Isl1nlacZ recapitulates endogenous Isl1 protein expression, we examined the co-localization of Isl1nlacZ and Isl1 protein by coimmunostaining with antibodies to Isl1 and β -galactosidase at E11.5. In the central nervous system, a subpopulation of neurons in the midbrain/hindbrain junction (isthmus) expressed Isl1 in a manner completely overlapping with Isl1nlacZ expression (Fig 4A). In the basal forebrain and diencephalon, expression of Isl1 and Isl1nlacZ largely overlapped in the outer mantle layer where differentiated neurons reside. However, a significant number of migrating neurons just leaving the subventricular zone were clearly labeled by Isl1nlacZ, but expressed barely detectable Isl1 protein (Fig 4B, C), suggesting greater sensitivity of detection by the nlacZ transgene. In the hindbrain, there are three distinct types of motor neurons, the ventrally localized somatic motor neurons and dorsal-lateral visceral and branchial motor neurons. Previous *in situ* analyses have revealed dynamic expression of Isl1 in all motor neurons of the hindbrain (Varela-Echavarría et al., 1996; Chandrasekhar, 2004). We observed that the somatic motor neurons in ventral hindbrain expressed Isl1nlacZ in a manner overlapping with Isl1 protein expression. However, visceral motor neurons and branchiomotor neurons in dorsal-lateral hindbrain were also labeled by Isl1nlacZ, but these neurons expressed no or only barely detectable Isl1 (Fig 4D). In cranial ganglia (V, VII–X) (Fig 4E–H), dorsal root ganglia and spinal motor neurons (Fig 4I), expression of Isl1nlacZ was largely overlapping with that of Isl1 protein, except that a small subset of Isl1 expressing cranial ganglion neurons expressed no or barely detectable Isl1nlacZ (Fig 4E–H, green only). It might be relevant to note that these cranial ganglion neurons are derived from two distinct lineages, the neural crest and cranial placodes, and it is possible that the knocked-in beta-galactosidase transgene has disrupted regulatory regions required for expression in one of these lineages. In pharyngeal mesoderm and endoderm, arytenoid swelling and carotid artery, expression of Isl1nlacZ and Isl1 protein was largely overlapping. Consistent with our previous data (Sun et al., 2007), we observed that a significant number of myocardial cells of the distal outflow tract continued to express Isl1, albeit at lower levels. However, few if any endocardial cells or outflow cushion mesenchymal cells exhibited Isl1 expression. In contrast, a significant number of outflow tract cells including those of the proximal outflow tract, endocardium and cushion mesenchyme, were clearly labeled by Isl1nlacZ expression (Fig 4K, red only), likely due to perdurance of Isl1nlacZ. The foregoing results suggest that in some contexts Isl1nlacZ can act as a sensitive indicator of Isl1 expression when Isl1 is beginning to be expressed, or as a lineage marker in cells where Isl1 expression has been downregulated.

2. Experimental procedures

2.1. Transgenic mice

Isl1 nuclear lacZ (nlacZ) knockin mouse line was generated as described (Sun et al., 2007). All the experiments involving mice were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee of USCD, USA (A3033-01) and by the Animal Committee of Tongji University School of Medicine, China (TJmed-010-10).

2.2. Immunohistochemistry and β -gal staining

Females with copulation plugs were considered to be at embryonic development day 0.5 (E0.5) of gestation. Pregnant females were sacrificed at different stages of gestation, and embryos were dissected for histological analysis as described (Liang et al., 2007). For β -gal staining, embryos expressing β -gal were harvested in cold PBS and fixed for 1–2 hours in 4% PFA and incubated in β -gal substrate as described (Liang et al., 2007). For high-resolution analysis of β -gal activity, embryos were paraffin embedded, sectioned, and counterstained with nuclear Fast Red. Immunostaining was performed as described (Sun et al., 2007; Sun et al., 2008). 10 μ m sections were incubated with primary antibodies to Isl1 (DSHB) and β -gal (Cappel, #55976) overnight at 4°C. After washing with 0.25% TritonX-100 in PBS, sections were incubated with the appropriate secondary antibodies fluorescently labeled with Alexa 488 or 594 (Invitrogen), and mounted in Vectashield DAPI medium (Vector Laboratories).

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Highlight

- * Isl1 nuclear LacZ is expressed in a pattern that recapitulates its endogenous protein expression.
- * In some cases, Isl1nlacZ appears to be more readily detectable than Isl1 protein when expression level is low,
- * In some cases, Isl1nlacZ appears to act as a lineage tracer, likely owing to perdurance of the nuclear localized beta-galactosidase.

Figure 1. Expression of Isl1 nuclear LacZ (nLacZ) during development revealed by wholemount Xgal staining

A, B) At E8.5 and E9.5, Isl1nLacZ is expressed in pharyngeal mesoderm (ph), cardiac outflow tract (oft), foregut endoderm (g), umbilical cord and surrounding mesenchyme (uc). C, D) At E10.5 and E11.5, Isl1nLacZ is highly expressed in aforementioned structures, and in regions of midbrain (mb), forebrain (fb), all cranial ganglia, spinal motor neurons (mn), dorsal root ganglia (drg), sinus venosus (arrowhead) and the posterior hindlimb (hl). E, F) Expression of Isl1nLacZ at E12.5 and E15.5. Isl1nLacZ expression in hindlimb is downregulated at E12.5 (E), but reexpressed at E15.5 in the posterior hindlimb. Besides aforementioned structures, at E15.5, Isl1nLacZ is also expressed in cranial skeletal structures and muscle of the body wall (F, arrowhead). G–K) Isl1nLacZ expression at E15.5 in: (G) forebrain (fb), midbrain (mb), diencephalon (di), motor columns of the hindbrain (hb) and spinal cord, and cranial ganglia V, VII/VIII; (H) in superior cervical ganglia (scg), stellate ganglia (sg), thymus (th) and outflow tract (oft); (I) in cardiac ganglia (cg), sinoatrial node (san); (J) in dorsal root ganglia, medulla of adrenal gland (ad), and prevertebral sympathetic chain (psc); (K) in upper gastrointestinal tract, including esophagus (es), the pyloric end of the stomach and duodenum, and pancreas.

Figure 2. Xgal staining and histological analysis of Isl1nLacZ expression at E9.5 and E11.5
 A–E) At E9.5, Isl1nLacZ is expressed in subdomains of the outer mantle layer of midbrain (mb), hindbrain (hb), Rathke's pouch (rp), trigeminal ganglia (v) (A–C), mesoderm core of the first and second pharyngeal arch, pharyngeal pouch (arrow) (C) and foregut endoderm (e) (C, D). Isl1nLacZ is also expressed in thyroid primordium (t), outflow tract (D), sinus venosus (sv), lung bud (lb) and tracheal epithelium (tr) (E). F–M) At E11.5, Isl1nLacZ+ cells were observed in mantle layer of midbrain (F–G) and diencephalon, trigeminal motor nucleus (tmn) of midbrain (H), mantle layer of basal forebrain (fb), Rathke's pouch (rp) (I), cranial ganglia V, VII/VIII, XI, ventral and dorsal pole of otic vesicle (ov), hindbrain and spinal motor neurons (I). Isl1nLacZ is expressed in outflow tract, arytenoid swelling (asw), tracheal epithelium (tr) and sympathetic chain (sc) (J). Isl1nLacZ continues to be expressed in sinus venosus, sinoatrial node (san) and atrial septum (as) and the mesenchyme ventral to the trachea (K). Isl1nLacZ is expressed in sympathetic chain, dorsal root ganglia, the middle part of the stomach (st), pancreas (pan) and the surrounding mesenchyme, superior mesenteric vein (smv) and umbilical artery (L, M).

Figure 3. Expression of Isl1nLacZ at E13.5

A–C) At E13.5, Isl1-nLacZ is expressed in cranial ganglia (v, vii/viii, ix/x, xi), the basal forebrain (caudate nucleus) (cn), diencephalon (di), otic vesicle/inner ear (ie) and retina (ret). D) Isl1nLacZ is expressed in ventral tracheal epithelium and surrounding mesenchyme (arytenoid swelling, asw), ultimobranchial bodies (ubb), thyroid glands (thy), thymus (th) and carotid arteries (ca, insert). E–G) Isl1nLacZ continues to be expressed in aortic arch (E), the right ventricular outlet, sinoatrial node (SAN) and mesenchyme lateral to the left (lmb) and right (rmb) main bronchi (F, G), cardiac ganglia and atrial septum (as) (G). H) Isl1 is expressed in the medulla of the adrenal gland (ad), the middle part of the stomach (st), lesser omentum (lo), pancreatic islets (pan) and the dorsal esophageal-mesentery (dem), umbilical artery (ua) and surrounding mesenchyme. In addition, Isl1nLacZ continues to be in hindbrain and spinal motor neurons, DRG, sympathetic ganglia (scg, sc) (A–H).

Figure 4. Isl1nLacZ recapitulates endogenous Isl1 expression revealed by coimmunostaining with Isl1 and β gal antibodies

Co-localization of Isl1nLacZ and Isl1: A) At the midbrain/hindbrain junction (isthmus, ism); B) Basal forebrain; C) Diencephalon (di). Note that the migrating neurons just leaving the subventricular zone expressed barely detectable Isl1 protein, but they were clearly labeled by Isl1nLacZ (B, C); D) In the hindbrain, the ventral motor neurons express both Isl1nLacZ and Isl1 protein and the dorsal-lateral motor neurons express Isl1nLacZ, but barely detectable Isl1. E–H) Co-expression of Isl1 and Isl1nLacZ in cranial ganglia (V, VII/VIII, IX and X). Note that a subset of cranial neurons express only Isl1, but not Isl1nLacZ (green only). H, J) Co-expression of Isl1nLacZ and Isl1 protein in carotid artery (ca), the pharyngeal arch mesoderm (*) and endoderm (e), Drg, spinal motor neurons (MN) and arytenoid swelling (asw). K) Expression of Isl1nLacZ in outflow tract (oft).