Caudal dysgenesis in Isl-1 transgenic mice.

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SPECIFIC AIM

The long-term goal of our research is to understand the role of homeodomain transcription factors in development and disease. To this end, we generated transgenic mice that express the Isl-1 transcription factor under control of a developmentally regulated promoter from the Hoxc-8 gene. The specific aims of the current report were to determine the basis for growth defects in Isl-1 transgenic mice that resemble the human syndrome caudal regression/sacral agenesis, which is characteristically found in diabetic embryopathy. Information about the pathogenesis of Isl-1-induced developmental defects is a prerequisite for molecular studies of the transcriptional targets of Isl-1 in vivo, and for studies of the regulation of developmental control genes by metabolic factors.

EXPERIMENTAL APPROACH

Isl-1 transgenic mice were generated using a binary transgenic system, in which the one parental strain expresses the viral transactivator VP16 under control of the Hoxc-8 promoter, and the other strain contains the Isl-1 transgene linked to the VP16-responsive IE promoter, which is silent in the absence of VP16. Neither transgenic parent expresses the Isl-1 transgene, which only becomes transactivated in double transgenic offspring. By breeding double transgenic mice that are hemizygous for each transgene locus to each other or to transgenic parents, gene dosage at either transgene locus, or both, can be increased in combinatorial fashion simply by breeding. Progeny and embryos from these crosses were characterized by morphological and histological methods, in situ hybridization and immunohistochemistry for markers of embryonic patterning in the developing neural tube and mesodermal derivatives, and for markers of apoptosis.

PRINCIPAL FINDINGS

1) Transgenic expression of the pancreatic transcription factor Isl-1 in the posterior region causes caudal growth defects in developing mice (Figure 1).

2) The severity of the caudal growth defect phenotype is dependent on transgene dosage, with increased perinatal mortality at the highest dose.

3) While surviving Isl-1 transgenic mice are smaller at birth, they grow to normal size as adults. No indication of abnormal glucose metabolism was found.

4) The caudal growth deficiency is sometimes accompanied by spina bifida or sacral mass, composed of extruding neuroepithelium (Figure 2).

5) By virtue of histology and immunohistochemistry, notochord development and patterning of the neural tube appeared normal, arguing against a notochord growth or signaling defect in Isl-1 transgenic mice.

6) Increased apoptosis was found in posterior mesoderm, suggesting mesodermal insult as the cause of pathogenesis. Concurrently, the skeletal precursor cell marker Pax-1 was downregulated.

7) Isl-1 transgenic mice resemble the human phenotype of caudal regression/sacral agenesis, which is highly associated with maternal diabetes and characteristic for diabetic embryopathy.

CONCLUSIONS AND SIGNIFICANCE

In our transgenic mice, Isl-1 is expressed in neuroepithelial progenitors and mature cells in the developing posterior spinal cord. Yet, no evidence for altered neural tube patterning was found in this transgenic mouse model; ventral and dorsal neural tube markers were expressed normally. These results indicate that overexpression of Isl-1 is not sufficient for inducing changes in neural patterning.

Isl-1 expression in mesoderm results in increased apoptosis, indicating a role for the Isl-1 transcription factor in regulation of cell growth and cell cycle progression. In motorneurons and pancreas, Isl-1 expression is correlated with exit from the cell cycle, suggesting that arrest of mesodermal cell proliferation could be a direct effect of Isl-1 expression. Our results support the general hypothesis of mesodermal insult as the cause for caudal growth defects.

The phenotype of caudal growth defects in Isl-1 transgenic mice implicates de-regulation of Isl-1 expression in the pathogenesis of caudal regression/sacral agenesis as found in diabetic embryopathies. A postulate from this hypothesis is that the Isl-1 gene, under conditions of maternal diabetes, becomes activated in mesoderm in the posterior region of the developing embryo. We have recently discovered a regulatory element in the Isl-1 gene (unpublished data) with specificity for posterior mesoderm, providing support for this hypothesis. The Isl-1 transgenic mice thus constitute a genetic model for diabetic embryopathy and enable detailed investigations into the pathogenesis of disorders associated with maternal diabetes.

The phenotype of caudal regression/sacral agenesis with sacral mass is also similar to human inherited sacral agenesis as found in Currarino syndrome. Interestingly, the gene mutated in Currarino syndrome is HLXB9, a homeobox transcription factor downstream of Isl-1 in pancreatic development and motorneuron differentiation. Our results provide support for the hypothesis that two transcription factors from the same cellular pathway are involved in similar pathologies.

The Isl-1 transgenic mouse model now allows detailed investigation of genes that are regulated by the Isl-1 transcription factor. Ongoing experiments indicate that the number of transcriptional targets is limited, implicating a specific developmental pathway in sacral agenesis and diabetic embryopathy.

Legend to Summary Figure 1

Summary Figure 1: Caudal growth defects in Isl-1 transgenic mice.

The VP-16-based binary transgenic system (**A**) was used to overcome perinatal lethality, and to consistently generate Isl-1 transgenics from stable parental transgenic mouse lines (22, 23). The transactivator (TA) lines express the viral transactivator VP16 under control of the Hoxc-8 promoter (25), and transresponder (TR) lines carry the cDNA from the rat Isl-1 gene linked to the VP16-responsive immediate early (IE) gene promoter from Herpes Simplex Virus (26). Mice double transgenic for both the TA and TR transgenes were obtained by crossing the two parental transgenic lines. Some double transgenic mice were viable and were further bred to generate mice homozygous for one or both transgene loci. (**B**,**C**) The domain of VP16 expression under control of the Hoxc-8 promoter is revealed by crossing the Hoxc-8-VP16 transactivator to IE-LacZ transresponder mice. Blue staining for β-galactosidase activity indicates the region of transgene activation in the posterior region at E9.5 (**B**) in mesoderm and neural tube, and at E12.5 (**C**), in the tail and mesodermal derivatives. (**D**) Ectopic Isl-1 protein (arrows) in posterior mesoderm of an Isl-1 transgenic embryo at E11.5. As expected for a transcription factor, Isl-1 protein is localized to nuclei. This picture was composed from photographs taken at 1000x magnification. (**E**) Isl-1 transgenic newborn mouse with open eyes (open arrow) and absence of tail (closed arrow). (**F**) Skeletal preparation of an Isl-1 transgenic newborn mouse shows absence of the tail, of caudal and sacral vertebrae, and unclosed neural arches of sacral vertebrae.

Legend to Summary Figure 2

Summary Figure 2: Pre-sacral mass in Isl-1 transgenic embryos.

An Isl-1 transgenic embryo isolated at E13.5 shows absence of the tail and presence of pre-sacral mass (**A**). E14.5 Isl-1 transgenic embryo with a pre-sacral mass. Skeletal cartilage was stained with Alcian blue. The truncation of the vertebral column is obvious (**B**). Sagittal section through an embryo at 13.5 days (**C**), and magnification of the area of the pre-sacral mass (**D**). The pre-sacral mass consists of neuroepithelium in continuity with the neural tube.

Legend to Schematic Diagram

Isl-1 transgenic mice as a model for caudal growth defects in diabetic embryopathy

Normally, Isl-1 is expressed in motorneurons in the spinal cord, dorsal root ganglia, and in the developing pancreas. Under control of the regions-specific promoter from the Hoxc-8 gene, Isl-1 expression becomes deregulated in Isl-1 transgenic mice, with widespread expression of Isl-1 in posterior tissues (except for notochord and floor plate). Isl-1 inhibits growth of the caudal region, resulting in increased apoptosis. The functional consequences of expression of the Isl-1 transgene in mice are caudal growth defects, shorter or absent tail (with perinatal lethality of aniamls with the highest transgene dosage), or, in milder cases, reduced mesodermal tissue development and failure to close the neural tube. These developmental defects mimick those found in human offspring born to diabetic mothers, advancing the hypothesis that growth defects in diabetic pregnancies may be caused by deregulated Isl-1 expression.