The pancreatic homeodomain transcription factor IDX1/IPF1 is expressed in neural cells during brain development

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Abstract. Expression of the homeodomain transcription factor IDX1/IPF1 has been shown to be restricted to cells in the developing foregut that form the pancreatic primordium. In the adult, IDX1/IPF1 is expressed in the duodenum and pancreatic islets. The IDX1/IPF1 gene is required for pancreatic development, and in the human, heterozygous mutations have been linked to diabetes mellitus. In the present communication, we report that IDX1/IPF1 is expressed in discrete cells of the rat central nervous system during embryonic development. Using RT-PCR, IDX1/IPF1 mRNA was detected in neural precursor RC2.E10 cells, as well as in both forebrain and hindbrain of developing rats at embryonic day 15 (E15). The presence of IDX1/IPF1 protein was confirmed by Western immunoblotting. Immunohistochemical analyses of sagittal sections of E15 rats demonstrated the presence of scattered IDX1/IPF1-immunopositive cells in the forebrain. Finally, electrophoretic mobility shift assays using nuclear extracts from neural cells revealed the presence of IDX1/IPF1 bound to a putative homeodomain protein DNA-binding site present in the promoter of the glial fibrillary acidic protein gene. Our results suggest that IDX1/IPF1 may have previously unsuspected extrapancreatic functions during development of neural cells in the central nervous system.

Transcription factors of the homeotic selector class are involved in the regulation of genetic mechanisms that determine the developmental fates of different segments of the embryo. According to the relative degree of conservation of their DNA-binding homeodomain, these transcription factors are grouped into different families, prominent among which is that of Hox-like homeodomain proteins (1).

IDX1/IPF1 (also known as STF-1 and PDX-1) is a Hox-like homeodomain transcription factor whose expression has been described to be restricted to the endocrine pancreas and duodenum. An essential role for IDX1/IPF1 in pancreas development has been demonstrated by genetic inactivation following homologous recombination of a mutated allele in mice (2, 3), and by the identification of a human subject with pancreatic agenesis and a homozygous mutation in the IPF-1 gene (4). In addition, several studies have provided evidence indicating that IDX1/IPF1 regulates the expression of a number of pancreatic genes, including insulin, somatostatin, islet amyloid polypeptide and glucagon transporter type 2 (5-8), supporting the notion that this transcription factor is required for normal islet function. This notion has been recently confirmed by the finding that heterozygous mutations in the human IPF-1 gene are linked to a type of autosomal dominant type of diabetes known as maturity onset diabetes of the young (MODY4) (9).

In the present communication, we report that IDX1/IPF1 is expressed in neural forebrain cells during the development of the rat central nervous system, suggesting the existence of hitherto unsuspected extrapancreatic developmental functions for this Hox-like transcription factor.

Materials and Methods

RT-PCR/Southern blot hybridization analyses. Developing brains from rat embryos removed at 15 days of gestation (E15) from timed-pregnant Sprague-Dawley rats were dissected under the microscope and divided into two parts (forebrain and hindbrain) by a transverse section at the level of the caudal edge of the cerebral cortex. Total RNA from these samples, adult rat pancreas, or cell lines (see below), was purified by CsCl gradient centrifugation. RNA (10 μg) from individual samples was primed with poly-(dT)15 and incubated with avian myeloblastosis virus reverse transcriptase to synthesize cDNA. For PCR amplification, a forward primer (nucleotides 96 to 117; 5'-CCACCATGATAGTGAGGACA-3'), and a reverse primer (nucleotides 305 to 322; 5'-GCGGAGGCCGGGGCGACCTTC-3') taken from the published IDX1/IPF1 cDNA sequence (7) were used. PCR conditions were: 98°C for 1 min, followed by 30 cycles of 96°C for 30 sec, 55°C for 30 sec, and 75°C for 1 min, after which a 5 min incubation at 75°C followed. After PCR, an aliquot of the reaction was resolved in a 1% agarose gel, blotted onto a nylon membrane, probed with a 32P-labeled internal primer (nucleotides 216 to 236; 5'-GCCGAGCGCCCGACCTTCGCCG-3'), and autoradiographed at -80°C.

For PCR amplification of Dlx-2 (GenBank accession S81929), the primers used were: Forward, 5'-ACAGCCA-TGTCTCGTATTAGCAGCCG-3' (nucleotides 1 to 23); reverse, 5'-TTCACGCGTATCTGACTGTCG-3' (nucleotides 298 to 321). PCR conditions were identical to those described for IDX1/IPF1, except that annealing temperature was 60°C. As a probe to detect the PCR product after blotting onto nylon, the following 32P-labeled internal primer was used: 5'-ACTTCCAAGCTTCCGTGGGGCCGG-3'.

For PCR amplification of actin, the primers used were: Forward, 5'-GACATATGGGAAAGTCTTGTGGCAG-3', (anneals to exon 2); Reverse, 5'-CCATCTCTTGTGTGGGAATCTAGG-3', (anneals to exon 3). PCR conditions

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Fig. 1. Detection of IDX1/IPF1 transcripts by RT-PCR/Southern blot hybridization in neural cells. A, IDX1/IPF1 was found expressed in immortalized cerebrocortex-derived RC2.E10 cells. RNA obtained from rat pancreas and pancreatic islet-derived RIN-1027-B2 cells (RIN-B2) was used as positive control. B, Expression of IDX1/IPF1 in developing E15 rat brain. IDX1/IPF1 was found both in forebrain (F) and hindbrain (H). Dlx2, an unrelated homeodomain gene preferentially expressed in the forebrain, was used for comparison. Identical results were obtained from two independent experiments.

Fig. 2. Western immunoblot carried out with anti-IDX1/IPF1 α253 antiserum. IDX1/IPF1 (arrows) was detected in pancreatic islet-derived RIN-1027-B2 cells (RIN-B2), in rat cerebral cortex, and in cerebrocortex-derived RC2.E10 cells. RC2.E10 cells were either untransfected (−) or transfected (+) with 5 μg of an expression plasmid encoding IDX1/IPF1 (pcDNA3-IDX1). Transfection protocol was exactly as described in ref. 10. Note the comigration of endogenous and recombinant IDX1/IPF1 in RC2.E10 cells.

Fig. 3. Immunohistochemical detection of IDX1/IPF1 in developing rat brain. A parasagittal section corresponding to the cerebral cortex of an E15 rat at low (A) and high (B) magnification is shown. Note the scattered distribution of IDX1/IPF1-containing cells throughout the thickness of the cortex. No staining was detected when control non-immune serum was used. Arrows indicate IDX1/IPF1 immunopositive cells clustered in close proximity to the ventricular surface. V, ventricle. Scale bar represents 50 μm.

Fig. 4. EMSA with nuclear extracts of cerebrocortex-derived RC2.E10 cells (A) or E17 rat forebrain (B), showing binding of protein complexes to a TAAT-containing site of the GFAP gene. Binding reactions were carried out in the absence (+) or presence of competing oligonucleotides (10-, 50-, or 100-fold molar excess) of identical probe sequence, or in the presence of a nonspecific competing (NSC) oligonucleotide of unrelated sequence (100-fold molar excess). Where indicated, binding reactions were carried out in the presence of anti-IDX1/IPF1 α253 antiserum (Anti-IDX1) or normal rabbit serum (NRS). Arrows indicate the absence of a protein-DNA complex in the presence of the anti-IDX1/IPF1 antiserum.
were: 94°C for 5 min, followed by 20 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, after which a 5 min incubation at 72°C followed. As a probe to detect the PCR product, the following 32P-labeled internal primer was used: 5'-CACACCGACCTATTGTATAAAAGT-3'.

In all cases, controls were carried out in which samples were treated with RNase A prior to cDNA synthesis to rule out amplification from contaminating DNA.

Cell lines. Neural RC2.E10 cells derived from rat E16 embryonic cortex, and pancreatic islet RIN-1027-B2 cells, have been described and were cultured without modification (10).

Western immunoblot. Cells or tissue samples were lysed and processed for Western immunoblotting as described (10). Immunoreactivity was detected using an antiserum (α253) (1:10,000 dilution) that specifically recognizes the C-terminal 12 amino acids of IDX1/IPF1 (4, 11), and was visualized by enhanced chemiluminescence (ECL, Amersham, Buckinghamshire, England).

Immunohistochemistry. E15 rat fetuses were removed from timed-pregnant Sprague-Dawley rats and fixed overnight in 4% paraformaldehyde. After fixation, they were washed with PBS, transferred to PBS containing 20% sucrose, and kept at 4°C for at least 24 hours. Sagittal cryostat sections (10 μm) were cut and kept at -80°C until used. Sections were brought to room temperature, and after blocking with normal goat serum, they were incubated overnight with the α253 anti-IDX1/IPF1 antiserum (1:1500 dilution) at 37°C. Immunodetection was carried out with a secondary biotinylated goat antirabbit antiserum (Bio-Rad Laboratories, Hercules, CA) using nickel-intensified immunoperoxidase staining with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA).

DNA-Protein binding assays. Electrophoretic mobility shift assays (EMSAs) were carried out as described (10), using nuclear extracts of RC2.E10 cells or E17 rat forebrain. The probe was a synthetic double stranded oligonucleotide corresponding to a segment of the rat glial fibrillary acidic protein (GFAP) promoter spanning nucleotides 1079 to 1096 (GenBank accession Z48978), to which 5' GATC overhangs had been added, labeled with α-32P-dATP and Klenow enzyme. The sequence of the sense-strand is: 5'-GATCCGAGATTTAGGGTCA-3'.

Results

To investigate whether IDX1/IPF1 is expressed in neural cells during development, we carried out RT-PCR/Southern blot analyses using RNA from both RC2.E10 cells and from E15 rat brain. IDX1/IPF1 transcripts were detected in RC2.E10 neural cells, as well as in pancreas and pancreatic islet-derived RIN-1027-B2 cells, used as positive controls (Fig. 1A). In addition, IDX1/IPF1 transcripts were detected in both forebrain and hindbrain of E15 rats (Fig. 1B). This distribution contrasts with that of the homeodomain transcription factor Dlx-2, used for comparison, which was found preferentially expressed in the forebrain (Fig. 1B), as has been described (12). No signal was found in any of the samples after treatment with RNase prior to RT-PCR (not shown).

The presence of IDX1/IPF1 protein was confirmed in rat cerebral cortex and RC2.E10 cells by Western immunoblot. Cortical IDX1/IPF1 was found to comigrate with IDX1/IPF1 present in pancreatic islet RIN-1027-B2 cells (Fig. 2). Endogenous IDX1/IPF1 in RC2.E10 cells comigrated with recombinant IDX1/IPF1, overexpressed in RC2.E10 cells transfected with an IDX1/IPF1-encoding expression vector (Fig. 2).

To evaluate the distribution of neural cells that express IDX1/IPF1 in the developing cortex, we carried out immunohistochemistry on sagittal sections of E15 rats. In the periphery, IDX1/IPF1 immunoreactivity was found in the developing duodenum and pancreatic mesenchyme (not shown). In the central nervous system, scattered IDX1/IPF1 immunopositive cells were found throughout the cerebral cortex, although a higher density was detected in regions close to the ventricular surface (Fig. 3).

To investigate whether IDX1/IPF1 present in neural cells binds DNA, we carried out EMSA using an oligonucleotide containing a homeodomain-binding TAAT consensus motif (13), present in the promoter of the GFAP gene, which is expressed throughout the central nervous system. Specific protein-DNA complexes were detected when nuclear extracts of RC2.E10 cells or E17 rat forebrain were used (Fig. 4). Addition of the anti-IDX1/IPF1 α253 antiserum to the protein-DNA binding reaction resulted in the disappearance of one of these complexes, indicating the presence of IDX1/IPF1 bound to the TAAT-containing site of the GFAP gene.

Discussion

In the developing forebrain, expression of Hox-like homeodomains has been difficult to demonstrate. It has been proposed that development of the anterior segments of the central nervous system is directed by members of other families of homeodomain transcription factors, including Distal less (12), Orthodenticle (14), Empty spiracles (15), Pou (16), and Paired (17) homeoproteins. However, some Hox-like homeoproteins with patterns of expression preferentially restricted to the forebrain have been described (18, 19), supporting the notion that Hox-like transcription factors may play developmental roles in discrete populations of neural forebrain cells.

Our study shows that IDX1/IPF1, whose expression was thought to be restricted to the duodenum and pancreas, is present in neural cells in the forebrain during development. This finding raises the possibility that IDX1/IPF1 may participate in mechanisms of cellular differentiation in the central nervous system. The target genes whose expression may be regulated by IDX1/IPF1 are unknown. In this regard, we would like to emphasize that our experiments described in Fig. 4 provide no direct evidence that the GFAP gene is a target for regulation by IDX1/IPF1 in neural cells. Those experiments were intended to demonstrate the presence of IDX1/IPF1 in nuclear extracts from neural cells, and its ability to bind to a canonical homeodomain binding site present in the promoter of a neural gene. Nonetheless, we have observed
that the activity of a GFAP-luciferase reporter plasmid increases when cotransfected in RC2.E10 cells with an expression plasmid encoding ID1/IPF1 (data not shown).

A possible candidate gene that may be regulated by ID1/IPF1 is the gene encoding somatostatin. In the pancreas, somatostatin is expressed in islet $\delta$ cells, where ID1/IPF1 appears to regulate its expression (7, 20). In the central nervous system, somatostatin acts as a neurotransmitter synthesized in small interneurons of the cerebral cortex and other areas of the brain (21). We have previously shown that neural RC2.E10 cells, found to express ID1/IPF1 in this study, also express somatostatin (10). Thus, the possibility that ID1/IPF1 regulates somatostatin gene expression in neural cells merits further investigation and is currently under study.

Although the effects of the inactivation of the ID1/IPF1 gene on pancreas development have been studied in detail in mutant mice (2, 3), no phenotypic changes in the central nervous system of those animals has been reported. However, it is important to bear in mind that some of the alterations resulting from the deletion of homeodomain-encoding genes specifically expressed in the forebrain are subtle and would escape detection if they are not the subject of a detailed study (12, 14, 15, 17). An additional difficulty that may have prevented the detection of possible neural deficits in mutant mice is that ID1/IPF1 gene inactivation leads to early postnatal lethality due to the resulting pancreatic agenesis.

In summary, our study provides evidence consistent with the notion that ID1/IPF1 may regulate neural gene expression during development in the central nervous system. Thus, possible extrapancreatic functions for ID1/IPF1 may include participation in the mechanisms of differentiation of specific types of neural cells, and/or regulation of neural cell-specific neuropeptide gene expression.

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