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Eph receptors and their ephrin ligands are expressed in developing mouse pancreas

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Abstract

Pancreas development involves branching morphogenesis concomitantly to differentiation of endocrine, exocrine and ductal cell types from a single population of pancreatic precursors. These processes depend on many signals and factors that also control development of the central nervous system. In the latter, Eph receptors and their class-A (GPI-anchored) and class-B (transmembrane) ephrin ligands control cell migration and axon-pathfinding, help establish regional patterns and act as labels for cell positioning. This raised the question as to whether and where Ephs and ephrins are expressed during pancreas development. Here we have identified the Eph and ephrin genes that are expressed in mouse embryonic pancreas, as detected by RT-PCR analysis. In situ hybridization experiments showed that Ephs and ephrins are mainly expressed in the burgeoning structures of the epithelium which differentiate into exocrine acini. Binding experiments on whole pancreas demonstrated the presence of functional Eph receptors. They showed that EphBs are expressed by the pancreatic epithelium at embryonic day (e) 12.5 and that, from e14.5 on, Ephs of both classes are expressed by the pancreatic epithelium and then become restricted to developing acini. We conclude that specific members of the Eph/ephrin family are expressed in embryonic pancreas according to a dynamic temporal and regional pattern.

Keywords: Eph; Ephrin; Pancreas development; Mouse embryo

1. Results and discussion

The adult pancreas is a complex organ which fulfils exocrine and endocrine functions that control digestion and glucose homeostasis, respectively. The pancreatic cell types include exocrine cells arranged into acini which secrete digestive enzymes, ductal cells which produce bicarbonate and line the ducts that drain the exocrine fluid to the duodenum, and endocrine cells. The latter comprise glucagon- (α), insulin- (β), somatostatin- (δ) and pancreatic polypeptide- (PP) producing cells, which are organized into islets of Langerhans and secrete these hormones into the blood. All these pancreatic cell types originate from a single population of pluripotent precursors. In the mouse, these pancreatic precursors, which express the transcription factor Pdx-1, arise at embryonic day (e) 8.5 from specified dorsal and ventral territories of the posterior foregut endoderm, and differentiate according to a multistep process into the various pancreatic cell types (Jensen, 2004). Concomitantly, morphogenesis of the pancreas takes place with proliferation and budding of the epithelium from the specified endoderm, branching of the epithelial cells within the surrounding mesenchyme, and delamination of endocrine cells from the epithelium and their clustering into islets (Pictet and Rutter, 1972; Kim and MacDonald, 2002). All these processes depend on transcription factors cascades (Wilson et al., 2003) and on extracellular signals (Kim and Hebrok, 2001; Kim and MacDonald, 2002) that mediate these precisely timed events. Interestingly, many of the developmental mechanisms and factors that control pancreas differentiation are also involved in differentiation of the nervous system. For example, the neural transcription factors Isl-1, NeuroD, Pax-4, Pax-6, Nkx, and neurogenin3 (Ngn-3) are essential for pancreatic endocrine differentiation (Wilson et al., 2003). This similarity does not relate only to endocrine differentiation, as netrins, which are axon guidance factors, control migration of putative pancreatic precursors during pancreas development (Yebra et al., 2003; Hebrok and Reichardt, 2004). Another important class of factors involved

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in the development of the central nervous system is that of the Eph receptors and their ephrin ligands.

Eph receptors represent the largest family of receptor tyrosine kinases with, in the mouse, 13 members which bind eight ephrins. These receptors are divided into two classes, based on their ligand binding specificity. EphA receptors bind GPI-anchored ephrin-A ligands and EphB receptors bind transmembrane ephrin-B ligands (Flanagan and Vanderhaeghen, 1998; Himanen and Nikolov, 2003). As receptors and ligands are anchored to the plasma membrane, their interactions involve cell–cell contacts, upon which bidirectional signaling can occur in each interacting cell. This includes forward signaling downstream of the Ephs and reverse signaling downstream of the ephrins. Diverse signaling pathways have been described, depending on the biological process, but they often result in modifications of cytoskeleton, cell motility, and guidance of migration (Schmucker and Zipursky, 2001; Palmer and Klein, 2003). Most of the current knowledge on Eph and ephrin signaling stems from studies on the central nervous system, but recent work showed that this family is involved in other developmental events, such as intestinal cell positioning along the crypt-villus axis (Batlle et al., 2002) and urorectal development (Dravis et al., 2004). As developing pancreatic cells share differentiation mechanisms with neural cells and because mRNAs for EphB2 (Ikegaki et al., 1995) and EphB3 (Bohme et al., 1993), and for ephrin-B1 (Beckmann et al., 1994), were detected in adult human pancreas, we have investigated whether Ephs and their ligands are expressed during pancreas development. Here we show that several Ephs and ephrins are expressed throughout pancreas development with a dynamic temporal pattern and that their expression becomes restricted to the burgeoning structures of the pancreatic epithelium that give rise to the acini.

1.1. Expression of Eph and ephrin genes during pancreas development

To determine which Eph and ephrin genes are expressed during pancreas development, we performed RT-PCR experiments on RNA from mouse embryonic tissue. Primers were designed to specifically amplify a fragment encompassing an exon–exon boundary (to avoid amplification of genomic DNA) for each Eph or ephrin mouse gene (Table 1). The primers were validated on cDNA from mouse central nervous system as a control (data not shown). We then assessed the expression of these genes by using cDNA from e12.5, e14.5 and e16.5 developing pancreas. To offset individual variations, cDNA was pooled from five embryos. As shown in Table 2, all Eph and ephrin genes, except EphA4 which we could not detect at any of the stages studied, were expressed at e12.5. At e14.5 and e16.5 we no longer detected the expression of EphA6 and EphA8, and of ephrin-A5 and ephrin-B3, but all the other genes were still expressed.

In view of these results and because there is functional redundancy in the ephrin family (Flanagan and Vanderhaeghen, 1998), we investigated whether some members might be more expressed than others and thus be of greater functional relevance. We therefore determined by quantitative RT-PCR, the relative expression levels of all these transcripts at e12.5, a critical time at which a burst of differentiation occurs and branching morphogenesis starts (Jensen, 2004). Data were obtained on individual pancreatic cDNA samples from five embryos (Fig. 1). Within the ephrin-A class, the A1, A2, A4 and A5 ligands were the most expressed, in contrast to A3, which was barely detectable (ratio to actin mRNA ≤ 0.0001). In the corresponding class of receptors, only EphA3 and EphA7 were expressed at high levels, making up about 60% of all EphA transcripts. Within the ephrin-B class, expression of the B2 and B3 ligands was 2- to 3-fold higher than that of

Table 1
Eph and ephrin primer sequences

Primer	Sequence	Product size (bp)
ephrinA1S	5′-AGTTCAAGGAAGGACACAGC-3′	139
ephrinA1AS	5′-CTCTTCTCCTGTGGGTTGAC-3′	
ephrinA2S	5′-TCTTCACCCCCTTTTCCCTG-3′	108
ephrinA2AS	5′-GCACATAAACCTTGAGTCGC-3′	
ephrinA3S	5′-CCACGCCCACTCACAACCTG-3′	156
ephrinA3AS	5′-CCTCAAAGTCTTCCAACACG-3′	
ephrinA4S	5′-CAGCGCTACACACCCTTCCC-3′	142
ephrinA4AS	5′-GTGATGACCCGCTCTCCTTG-3′	
ephrinA5S	5′-CCCAGACAACGGAAGAAG-3′	202
ephrinA5AS	5′-ACAGGCGGACGGGAGGAG-3′	
EphA1S	5′-GCCTGGCCCTTTCTCCCCTG-3′	240
EphA1AS	5′-TCTCTGTCTCTGGCCTCTCC-3′	
EphA2S	5′-TGGATGGCGAGTGGCTGGTG-3′	250
EphA2AS	5′-TTGGGGGCAGAGGGTGGACG-3′	
EphA3S	5′-AAGCAGGAGCAAGAGACGAG-3′	183
EphA3AS	5′-CACCGGAGATGGAGAAAGAG-3′	
EphA4S	5′-TCTTTTCGTTTCTCTTTGG-3′	222
EphA4AS	5′-GTTGTTCTGGCTGGCTTCC-3′	
EphA5S	5′-AAGGGCAAAGAAGCGGGAC-3′	244
EphA5AS	5′-GGAAGGGGCGAGAGAGACG-3′	
EphA6S	5′-ATTCTTCCTCTTTGGTTG-3′	298
EphA6AS	5′-GGTGGGTCTTTTTCTGCC-3′	
EphA7S	5′-TCTGGCTGCTTGGCTTTGC-3′	86
EphA7AS	5′-TCTGTTTGTTGTGCTTTCG-3′	
EphA8S	5′-TCACCACGAACCAGGCAG-3′	386
EphA8AS	5′-GAGAAGCAAGAGGAGCAC-3′	
ephrinB1S	5′-AAGCCACACCAGGAAATCCGC-3′	600
ephrinB1AS	5′-CGGTGCCCGCTGTACCACTAC-3′	
ephrinB2S	5′-CTGTGCCAGACCAGACCAAGA-3′	214
ephrinB2AS	5′-CAGCAGAACTTGCATCTTGTC-3′	
ephrinB3S	5′-AAGGTGCTTCTGCGAGTGG-3′	155
ephrinB3AS	5′-GGAGGTTGCATTGCTGCTGG-3′	
EphB1S	5′-CCCTGGATTGCTTGCTGCTC-3′	216
EphB1AS	5′-AGTTGTTCTGGTTGGGTTCG-3′	
EphB2S2	5′-CCAACCAAGGGGACGAAGCC-3′	197
EphB2AS	5′-CCACTCTAGCATGAGGGACG-3′	
EphB3S	5′-TCATCTCTGTGCGTGCCTTC-3′	291
EphB3AS	5′-CTTCTCCTTGCTTTGCTTTG-3′	
EphB4S	5′-CAGGTGGTCAGCGCTCTGGAC-3′	387
EphB4AS	5′-ATCTGCCACGGTGGTGAGTCC-3′	
EphB6S	5′-CAGTCTGTGGCTCCTGGTTC-3′	251
EphB6AS	5′-TCTGGGCCCCTCGCCGTTCC-3′	