Insulin-like growth factor II in human fetal pancreas and its co-localization with the major islet hormones: comparison with adult pancreas

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Abstract

Insulin-like growth factor II (IGF-II) appears to play an important role during fetal life in cell growth and differentiation in several organs, including the pancreas.

In the present study we investigated the cellular localization of IGF-II in human fetal pancreas at 16, 18 and 22 embryonic weeks and compared it with adult pancreas. Single and double immunofluorescence methods were used to study co-localization of IGF-II with the four major islet hormones – insulin, glucagon, somatostatin, pancreatic polypeptide – and with islet amyloid polypeptide (IAPP).

Distinct IGF-II immunoreactive (IR) cells were found in the endocrine, but not in the exocrine, pancreas. The intensity of IGF-II immunoreactivity was more pronounced in the fetal than in the adult pancreas. In fetal pancreas IGF-II immunoreactivity was observed in virtually all insulin-IR cells and in subsets of the glucagon, somatostatin and IAPP cells. In the adult pancreas, IGF-II immunoreactivity was found in insulin/IAPP cells only.

Our results suggest a broader effect of IGF-II in fetal endocrine pancreatic cells than in the adult.

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Introduction

The human adult endocrine pancreas contains four major cell types: A-, B-, D- and PP-cells synthesizing glucagon, insulin, somatostatin and pancreatic polypeptide, respectively (Klöppel *et al.* 1998). The B-cells also co-store islet amyloid polypeptide (IAPP or amylin) (Westermarck *et al.* 1986). During fetal development of the pancreas, endocrine cells are believed to differentiate from progenitor cells, forming clusters close to the ducts (Yamaoka & Itakura 1999). In fetal pancreas the endocrine cells may co-store more than one of the four 'classical' islet hormones (De Krijger *et al.* 1992, Lukinius *et al.* 1992).

During fetal life, insulin-like growth factor II (IGF-II) appears to play an important role in cell growth and cell differentiation in the pancreas, as well as in other tissues and organs (Gray *et al.* 1987, Sara & Hall 1990, Hedborg *et al.* 1994). This polypeptide may also be involved in carbohydrate metabolism (Lewitt 1994). Other family members are proinsulin/insulin, insulin-like growth factor I (IGF-I) and relaxin (Gammeltoft 1989).

In human adults, detectable levels of IGF-II have been found in many tissues including the central nervous system (Gray *et al.* 1987, Sara & Hall 1990) and adrenal medulla

(Suzuki *et al.* 1989). This peptide has also been detected in fetal (Han *et al.* 1987, Bryson *et al.* 1989, Hill 1990, Miettinen *et al.* 1993), as well as in adult pancreas (Bergmann *et al.* 1996). In the adult pancreas, IGF-II has been found in B-cells (Höög *et al.* 1993, Maake & Reinecke 1993).

The purpose of the present study was to investigate the human fetal pancreas with regard to the expression of IGF-II and its distribution in the various islet cell types.

Materials and Methods

Pancreatic tissue from three human female fetuses obtained for routine histopathological examination in connection with autopsy examination was included in the study. The gestational ages were determined to be 16, 18 and 22 weeks (w). The youngest fetus was from a prostaglandin-induced abortion warranted by ultrasound findings of hygroma colli and hydrothorax, while the two older fetuses were obtained from spontaneous abortions. The fetuses measured 12.0, 19.5 and 25.5 cm, respectively (crown–rump length), and their weights were 56, 180 and 337 g. Autopsy findings regarding the 16 w fetus

Table 1 Antibodies used for immunofluorescence

Antibody raised against	Code no.	Directed against	Working dilution	Source
Synthetic porcine glucagon	A565	Rabbit	1 : 60	DAKO Corp., Santa Barbara, CA, USA
Synthetic human insulin	Ma 47	Guinea-pig	1 : 80	Dr. Per Westermark, Dept. Genetics & Pathology, Uppsala, Sweden
Synthetic human insulin-like growth factor II	128	Mouse	1 : 10*	Pharmacia & Upjohn, Stockholm, Sweden
Synthetic human islet amyloid polypeptide	RAS 7321	Rabbit	1 : 200	Peninsula Laboratories, Belmont, CA, USA
Synthetic human pancreatic polypeptide	A619	Rabbit	1 : 50	DAKO Corp.
Synthetic human somatostatin (1–14)	A566	Rabbit	1 : 100	DAKO Corp.

* = 20 µg/ml.

confirmed hygroma colli and hydrothorax; a malformed right ear and horse-shoe kidney were also found; the other two fetuses displayed asphyxial hemorrhages in the lungs, due to placental ablatio. None of the mothers had a history of diabetes mellitus. Adult human pancreas was collected from three patients undergoing surgery for pancreatic adenocarcinoma. The tissue specimens were from grossly normal glandular regions and the microscopical examination revealed normal histology. This study was approved by the Medical Ethics Committee at Huddinge Hospital, Stockholm.

The pancreatic specimens obtained were fixed in 4% buffered formaldehyde solution for 18–20 h at room temperature (RT) and embedded in paraffin. Sections, 5 µm thick, were cut and attached to poly-L-lysine coated glass slides. The sections were stained with hematoxylin–eosin, or immunostained to demonstrate various islet hormones (see Table 1). The streptavidin–biotin complex (ABC) technique (Hsu *et al.* 1981), with diamino-benzidine as chromogen, was used as a single immunostain, mainly to demonstrate the distribution of endocrine cell types in the pancreas, but also to serve as a control for the stainings mentioned below. Immunofluorescence methods were used in single or double stainings; the latter staining procedure was applied to the same section to investigate co-localization of IGF-II with the major islet hormones.

For double immunofluorescence staining, the following procedure was performed: the sections were incubated overnight with a cocktail of antibodies (one monoclonal+

one polyclonal (rabbit IgG or guinea-pig IgG)), at RT. Thereafter the sections were incubated with biotinylated goat anti-mouse IgG for 30 min at RT and then transferred to a mixture of fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG, or goat anti-guinea-pig IgG and Texas Red (TXRD)-conjugated streptavidin for 30 min at RT. The sections were carefully rinsed in phosphate-buffered saline (PBS) after each incubation step. Before the respective primary antibodies were applied, the sections were incubated with non-immune serum diluted 1:10 from the animal species producing the secondary antibodies.

The control stainings included omission of the primary antibody and replacement of the first layer of antibody by non-immune serum diluted 1:10 or by the diluent alone. To confirm the specificity of the IGF-II antibody, absorption tests were carried out by overnight incubation at +4°C, with 0.5, 2 and 10 nmol IGF-II per ml diluted antibody, before application to the sections (Höög *et al.* 1993, 1997).

The primary antibodies are characterized in Table 1. The IGF-II antibody displayed less than 1% cross-reactivity to IGF-I (Höög *et al.* 1993). The labeled secondary antisera were: biotinylated goat anti-mouse IgG (DAKO, Glostrup, Denmark), TXRD-labeled streptavidin (Vector Laboratories, Burlingame, CA, USA), FITC-conjugated goat anti-rabbit IgG or goat anti-guinea-pig IgG (Sigma, St Louis, MO, USA). The ABC kit used was from DAKO. The IGF-II antigen used in the

absorption tests was from two different sources: Pharmacia & Upjohn, Uppsala, Sweden, and Gropep, Adelaide, Australia.

The sections were examined using an Olympus Vanox AHBS3 fluorescence microscope (Olympus, Tokyo, Japan) equipped with filters (Olympus) giving excitation at wavelength band-widths of 475–555 nm for TXRD (filter no. 32821, dichroic mirror BH2-DMG) and 453–488 nm for FITC (no. 32822, BH2-DMIB), respectively. For simultaneous visualization of TXRD- and FITC-labeled cells a double-band filter set (no. 39538, BH2-DFC5) was used, giving excitation at 550–570 nm and 480–495 nm, respectively. Photomicrographs were taken with Fujicolor 400 film.

Results

Numerous distinct IGF-II immunoreactive (IR) cells were seen in the endocrine pancreas of the three fetuses and adults examined. The cells belonging to the exocrine parenchyma did not show any immunoreactivity. All control tests turned out as expected. The reactions of the double immunostainings did not influence one another. In the neutralization test, 0.5 nmol gave a weak staining reaction, and was abolished when 2–10 nmol IGF-II antigen/ml was used.

In the 16 w fetus the IGF-II-IR cells were distributed in small groups, mainly connected to ducts; in the 18 w fetus these cells also assembled in larger clusters, while in the 22 w fetus most of the cells were concentrated into islet-like structures, with a central localization similar to that found in adult pancreas. The intensity of the IGF-II-IR cells was generally stronger in the fetal than in the adult pancreas.

The immunoreactivity in IGF-II-IR cells in the 16 w fetus varied from weak to strong, but the intensity of the IGF-II immunoreactivity was generally stronger in the two older fetuses.

IGF-II immunoreactivity was found in virtually all insulin-IR cells and vice-versa (Figs 2, 5, 8). The intracytoplasmic distributions of the IGF-II and insulin immunoreactivities were in close agreement, but the fluorescence intensity of IGF-II was usually stronger than that of insulin. In the 18 w fetus, virtually all IGF-II cells displayed IAPP immunoreactivity and vice versa, while in the 16 and 22 w fetuses, IGF-II was seen in only a few of the IAPP-IR cells, usually in different cytoplasmic areas (Fig. 9). IGF-II immunoreactivity appeared in a few glucagon-IR cells (Figs 1, 4, 7). In both fetuses, a few somatostatin-IR cells also displayed IGF-II immunoreactivity (Figs 3, 6, 10), appearing only in small cytoplasmic areas. IGF-II immunoreactivity was not present in any of the PP cells.

In the adult pancreas, IGF-II-IR cells displayed insulin/IAPP immunoreactivity and vice versa; the IGF-II

immunoreactivity often appeared weaker than that of insulin (Fig. 11). IGF-II immunoreactivity was not observed in the glucagon-, PP- or somatostatin-IR cells.

Discussion

Several investigators have established the presence of IGF-II in adult and fetal human pancreas by using immunohistochemical, biochemical and *in situ* hybridization techniques (Han *et al.* 1987, Bryson *et al.* 1989, Hill 1990), whereas the present study was intended to ascertain the extent to which IGF-II might be co-localized with the major islet hormones. IGF-II has also been reported to occur in isolated islets or cell clusters (Bryson *et al.* 1989, Miettinen *et al.* 1993) or in insulin-producing cell lines (Asfari *et al.* 1995, Katz *et al.* 1997). The reason for assuming the location of IGF-II to be B-cells is the finding of IGF-II peptide and IGF-II mRNA centrally in the islets, i.e. a location known to contain insulin cells (Miettinen *et al.* 1993). Previous studies have confirmed that IGF-II immunoreactivity was located in B-cells in the human adult pancreas (Höög *et al.* 1993, Maake & Reinecke 1993). Maake & Reinecke (1993) were also able to demonstrate IGF-II immunoreactivity in dog and rat insulin cells.

In the present study we confirmed previous findings in adult pancreas that only insulin cells display IGF-II immunoreactivity (Höög *et al.* 1993, Maake & Reinecke 1993).

The intensity of IGF-II immunoreactivity was more pronounced in the fetal than in the adult pancreas. In agreement with earlier reports showing greater expression of IGF-II mRNA in fetal than in adult tissue (Sara & Hall 1990, Hedborg *et al.* 1994), our findings indicate that the fetal pancreas synthesizes more IGF-II. This suggestion is also underlined by the finding that the IGF-II peptide level declined 2 weeks after birth (Petrik *et al.* 1998).

Co-localization of the four major islet hormones in early fetal pancreas is well known (De Krijger *et al.* 1992, Lukinius *et al.* 1992), whereas in mature adult endocrine cells these hormones are produced and stored in separate cell types (De Krijger *et al.* 1992, Lukinius *et al.* 1992). This is the first report on the expression of IGF-II at the protein level in the major islet cell types of the human fetus. In the present study on the fetal pancreas, IGF-II-IR cells were thus found in all insulin-IR cells, as well as in some glucagon and somatostatin-IR cells.

In the oldest fetus some IAPP-IR cells did not display any IGF-II immunoreactivity. Lukinius *et al.* (1996) showed that a proportion of glucagon cells contained IAPP immunoreactivity in the secretory granules. This finding of co-localization of IAPP with glucagon has also been demonstrated with double immunofluorescence (Portela-Gomes *et al.* 1999). This IAPP cell fraction may constitute the cell population not containing IGF-II.

Figure 1 Fetal pancreas (16 w), double immunostained for IGF-II (Texas Red) (A) and glucagon (FITC) (B). Distinct co-localization of these two substances is shown with the double-band filter set in one cell (yellow) (C). Bar=32 μ m.

Figure 2 Fetal pancreas (16 w), double immunostained for IGF-II (Texas Red) and insulin (FITC), demonstrating varying degrees of co-localization between IGF-II and insulin (double-band filter set). The yellow colour indicates a similar amount of the two fluorescent substances, while the orange colour is indicative of a predominant IGF-II fluorescence.

Figure 4 Fetal pancreas (18 w), double immunostained for IGF-II (Texas Red) and glucagon (FITC). In the more central part of the islet, IGF-II is seen as red. In the periphery, glucagon cells predominate. Some glucagon cells probably contain a small amount of IGF-II (yellow-green). One cell shows yellow colour, indicating a co-localization of these two peptides in more equivalent amounts. Bar=53 μ m.

Figure 3 Fetal pancreas (16 w), double immunostained for IGF-II (Texas Red) and somatostatin (FITC), showing co-localization only in one cell (yellow). Bar=32 μ m.

Figure 5 Fetal pancreas (18 w), double immunostained for IGF-II (Texas Red) and insulin (FITC). Co-localization of these two peptides, illustrated by the yellow colour, is observed in all immunostained cells. Bar=32 μ m.

Figure 6 Fetal pancreas (18 w), double immunostained for IGF-II (Texas Red) and somatostatin (FITC), showing co-localization in some cells (yellow). Bar=53 µm.

Figure 7 Fetal pancreas (22 w), double immunostained for IGF-II (Texas Red) and glucagon (FITC) demonstrating co-localization in a fraction of cells. Bar=32 µm.

Figure 8 Fetal pancreas (22 w), double immunostained for IGF-II (Texas Red) and insulin (FITC). The orange colour indicates that IGF-II fluorescence is predominant. Bar=32 µm.

Figure 9 Fetal pancreas (22 w), double immunostained for IGF-II (Texas Red) and islet amyloid polypeptide (IAPP) (FITC). Co-localization of IGF-II and IAPP, identified by the yellow colour (double-band filter set), is present in a fraction of immunoreactive cells. In some cells, IGF-II immunoreactivity (green) and IAPP (red) appeared to occur separately. Bar=32 µm.

Figure 10 Fetal pancreas (22 w), double immunostained for IGF-II (Texas Red) and somatostatin (FITC), showing co-localization of these peptides in two cells (yellow colour). Bar=32 µm.

Figure 11 Adult pancreas, double immunostained for IGF-II (Texas Red) (A) and insulin (FITC) (B). Insulin immunoreactivity (B) is often stronger than that of IGF-II (A). (C) With the double-band filter set, co-localization of these two peptides is visualized as yellow colour in virtually all cells. Some cells have a yellow-green colour which probably indicates that insulin fluorescence is predominant in these cells. Bar=46 µm.

Little is known about the function of IGF-II in islet cells. In a previous study in rat, using immunocytochemistry at the ultrastructural level, the IGF-II peptide has been localized to a subset of insulin containing secretory granules (Höög *et al.* 1997). In these animals, IGF-II peptide may play a role in the lysosomal degradation of non-released 'old' secretory granules (crinophagia) (Borg *et al.* 1986, Sawano *et al.* 1986, Nissley *et al.* 1991). These two findings could explain how the cell can select only older granules for degradation.

IGF-II may induce B-cell proliferation (Rabinovitch *et al.* 1982, Rafaeloff *et al.* 1993, Hill *et al.* 1998). Recent studies in fetal and neonatal rat pancreas suggest that endogenous IGF-II can protect islet cells from cytokine-induced apoptosis, at least *in vitro*. Such apoptosis is a part of the late gestational and postnatal development of the endocrine pancreas (Tornehave & Larsson 1997, Petrik *et al.* 1998, Hill *et al.* 1999). Blocking of endogenous IGF-II with an IGF-II antibody inhibited this protective effect (Petrik *et al.* 1998). A decrease in apoptosis in islet cells has also been found in transgenic mice overexpressing IGF-II, giving rise to a marked islet hyperplasia (Petrik *et al.* 1999). Ilieva *et al.* (1999) have recently reported that hamster pancreatic duct epithelium contained IGF-II and that this secretion has an anti-apoptotic effect on B-cells; these results are not in line with ours but raise the question about species dependent differences in IGF-II localization.

These experimental studies indicate that IGF-II exerts a growth-regulating effect on the fetal pancreatic islets. Whether or not this applies to the human fetus is an open question. However, the present study did reveal differences between fetal and adult pancreas in the expression of IGF-II, suggesting that this peptide plays a broader and probably more important role in fetal endocrine pancreas than in the adult.

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