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PDZ-domain containing-2 (PDZD2) is a novel factor that affects the growth and differentiation of human fetal pancreatic progenitor cells

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Abstract

Early-trimester human fetal pancreas is a promising potential source of pancreatic progenitor cells. However, the ethical controversy associated with the source of these cells, and technical difficulties associated with their differentiation into insulin-producing cells have limited both their availability and utility. This study aimed to characterize a population of pancreatic progenitor cells (PPCs) isolated from human fetus and describe the effects of a novel factor, PDZ-domain containing-2 (PDZD2), and its secreted form (sPDZD2), on PPC proliferation and differentiation. In particular, we examined and characterized the expression of several stem cell (nestin, ABCG2, c-kit), growth and differentiation markers (GLP-1R, c-met, erbB1), and PDZD2 in PPCs by RT-PCR, Western blot, and immunocytochemistry. We also examined the effects of sPDZD2 on PPC proliferation and differentiation by examining BrdU incorporation, MTT, cell number, and real-time PCR as well as ELISA. PPCs were isolated, cultured and characterized from human fetal pancreas. PDZD2 and sPDZD2 were detected at high levels in both human fetal pancreas and in PPCs. sPDZD2 acted as a potent mitogen on PPCs, and inhibited the differentiation of PPC-derived islet-like cell-clusters (ICCs), evidenced by the downregulation of Isl-1, Pdx-1, and insulin mRNA levels. sPDZD2 treatment also reduced levels of C-peptide in ICCs. These results show that a novel pancreatic developmental factor, PDZD2, is sufficient to promote the proliferation of human fetal PPCs while limiting differentiation of ICCs into islet/endocrine cells. Findings from this study will contribute to the development of improved methods for islet transplantation therapy in the treatment of diabetes.

Keywords: Diabetes; Human fetus; Nestin; Pancreatic stem cells; PDZD2

1. Introduction

Despite the successful release of the Edmonton protocol, there are two major hurdles yet to be overcome in the development of successful strategies for islet transplantation in patients with Type 1 (T1DM) and severe forms

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of Type 2 (T2DM) diabetes mellitus: an inadequate supply of pancreatic islets and toxic immunosuppression (Hirshberg, Rother, Digon, Venstrom, & Harlan, 2003). Recent reports have described the production of pancreatic islets from a variety of sources, including embryonic stem cells (ES cells) (Soria et al., 2000), bone marrow (Choi et al., 2005), intestinal epithelium (Suzuki, Nakauchi, & Taniguchi, 2003), and liver (Ferber et al., 2000), suggesting alternatives to otherwise limited sources of islet cells. Nevertheless, both fetal, and even adult, pancreas appears to be the best place to search for potential pancreatic stem cells or pancreatic progenitor cells (PPCs) (Bodnar et al., 2006; Choi, Ta, Atouf, & Lumelsky, 2004; Gao et al., 2003; Huang & Tang, 2003; Seaberg et al., 2004), from which insulin-producing cells can be derived.

Currently, possible progenitors have been hypothesized to reside within the nonendocrine fraction of the pancreas which contains enriched proportion of ductal epithelium, acinar tissue (Hao et al., 2006) and/or mesenchymal stem cells (Seeberger et al., 2006). They have also been reported to cross the mesoderm lineage and give rise to endoderm (Seo, Suh, Bae, & Jung, 2005). Notwithstanding, the lack of definitive pancreatic stem cell markers has made studies of pancreatic progenitors/precursors difficult. Nestin, an intermediate filament protein was first identified in neuroepithelial stem cells as a marker of stem cells isolated from the adult pancreatic islets (Zulewski et al., 2001). Subsequent reports have shown that nestin expressing stem cells can be isolated from both islets and ductal origin (Bonner-Weir et al., 2004; Maria-Engler et al., 2004; Wang, Li, Yashpal, & Gao, 2005). Despite expression of islet-associated genes after differentiation in the conventional protocols, these cells remain mostly immature. New growth factors or regulators for enhancing proliferation and differentiation in these cells thus await to be explored.

The fetal pancreas, which is rich in undifferentiated ductal epithelial and/or mesenchymal precursor cells, has been shown to have a better immune privilege status over the adult pancreas (Si, Tuch, & Walsh, 2001; Simpson, Tuch, & Vincent, 1991). Here, we describe the isolation and characterization of a group of nestin-positive progenitor cells from the early-trimester human fetus. In addition, we have identified a novel protein, PDZ-domain containing-2 (PDZD2), which is detectable in both the fetal pancreas and our isolated PPC. PDZD2 is a multi-PDZ-domain protein, with homology to pro-interleukin-16 (pro-IL-16). Caspase-3-mediated proteolytic cleavage at the C-terminus results in a 37 kDa secreted PDZD2 (sPDZD2) peptide (Yeung, Tam, Tsang, & Yao, 2003). Similarly to that of IL-16, PDZD2/sPDZD2 might function as a growth and differentiation factor in various cell types (Cruikshank, Kornfeld, & Center, 2000). We previously reported the detection of sPDZD2 in rodent pancreatic β-cells and its concentration-dependent mitogenic effects in INS-1E cells (Ma et al., 2006). Nevertheless, its role in pancreatic precursors or PPC biology has never been reported. In this study, we aimed to investigate the role of sPDZD2 in the proliferation and differentiation of PPC-derived islet-like cell-clusters (ICCs). We present evidence that sPDZD2 may represent a cardinal β-cell growth and differentiation factor involved in these putative pancreatic progenitors' maturation and function. Present study provides better insight of the molecular mechanisms underlying the growth and differentiation of these PPCs which is essential for guiding them into the fully functional insulin-producing cells.

2. Materials and methods

2.1. Tissue procurement

Human fetal pancreas specimens used in these experiments were provided by the Department of Obstetrics and Gynecology, The Prince of Wales Hospital, The Chinese University of Hong Kong after termination of pregnancy by dilation and extraction. Specimens were collected during early gestational age between 11 and 13 weeks. Informed consent was obtained by the outpatient clinic of the Prince of Wales Hospital before procedures were performed. Approval for the use of fetal tissue was obtained from the Clinical Research Ethics Committee (CREC-2005.461).

2.2. Tissue processing and cell culture

PPCs were isolated as reported previously (Zou et al., 2006; Zulewski et al., 2001). Each fetal pancreas was minced and digested with collagenase P at 37 °C. After thorough washing, cell-clusters were resuspended in modified RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES buffer, 1 mM sodium pyruvate, 100 U of penicillin G, 100 μg of streptomycin sulfate per ml (Gibco Life Technologies, CA, USA, and 71.5 μM β-mercaptoethanol (Sigma, MO, USA) in 60 mm culture dishes. Rounded, non-adherent cell-clusters formed within 48 h of incubation, and were transferred to a new culture dish and incubated for another 48 h to remove fibroblasts. After 96 h, medium was replaced with modified RPMI 1640 media supplemented with 20 ng/ml each of basic fibroblast growth factor (bFGF) (Sigma) and epidermal growth