Nuclear Protein p8 Is Associated With Glucose-Induced Pancreatic β-Cell Growth

Günter Päth, Anne Opel, Anita Knoll, and Jochen Seufert

On its own, glucose is a major factor for proliferation of pancreatic β-cells and is also an essential prerequisite for IGF-I and growth hormone–induced growth of these cells. p8 was originally identified as an emergency gene product upregulated in pancreatic acinar cells in response to acute pancreatitis. p8 was further shown to be involved in a broad range of biological functions, including cell growth, growth arrest, apoptosis, and tumor development. These in part opposite actions may be related to distinct stimuli and pathways in certain conditions and cell types. Here we demonstrate that p8 is widely expressed in human pancreatic islets in vivo and in several β-cell lines in vitro. Based on this observation, we tested the hypothesis that p8 production in pancreatic β-cells is regulated by glucose. Incubation of rat INS-1 β-cells with 25 mmol/l glucose resulted in a continuous increase of proliferating cell numbers. This was accompanied by a strong upregulation of p8 mRNA and protein expression, indicating that p8 is a physiological mediator of glucose-induced pancreatic β-cell growth. Binding of glucose-activated protein kinase C (PKC) to two PKC sites within a highly conserved region of the p8 protein may be a possible mechanism linking glucose and p8 pathways leading to proliferation. *Diabetes* 53 (Suppl. 1):S82–S85, 2004

Glucose is a major factor for proliferation of pancreatic β-cells. In vivo, glucose infusion for 4 days resulted in a 50% increase in pancreatic β-cell mass in rats compared with saline-infused control animals (1). Results from inhibitor studies indicate that this effect may be attributed to activation of protein kinase A and protein kinase C (PKC) (2,3). It was also demonstrated that glucose at concentrations >6 mmol/l independently activates insulin receptor substrates 1 and 2 as well as SH2-containing protein (Shc)-mediated signal transduction pathways resulting in downstream activation of mitogen-activated protein kinase isoforms, i.e., extracellular signal-regulated kinases ERK1 and ERK2, phosphatidylinositol 3-kinase, and 70-kDa S6 kinase ($p70^{S6K}$) (2–5). Moreover, pancreatic β-cell proliferation induced by IGF-I or growth hormone is glucose dependent. Further characterization of the existing and possible alternative molecular pathways of nutrient- and growth factor–induced proliferation is of importance for the understanding of regeneration and neogenesis of pancreatic β-cells.

p8 was first described by Mallo et al. (6) as a gene product that is strongly upregulated in rat pancreas during acute experimentally induced pancreatitis. In addition, p8 seems to play a role in development because the highest mRNA expression in rats was observed in the fetal pancreas and liver, with a progressive postnatal decrease during the first 2 months to a remaining constantly low level. In healthy control animals, p8 mRNA expression was high in salivary glands; moderate in stomach, colon, liver, and kidneys; slight in lungs, heart, duodenum, jejunum, and ileum; and absent in brain, spleen, testes, thymus, and skeletal muscles. In the animal model used, pancreatitis-induced p8 expression within the pancreas was limited to acinar cells and absent in duct cells or islets. Rat AR42J cells, derived from a chemically induced rat pancreatic acinar cell tumor, and human HeLa cervix epithelial cells doubled their growth after transfection with p8, demonstrating its proliferation-inducing character (6,7). In addition, p8 also seems to play a role in cell growth arrest, apoptosis, and tumor development (8–12).

Because sequence analysis demonstrated a conserved nuclear localization motif in the COOH-terminal region, a putative helix-loop-helix motif, and slight homology with most homeotic genes, it was suggested that p8 may represent a DNA-binding protein and probably a transcription factor (6,13). This consideration is supported by the detection of p8 within the nucleus of COS-7 cells transfected with a p8 expression plasmid, although it was also partly located to the cytoplasm (7). Further biochemical and biophysical analysis revealed that p8, despite its only 35% amino acid sequence homology, features many structural aspects of high mobility group (HMG) proteins and that weak DNA binding of the native protein is strongly enhanced after phosphorylation by protein kinase A on serine/threonine residues (14). Because HMG protein binding to DNA is also regulated by post-translational modifications (15), p8 is considered to be an HMG-I/Y-like protein despite low sequence homology.

Although p8 is demonstrated to be present in a broad range of tissues, its expression within the pancreas in vivo is only described in exocrine acinar cells. To date, no p8 production by pancreatic β-cells is reported. Because we detected p8 transcripts in many pancreatic β-cell lines and in human pancreatic islets, we tested the hypothesis that p8 expression in β-cells is inducible by glucose and thus

From the Division of Metabolism, Endocrinology, and Molecular Medicine, Medizinische Poliklinik, University of Würzburg, Würzburg, Germany.

Address correspondence and reprint requests to Jochen Seufert, Division of Metabolism, Endocrinology, and Molecular Medicine, Medizinische Poliklinik, University of Würzburg, KlinikstraBe 6-8, 97070 Würzburg, Germany. E-mail: j.seufert@mail.uni-wuerzburg.de.

Received for publication 14 March 2003 and accepted 30 May 2003.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Les Laboratoires Servier.

HMG, high mobility group; MEF, mouse embryonic fibroblast; PKC, protein kinase C.

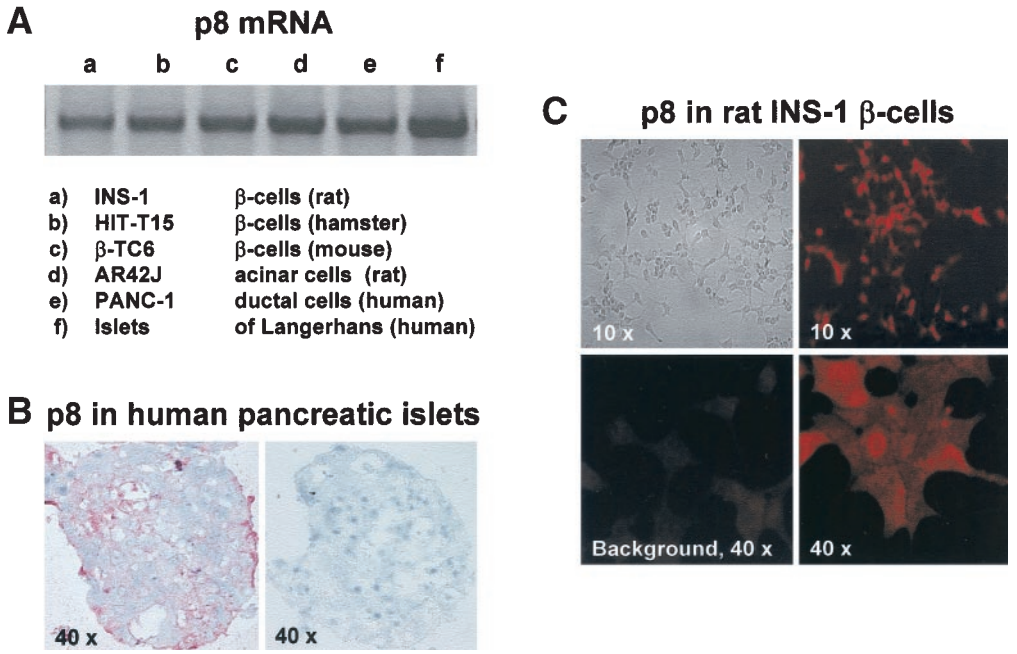

FIG. 1. p8 expression in human pancreatic islets and several pancreatic lines. Representative results of three independent experiments are demonstrated. *A*: Detection of p8 mRNA expression. *B*: Immunohistochemical detection of p8 protein expression revealed wide distribution in human pancreatic islets. Localization of p8 in the cytoplasm indicates absence of proliferation in cultured islets. *C*: Immunohistochemical staining of p8 protein expression in proliferating rat INS-1 β-cells demonstrates translocalization of p8 into the nucleus.

may be a novel mediator of glucose-induced pancreatic β-cell growth.

RESEARCH DESIGN AND METHODS

Cell culture. INS-1 cells as well human pancreatic islets were routinely maintained in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum, 10 mmol/l HEPES, 1 mmol/l sodium pyruvate, 2 mmol/l L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, and 50 μmol/l β-mercaptoethanol. The medium was further supplemented with 5.6 mmol/l (low), 11.1 mmol/l (standard), or 25 mmol/l (high) glucose. Cells were seeded in 60-mm dishes at a density of 2 million cells per dish. In the presence of 11.1 mmol/l glucose, the resulting cell layers were half confluent after ~3 days of culture. Standard medium was then exchanged with medium containing 25 mmol/l glucose to start induction of p8 mRNA expression. For analysis of glucose-induced p8 protein expression, cells were maintained for one passage in medium with 5.6 mmol/l glucose and then directly seeded in medium containing 25 mmol/l glucose.

Detection of proliferation. The amount of proliferating cells was analyzed using the 5-Bromo-2′-deoxy-uridine Labeling and Detection Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol.

Detection of p8 mRNA. RNA was isolated using Trizol and then reverse-transcribed into cDNA with Superscript II using oligo-(dT) primers according to the manufacturer's protocol (Life Technologies, Karlsruhe, Germany). PCR was performed using specific primers for p8. Resulting PCR products were size-fractionated in agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. Quantification was determined by densitometric scanning. (Oligo sequences and PCR conditions can be obtained from the authors on request.)

Detection of p8 protein. The primary antibody used for both immunohistochemistry and Western blotting was a polyclonal p8 antiserum generated in a rabbit with a bacterially expressed the p8 glutathione sulfonyl transferase fusion protein as antigen. Immunohistochemical staining of negative controls was performed without p8 antiserum. Western blotting was performed with whole cell extracts. Equal amounts of protein were size-fractionated on 10–20% Tris-Tricine Ready Gels (Bio-Rad, München, Germany). Visualization was achieved using Envision AP and Fast Red (both from DakoCytomation, Hamburg, Germany) or Cy3 fluorescently labeled goat anti-rabbit antiserum for immunohistochemistry and ECL Plus Western Blotting Detection Reagents (Amersham Biosciences Europe, Freiburg, Germany). After visualization, blotted p8 protein was quantified by densitometric scanning.

RESULTS

Expression of p8 in pancreatic islets and cell lines. p8 transcripts were detected in human pancreatic islets and several pancreatic cell lines including β-cells, acinar cells, and ductal cells as indicated in Fig. 1*A*. Immunohistochemical staining revealed a wide p8 expression in human islets (Fig. 1*B*), although other studies demonstrated p8 expression within the pancreas in vivo only in acinar cells (6,9). It is noteworthy that p8 is primarily located within the cytoplasm, which may indicate absence of cell proliferation in cultured islets. Immunohistochemical staining of proliferating rat INS-1 β-cells demonstrates translocalization of p8 into the nucleus (Fig. 1*C*). p8 protein expression in rat INS-1 β-cells was additionally detected by Western blot analysis (Fig. 3*B*).

Glucose-induced proliferation and p8 production in rat INS-1 β-cells. Effects of elevated glucose concentrations on proliferation and p8 production were established in glucose-sensitive rat INS-1 β-cells, a well-characterized in vitro model for analysis of β-cell function. In dishes with half-confluent cell layers, enhancement of glucose concentrations from 11.1 to 25 mmol/l continuously enhanced the amount of proliferating cells up to threefold (Fig. 2). This was paralleled by an increase in p8 mRNA expression reaching a maximum of threefold after 24 h (Fig. 3*A*). Elevation of glucose concentrations in the medium from 5.6 mmol/l (one passage) to 25 mmol/l resulted in a continuous rise in p8 protein expression up to 5.5-fold after 72 h (Fig. 3*B*).

DISCUSSION

The present study demonstrates that p8 is expressed in human islets and certain rodent pancreatic β-cell lines. Furthermore, proliferation and p8 expression in rat INS-1 β-cells is strongly inducible by high glucose concentrations in the medium. Our findings point to a possible new role of p8 as a mediator of glucose-induced pancreatic β-cell proliferation. This hypothesis is supported by studies demonstrating increased cell growth in p8 overexpressing rat AR42J pancreatic acinar cells (6) and human HeLa cervix epithelial cells (7). Moreover, glucose-induced pancreatic β-cell proliferation involves activation of PKC

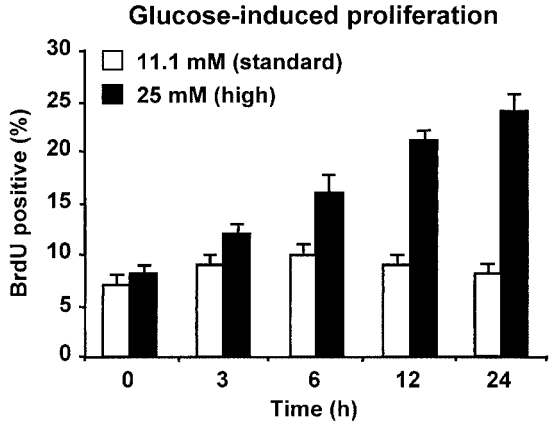

FIG. 2. Glucose-induced proliferation in rat INS-1 β-cells. INS-1 cells were stimulated by 25 mmol/l glucose for the indicated time intervals. Bars represent means ± SE of percentage of BrdU-positive cells at the indicated time points. Results are derived from three independent experiments.

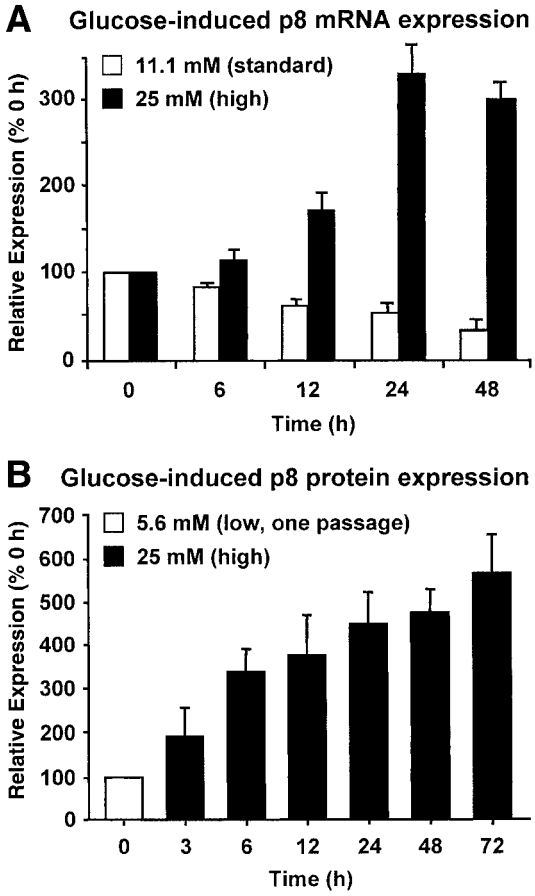

FIG. 3. Glucose-induced p8 expression in rat INS-1 β-cells. INS-1 cells were stimulated by 25 mmol/l glucose for the indicated time intervals. Bars represent means ± SE of percentage expression of time point 0 h. Results are derived from three independent experiments. *A*: p8 mRNA expression (RT-PCR). *B*: p8 protein expression (Western blot).

(2,3), and rat as well as human p8 amino acid sequences contain a highly conserved region with two PKC sites (16).

p8 was originally postulated as a pancreatic emergency gene product, which is exclusively upregulated in pancreatic acinar cells during stress caused by acute pancreatitis (6). The emergency gene character was underlined by the strong increase of pancreatic p8 expression in vivo and in rat AR42J acinar cells in vitro after endotoxin shock with lipopolysaccharide (17). Interestingly, former findings detect apoptosis during acute pancreatitis also only in acinar cells (18,19). Thus, Mallo et al. (6) tested several apoptosis-inducing conditions such as the addition of tumor necrosis factor-α, cycloheximide, dexamethasone, ceramide, or staurosporine or serum starvation with AR42J cells and observed upregulation of p8 mRNA expression. This observation led to the assumption that p8 expression is responsive to intracellular apoptotic signals.

Whereas p8-overexpressing HeLa cells displayed a twofold proliferation rate, tumor necrosis factor-α and cycloheximide alone or in combination did not alter survival (7). In contrast, p8-positive ($p8^{+/+}$) wild-type mouse embryonic fibroblasts (MEFs) grew less rapidly and were more sensitive to adriamycin-induced apoptosis than transgenic p8-deficient ($p8^{-/-}$) MEFs (10). Moreover, serum deprivation much more rapidly resulted in cell growth arrest in $p8^{+/+}$ than in $p8^{-/-}$ MEFs. The growth inhibitory action of p8 in $p8^{+/+}$ MEFs seems to involve p53, because both proteins were elevated in parallel and p53 was shown to suppress the p8 promoter in a dose-dependent manner. The finding that p8 mediates inhibition of cell growth in certain conditions is supported by increased 1,25-dihydroxyvitamin D_3–mediated suppression of colony formation by MCF-7 human breast cancer cells stably transfected with a p8 expression plasmid compared with p8-negative MCF-7 cells (12).

Besides the involvement of p8 in anti-tumorigenic processes such as apoptosis and growth arrest, several studies report a possible role in tumor development. p8 is highly expressed and negatively correlated to apoptosis in pancreatic cancers in vivo (8,9). Furthermore, p8 expression was found in MDF7/LCC2 cells, a highly tumorigenic subline of the low tumorigenic p8-negative MCF-7 cells (12), and p8 was demonstrated to be critical for tumor development in MEFs transfected with a ras^{V12} and E1A oncogene, because $p8^{+/+}$ MEFs were able to and $p8^{-/-}$ MEFs failed to form colonies (11). In contrast to its putative function as an oncogene, we observed a downregulation of glucose-induced cell growth and p8 expression in cultured INS-1 β-cells (data not shown), which may be a result of contact inhibition due to confluency. This absence of uncontrolled cell growth indicates that in pancreatic β-cells, p8 is regulated by intracellular signals and does not result in transformation leading to tumor development. Alternatively, it has to be considered that increased p8 expression in pancreatic cancer in vivo (8,9) is rather associated with proliferation but not with the development of tumors per se.

Taken together, these data show that p8 is involved in a broad range of biological functions, including cell growth, growth arrest, apoptosis, and tumor development. These in part opposite actions may be related to distinct stimuli and pathways in certain conditions and cell types. However, p8 is expressed in human pancreatic islets and pancreatic β-cell lines, and its expression is strongly upregulated in response to glucose, indicating that p8 may represent a physiological mediator of glucose-induced pancreatic β-cell growth. Binding of glucose-activated PKC to two PKC sites within a highly conserved region of

the p8 protein may be a possible mechanism linking glucose- and p8-dependent signaling pathways leading to proliferation.

ACKNOWLEDGMENTS

The study was supported by Bundesministerium für Bildung und Forschung (BMBF), grant FKZ 01GN0115.

Human pancreatic islets were kindly provided by R.G. Bretzel and M.D. Brendel from the Third Medical Department and Policlinic, University Hospital, Justus-Liebig University, Giessen, Germany.

REFERENCES

1. Bonner-Weir S, Deery D, Leahy JL, Weir GC: Compensatory growth of pancreatic beta-cells in adult rats after short-term glucose infusion. *Diabetes* 38:49–53, 1989
2. Cousin SP, Hugl SR, Myers MG Jr, White MF, Reifel-Miller A, Rhodes CJ: Stimulation of pancreatic beta-cell proliferation by growth hormone is glucose-dependent: signal transduction via janus kinase 2 (JAK2)/signal transducer and activator of transcription 5 (STAT5) with no crosstalk to insulin receptor substrate-mediated mitogenic signalling. *Biochem J* 344: 649–658, 1999
3. Hugl SR, White MF, Rhodes CJ: Insulin-like growth factor I (IGF-I)-stimulated pancreatic beta-cell growth is glucose-dependent: synergistic activation of insulin receptor substrate-mediated signal transduction pathways by glucose and IGF-I in INS-1 cells. *J Biol Chem* 273:17771–17779, 1998
4. Khoo S, Cobb MH: Activation of mitogen-activating protein kinase by glucose is not required for insulin secretion. *Proc Natl Acad Sci U S A* 94:5599–5604, 1997
5. Frodin M, Sekine N, Roche E, Filloux C, Prentki M, Wollheim CB, Van Obberghen E: Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting beta-cell line, INS-1. *J Biol Chem* 270:7882–7889, 1995
6. Mallo GV, Fiedler F, Calvo EL, Ortiz EM, Vasseur S, Keim V, Morisset J, Iovanna JL: Cloning and expression of the rat p8 cDNA, a new gene activated in pancreas during the acute phase of pancreatitis, pancreatic development, and regeneration, and which promotes cellular growth. *J Biol Chem* 272:32360–32369, 1997
7. Vasseur S, Vidal Mallo G, Fiedler F, Bodeker H, Canepa E, Moreno S, Iovanna JL: Cloning and expression of the human p8, a nuclear protein with mitogenic activity. *Eur J Biochem* 259:670–675, 1999
8. Su SB, Motoo Y, Iovanna JL, Berthezene P, Xie MJ, Mouri H, Ohtsubo K, Matsubara F, Sawabu N: Overexpression of p8 is inversely correlated with apoptosis in pancreatic cancer. *Clin Cancer Res* 7:1320–1324, 2001
9. Su SB, Motoo Y, Iovanna JL, Xie MJ, Mouri H, Ohtsubo K, Yamaguchi Y, Watanabe H, Okai T, Matsubara F, Sawabu N: Expression of p8 in human pancreatic cancer. *Clin Cancer Res* 7:309–313, 2001
10. Vasseur S, Hoffmeister A, Garcia-Montero A, Mallo GV, Feil R, Kuhbandner S, Dagorn JC, Iovanna JL: p8-deficient fibroblasts grow more rapidly and are more resistant to adriamycin-induced apoptosis. *Oncogene* 21:1685–1694, 2002
11. Vasseur S, Hoffmeister A, Garcia S, Bagnis C, Dagorn JC, Iovanna JL: p8 is critical for tumour development induced by rasV12 mutated protein and E1A oncogene. *EMBO Rep* 3:165–170, 2002
12. Bratland A, Risberg K, Maelandsmo GM, Gutzkow KB, Olsen OE, Moghaddam A, Wang MY, Hansen CM, Blomhoff HK, Berg JP, Fodstad O, Ree AH: Expression of a novel factor, com1, is regulated by 1,25-dihydroxyvitamin D3 in breast cancer cells. *Cancer Res* 60:5578–5583, 2000
13. Ree AH, Tvermyr M, Engebraaten O, Rooman M, Rosok O, Hovig E, Meza-Zepeda LA, Bruland OS, Fodstad O: Expression of a novel factor in human breast cancer cells with metastatic potential. *Cancer Res* 59:4675–4680, 1999
14. Encinar JA, Mallo GV, Mizyrycki C, Giono L, Gonzalez-Ros JM, Rico M, Canepa E, Moreno S, Neira JL, Iovanna JL: Human p8 is a HMG-I/Y-like protein with DNA binding activity enhanced by phosphorylation. *J Biol Chem* 276:2742–2751, 2001
15. Bianchi ME, Beltrame M: Upwardly mobile proteins. Workshop: the role of HMG proteins in chromatin structure, gene expression and neoplasia. *EMBO Rep* 1:109–114, 2000
16. Campagnoni CW, Landry CF, Pribyl TM, Schonmann V, Kampf K, Handley VW, Watson JB, Bongarzone ER, Campagnoni AT: Identification of genes in the oligodendrocyte lineage through the analysis of conditionally immortalized cell lines. *Dev Neurosci* 23:452–463, 2001
17. Jiang YF, Vaccaro MI, Fiedler F, Calvo EL, Iovanna JL: Lipopolysaccharides induce p8 mRNA expression in vivo and in vitro. *Biochem Biophys Res Commun* 260:686–690, 1999
18. Sandoval D, Gukovskaya A, Reavey P, Gukovsky S, Sisk A, Braquet P, Pandol SJ, Poucell-Hatton S: The role of neutrophils and platelet-activating factor in mediating experimental pancreatitis. *Gastroenterology* 111:1081–1091, 1996
19. Kaiser AM, Saluja AK, Sengupta A, Saluja M, Steer ML: Relationship between severity, necrosis, and apoptosis in five models of experimental acute pancreatitis. *Am J Physiol* 269:C1295–C1304, 1995