A Novel Gene Activated in Regenerating Islets*

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Kimio Terazono†, Hiroshi Yamamoto‡, Shin Takasawa§, Kiyoto Shiga§, Yutaka Yonemura¶, Yoshihiro Tochino¶, and Hiroshi Okamoto**

From the †Department of Biochemistry, Tohoku University School of Medicine, Sendai 980, Miyagi, Japan, the ‡Department of Surgery, Kanazawa University School of Medicine, Kanazawa 920, Ishikawa, Japan, and *Shionogi Research Laboratories, Shionogi & Co., Ltd., Osaka 553, Osaka, Japan

Administration of poly(ADP-ribose) synthetase inhibitors such as nicotinamide to 90% depancreatized rats induces regeneration of pancreatic islets, thereby ameliorating the surgical diabetes (Yonemura, Y., Takashima, T., Miwa, K., Miyazaki, L., Yamamoto, H., and Okamoto, H. (1984) Diabetes 33, 401–404). In screening the regenerating islet-derived cDNA library, we came across a novel gene encoding a 165-amino acid protein. The gene was expressed in regenerating islets but not in normal pancreatic islets, insulinomas, or regenerating liver. In 90% depancreatized and nicotinamide-injected rats, the expression of the gene was increased 1 month after the partial pancreatectomy and reached a peak 3 months after the operation. The increase in expression of the gene was temporally correlated with the increase in size of regenerating islets and the decrease in urinary glucose level. The gene was also found to be activated in hyperplastic islets of aurothioglucose-treated mice. Thus, the expression of the gene in both regenerating and hyperplastic islets suggests possible roles for this gene in replication, growth, and maturation of islet β-cells. We also found that a human pancreas-derived cDNA library contained a homologue to the gene.

Pancelial islets of Langerhans are the only organ of insulin production but have a limited capacity for regeneration (1), which predisposes to development of diabetes mellitus (2). Recently we demonstrated that administration of poly(ADP-ribose) synthetase inhibitors such as nicotinamide to 90% depancreatized rats induces regeneration of pancreatic islets, thereby preventing development of diabetes in the partially depancreatized rat (3–5). To elucidate the molecular mechanism of islet regeneration, we have looked for genes whose expression is altered in regenerating islets. We demonstrate

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† Present address: Dept. of Clinical Physiology, Setsunan University Faculty of Pharmaceutical Sciences, Hirakata 573-01, Osaka, Japan.

** To whom all correspondence should be addressed.

a novel gene that is activated in rat regenerating islets and the human homologue to the rat gene.

EXPERIMENTAL PROCEDURES

*Materials—Male Wistar rats weighing 180–200 g were 90% depancreatized (6), and beginning 7 days before the pancreatectomy and continuing postoperatively, nicotinamide at a dose of 0.5 g/kg of body weight was injected intraperitoneally every day (3). Regenerating islets were isolated by the collagenase digestion method (7) from remaining pancreases of 90% depancreatized and nicotinamide-injected rats. Insulinoma was induced by the combined administration of streptozotocin and nicotinamide (5). Regenerating liver was prepared as described by Higgins and Anderson (9). Male NON mice, a strain derived from ICR mice (10), received intraperitoneal injections of aurothioglucose (Sigma) at a dose of 0.25 g/kg of body weight on the 42nd and 56th days after birth. Six months after the aurothioglucose treatment, hyperplastic islets were isolated as described (7). Oligodeoxyribonucleotides were synthesized with an Applied Biosystems model 380B DNA synthesizer.

Construction of cDNA Libraries—RNA was extracted from rat regenerating islets and from human adult pancreas by the method of Chingwin et al. (11) using cesium trifluoroacetate. Poly(A)+ RNA was isolated by oligo(dT)-cellulose column chromatography (12). cDNA libraries were constructed according to the method of Wietgrefe et al. (13) using λgt10 and Escherichia coli Y1089.

DNA Sequencing—Cloned cDNA was cleaved with various restriction endonucleases (Fig. 1) and subcloned into M13 vectors mp10 and mp11 or a pBS vector (Stratagene, San Diego, CA). Nucleotide sequences of restriction fragments were determined by the dideoxy chain termination method (14–16).

Northern Blot Analysis—RNA from all tissues (Fig. 2) was isolated (11), electrophoresed on a 1.5% agarose gel, transferred onto a nitrocellulose filter (12), and hybridized to the antisense RNA which had been synthesized (18) from the PstI-DraI fragment (nucleotides –51 to 672) of the rat cloned cDNA (Fig. 3) in the presence of [α-32P]CTP (Amersham Corp.).

RESULTS AND DISCUSSION

Daily injection of nicotinamide to 90% depancreatized rats induces marked enlargement of islets of Langerhans in remaining pancreases, which is due to an increase in the number of insulin-producing β-cells (3–5). We isolated regenerating islets from remaining pancreases of 90% depancreatized rats, which had received nicotinamide for 3 months, and constructed a cDNA library of approximately 2.8 × 106 recombinants from the islet poly(A)+ RNA. From the cDNA library, ~2,000 recombinants were duplicated on nitrocellulose filters for differential screening (19). One set of filters was hybridized to 32P-labeled cDNA that had been reverse-transcribed from poly(A)+ RNA of regenerating islets. The other set of filters was hybridized to 32P-labeled cDNA synthesized from poly(A)+ RNA of normal islets. In the differential screening, we isolated a clone consistently hybridized preferentially to 32P-labeled cDNA from regenerating islets. The relative abundance of the cDNA clone in the regenerating islet-derived cDNA library was estimated to be 0.7%. We determined the nucleotide sequence of the cloned cDNA. As shown in Fig. 3, the cDNA was 748 nucleotides long plus poly(A)+ and had one large open reading frame encoding a 165-amino acid protein on the assumption that ATG at nucleotides 1–3 is the start codon and TGA at nucleotides 496–498 is the stop codon. At the amino terminus of the deduced 165-amino acid protein, there was a hydrophobic region similar to the signal sequence of many secretory proteins (20). The calculated molecular weight (18,656) of the deduced protein was in good agreement...
FIG. 1. Sequence strategy for cDNA corresponding to mRNA whose level was increased in rat regenerating islets and for the human homologue. Nucleotide numbers (see Fig. 3) are given at the top. Numbers of the nucleotide immediately on the 5' side of the restriction site are indicated in parentheses. The heavy line indicates the open reading frame. The open box indicates poly(A) tract. The complete overlapping sequences were determined using commercial M13 primer or synthetic primers (open circles). Arrows indicate the direction and the length of the region sequenced.

Fig. 2. Northern blot analysis of mRNA whose level was increased in regenerating islets. A: lanes 1 and 2, RNA from rat regenerating islets (4 and 2 µg, respectively); lanes 3 and 4, RNA from islets of normal untreated rats (4 and 2 µg, respectively); lanes 5–7, RNA from liver, kidney, and brain of normal rats (4 µg each); lane 8, RNA from rat insulinoma (4 µg); lane 9, RNA from rat regenerating liver (4 µg). B: lanes 1 and 2, RNA from hyperplastic islets of aurothioglucose-treated NON mice (4 and 2 µg, respectively); lane 3, RNA from islets of untreated ICR mice (2 µg). C: changes in mRNA level during islet regeneration in 90% depancreatized and nicotinamide-treated rats. RNA (lane 1, 2 µg; lane 2, 4 µg) from normal islets is shown. RNA from regenerating islets 1 month after partial pancreatectomy (lane 3, 2 µg; lane 4, 4 µg), 2 months after operation (lane 5, 2 µg; lane 6, 4 µg), 3 months after operation (lane 7, 2 µg; lane 8, 4 µg), and 1 year after operation (lane 9, 2 µg; lane 10, 4 µg) is shown. A trace amount of the 0.9-kilobase mRNA was noted in normal islets (lane 2); the mRNA amount was about 1% of that in regenerating islets 3 months after operation (lane 8), when the optical density of each band was measured with a Joyce-Loebl Chromoscan 3 densitometer. Bars indicate 28, 18, and 4 S RNAs on the same gel.

with that of the in vitro translation product of RNA which had been synthesized from the cloned cDNA (data not shown). The nucleotide and deduced amino acid sequences of the cDNA were screened for possible relationships to other genes and proteins stored in the nucleic acid and protein data banks of the European Molecular Biology Laboratory (Heidelberg, FRG), GenBank (Cambridge, MA), and the National Biomedical Research Foundation (Washington, D. C.) with a rapid similarity search algorithm (21). This screening indicated that there are neither genes nor gene products that are identical or statistically significantly homologous to the cDNA.

An antisense RNA was synthesized from the PstI-DraI fragment (nucleotides -51 to 672) of the cDNA (Fig. 3) and utilized to probe for levels of corresponding mRNA species in rat regenerating islets. As shown in Fig. 2A, the RNA probe hybridized to an mRNA of ~0.9 kilobase which was produced in large quantity in regenerating islets but not detected in normal islets, liver, kidney, brain, insulinoma, or regenerating liver. The level of the 0.9-kilobase mRNA was also increased in hyperplastic islets of aurothioglucose-treated NON mice (Fig. 2B). NON mice spontaneously display impaired glucose tolerance (10, 22). Aurothioglucose (23) administration to NON mice caused islet hyperplasia, thereby ameliorating the glucose intolerance, and immunohistochemical examination showed that the hyperplastic islets consisted predominantly of β-cells (data not shown). These results indicated that the
Regenerating Islet-specific Transcript

We isolated a human homologue to the rat cDNA; about 10^3 recombinants from a human pancreas-derived cDNA library were screened with a synthetic 60-base complement of nucleotide residues 76-135 of the rat cDNA (Fig. 3) as probe, yielding 70 hybridization-positive clones. The nucleotide sequence of the human clone with the longest cDNA insert was determined. As shown in Fig. 3, the human cDNA comprised 749 nucleotides plus poly(A) and had one large open reading frame encoding a 166-amino acid protein. Comparison of the nucleotide and deduced amino acid sequences between the rat and human cDNAs revealed a high degree of homology in the coding region (75 and 68% in nucleotide and amino acid sequences, respectively). There was also 75% homology in the 5'-untranslated region between the rat and human cDNAs. At the amino terminus of the deduced amino acid sequence of the human cDNA, there was a putative signal sequence which was one amino acid longer than that of the rat. The gene coding for the 0.9-kilobase mRNA was expressed specifically in regenerating or hyperplastic islets. We next examined changes in the level of the 0.9-kilobase mRNA during islet regeneration in 90% depancreatized and nicotinamide-administered rats. As shown in Fig. 2C, the mRNA level was significantly increased 1 month after the partial pancreatectomy, reached a peak 3 months after the operation, and then decreased to an almost undetectable level 1 year after the operation.

In this paper we have demonstrated the existence of a novel gene in rat regenerating islets and of the human homologue. Upper two lines show deduced amino acid and nucleotide sequences of cloned rat cDNA. Nucleotide residues in rat cDNA are numbered in the direction, beginning with the first residue of ATG triplet encoding initiator methionine; nucleotides on the 3' side of residue 1 are indicated by negative numbers. Amino acid residues are numbered beginning with Met. A high expression of the gene was found also in mouse brain. A high expression of the gene may be a general feature of pancreatic ß-cell regeneration. A pair of arginine residues at 108-109 was conserved in human sequences.

Fig. 3. Nucleotide and deduced amino acid sequences of cloned cDNA corresponding to mRNA whose level was increased in rat regenerating islets and of the human homologue. Upper two lines show deduced amino acid and nucleotide sequences of cloned rat cDNA. Nucleotide residues in rat cDNA are numbered in the 5' to 3' direction, beginning with the first residue of ATG triplet encoding initiator methionine; nucleotides on the 3' side of residue 1 are indicated by negative numbers. Amino acid residues are numbered beginning with initiator methionine in rat sequence. Nucleotide and amino acid differences found in human sequences are displayed beneath rat sequence. Putative signal sequence and polyadenylation signal are underlined.

[DNA sequence and amino acid sequence]
liver, suggesting that the expression of reg is highly specific to the normal replication of pancreatic β-cells but not related to malignant growth of β-cells nor regeneration of other tissues. In 90% depancreatized and nicotinamide-treated rats, the level of reg mRNA was higher 1 month after the partial pancreatectomy and reached a maximal level 3 months after the operation. The increase in reg expression was temporally correlated with the increase in size of regenerating islets and the decrease in urinary glucose level (3). Aurothioglucose administration to NON mice induced islet hyperplasia, ameliorating the glucose intolerance of the mice. Thus, the expression of reg in regenerating and hyperplastic islets suggests potential roles for this gene in normal replication, growth, and maturation of islet β-cells. In the present study, we also found that a human pancreas-derived cDNA library contained a reg homologue, which coded for a protein quite similar to that encoded by reg. Further studies of the expression and function of the protein encoded by the human homologue to reg may open a novel way for treatment of human diabetes.

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