Molecular and Cellular Endocrinology 229 (2005) 175–184

MCE Molecular and Cellular Endocrinology

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Hypoplasia of endocrine and exocrine pancreas in homozygous transgenic TGF-β1

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Received 1 April 2004; received in revised form 30 June 2004; accepted 11 August 2004

Abstract

We generated the homozygous transgenic mice with expression of the active form of TGF-β1 by the glucagon promoter (homozygous NOD-TGF-β1). The homozygous NOD-TGF-β1 showed severe diabetes in 84.6%, impaired glucose tolerance, and low serum insulin levels. The final size of endocrine and whole pancreas decreased, respectively, to 6 and 34%, compared to wild-type mice. The homozygous N_2 backcross to C57BL/6 (B6-TGF-β1) showed no diabetes, but impaired glucose tolerance and low serum insulin levels. In homozygous NOD-TGF-β1, the expression of $p15^{INK4b}$ was induced by 3.4-fold in pancreatic islets than that in wild-type mice. Based on these, we conclude first that excessive paracrine TGF-β1 signaling in islets results in endocrine and exocrine pancreatic hypoplasia, second that TGF-β1decrease the final size of endocrine and exocrine pancreas presumably through regulating cell cycle via $p15^{INK4b}$ at least in endocrine pancreas, and third that hypoplastic action of TGF-β1 of pancreatic islets is independent of the genetic background.

Keywords: TGF-β1 signaling; Hypoplasia; Endocrine pancreas; Exocrine pancreas; $p15^{INK4b}$; Glucagon promoter; Diabetes

1. Introduction

The optimal organ function requires a match between the organ size and physiologic demands of the host. Islet size, the number of islets, the relative ratio of islet β cells to other islet cells, and the balance between endocrine and exocrine cells must be at the right balance to maintain proper glucose homeostasis. TGF-β signaling components including TGF-β1, activin, their respective receptors, secretary factors including Notch, Hedgehog, FGF, and EGF likely regulate cell interaction necessary for proper histological development and functional maturation of the pancreas (Kim and Hebrok, 2001; Massague and Chen, 2000). TGF-β1, a multifunctional cytokine, regulates cell growth, differentiation courses in many systems, and other functions such as immune suppression (Massague, 1990; Roberts and Sporn, 1990).

The final endocrine islet size or exocrine pancreas in vivo is decreased by the decreased TGF-β signaling through defective type II TGF-β, type IIA or IIB activin receptors (Kim et al., 2000), or decreased receptor functions by transgenic expression of a dominant negative type I activin (Yamaoka et al.,

Abbreviations: NOD-TGF-β1, transgenic NOD mice expressing pTGF-β1 in islet α cells; B6-TGF-β1, backcrossed mice in N_2 generation which are homozygous for pTGF-β1 and generated by crossing NOD-TGF-β1 with C57BL/6 mice; ETCL, end trimming and cassette ligation; MoMuLV, Moloney murine leukemia virus; BG, blood glucose; ipGTT, intraperitoneal glucose tolerance test; HE, haematoxylin and eosin; Cdk, cyclin-dependent kinase

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doi:10.1016/j.mce.2004.08.007

1998) or type II TGF-β receptor (Bottinger et al., 1997). The final islet size or exocrine pancreas was also decreased by the increased TGF-β signaling through transgenic expression of a constitutively active type I activin receptor (Yamaoka et al., 1998). Thus, TGF-β signaling in vivo both in its excess and deficiency may determine the relative amount of endocrine and exocrine pancreas, and decrease islet size. But the mechanisms to control the relative ratios of endocrine and exocrine pancreas or the final islet size in vivo are not well understood.

Differentiation of pancreatic endocrine cells, especially islet β and PP cells, but not pancreatic exocrine cells were promoted by TGF-β1 in vitro in embryonic mouse pancreas (Sanvito et al., 1994). Transgenic TGF-β1 in vivo with the insulin promoter resulted in the accumulation of extracellular matrix (Lee et al., 1995), normoglycemia with unchanged β cell mass (Sanvito et al., 1995), or small islets to about 5% (Grewal et al., 2002), but the effect of excessive paracrine TGF-β1 with the glucagon promoter on the final size of the endocrine or exocrine pancreas remains unknown.

It was recently reported that increase in the final islet size might be determined by the cell cycle factors. Mice with a loss of cyclin-dependent kinase (Cdk) 4 expression develop diabetes associated with hypoplastic islets (Rane et al., 1999; Tsutsui et al., 1999), while Cdk 4 activation is associated with islet hyperplasia (Rane et al., 1999). These observations strongly suggest that the normal islet size requires regulated entry into, passage through, and exit from cell cycles. TGF-β1 induces the expression of $p15^{INK4b}$, a Cdk 4/6 inhibitor, causing G_1 arrest in many cell types including lung, thyroid, and mammary epithelial cells, astrocytes, and human keratinocytes (Massague et al., 2000; Hannon and Beach, 1994) by increasing mRNA levels or protein stability (Sandhu et al., 1997).

We generated homozygous transgenic mice with the expression of the active form of porcine TGF-β1 (pTGF-β1) under the glucagon promoter to determine the effects of excessive paracrine TGF-β1 in vivo first on the final size of endocrine α or β cells, second on the size of exocrine pancreas, and third on the expression of $p15^{INK4b}$ as the mediator of the hypoplastic action of TGF-β1 on endocrine pancreas.

2. Material and methods

2.1. Production of homozygous NOD-TGF-β1

Two lines of hemizygous NOD-TGF-β1 (Moritani et al., 1998) were intercrossed to generate homozygous NOD-TGF-β1. Hemizygous or homozygous transgene incorporation was quantitated with Southern blot analysis after PCR-based diagnosis.

2.2. Genetic backcross

To examine whether diabetes in homozygous NOD-TGF-β1 is limited only to the NOD genetic background, male hemizygous NOD-TGF-β1 were crossbred twice to female C57BL/6 mice. The resulting hemizygous N_2 mice were then intercrossed to obtain the homozygous N_2 backcrosses (B6-TGF-β1), which carry in average 75% genetic background of C57BL/6 mice.

2.3. Copy number assessment of the transgene with Southern blot analysis

Genome DNA was extracted from the tail at 4 weeks of age by phenol–chloroform method. Seven μg of genome DNA were digested with *EcoR*I and applied to 1% agarose gel, and transferred to a nylon membrane (Hybond-N; Amersham, Buckingamshire, England) by capillary transfer. The membrane was hybridized with the [^{32}P]-labeled 1300 bp pTGF-β1 cDNA probe obtained from a plasmid of pRB 601 (Moritani et al., 1998) with *Sal*I and *Not*I digestion. The hybridization was performed according to the published method (Sambrook et al., 1989). The copy number of the integrated transgene were semi-quantitated using the computer software of NIH image with the intensity of each radioactive band compared with the indication band of endogenous mouse TGF-β1.

2.4. Genetic diagnosis of transgene

Genotype of transgene in NOD-TGF-β1 or B6-TGF-β1 was determined by PCR. To discriminate the homozygous or hemizygous transgene with PCR, we determined the transgene's flanking sequence in the mouse genome with end trimming and cassette ligation (ETCL)-PCR method (Iwahana et al., 1994). Genomic DNA (0.5–1 μg) from hemizygous NOD-TGF-β1, NOD, or C57BL/6 mice was digested with three different groups (C1–C3) of restriction enzymes (Iwahana et al., 1994). The 3′-ends of DNA after digestion with restriction enzymes was end-trimmed by filling with one of four dNTPs using Klenow fragment. Three different cassettes were ligated with a DNA ligation kit (Takara, Kyoto, Japan). Ligated products were amplified with nested PCR. The amplified fragments were subcloned to TA vectors (Invitrogen Corp., Carlsbad, CA), and the DNA sequences were determined with the 3100 capillary sequencer (ABI, Foster City, CA). Genomic DNA from homozygous NOD-TGF-β1 was specifically amplified with a primer set of A1 + B2 (A1: 5′-ATGATATTGAATAGAGATAGAG-3′, and B2: 5′-GGTTTAGAGTTTGGCAACAT-3′), and genomic DNA from hemizygous NOD-TGF-β1 was amplified with both primer sets of A1 + A2 (A2: 5′-GCTCTTTGACAACCTCACAC-3′) and A1 + B2.

2.5. Detection of transgene and $p15^{INK4b}$ expression in islets

Mouse islets were isolated using collagenase (Moritani et al., 1996), and the total RNA was isolated with RNeasy 96 (QIAGEN GmbH, Hilden, Germany) and