Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Transcription factor AP-2β inhibits glucose-induced insulin secretion in cultured insulin-secreting cell-line

Shuichi Tsukada, Masa-aki Kobayashi, Shintaro Omori, Hiroyuki Unoki, Shiro Maeda *

Laboratory for Endocrinology and Metabolism, RIKEN Center for Genomic Medicine, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

ARTICLE INFO

Article history:
Received 3 March 2009
Received in revised form
12 June 2009
Accepted 15 June 2009
Published on line 10 July 2009

Keywords:
AP-2β
Insulin secretion
Type 2 diabetes

ABSTRACT

Aim: We previously identified the transcription factor activating enhancer-binding protein-2β (AP-2β) gene as a new candidate for conferring susceptibility to type 2 diabetes. To ascertain the possible involvement of AP-2β in the pathogenesis of type 2 diabetes we examined the effects of AP-2β on glucose-induced insulin secretion.
Methods: We measured the insulin secretion stimulated by glucose, tolbutamide, or KCl in the HIT-T15 cells infected with adenovirus vectors encoding AP-2β or LacZ (control).
Results: We identified clear expression of AP-2β in isolated rat pancreatic islets and in HIT-T15 cells. Glucose-induced increase in insulin secretion was significantly inhibited in AP-2β-overexpressing cells (LacZ, 5.0 ± 0.8 ng h^{-1} mg^{-1} protein; AP-2β, 1.7 ± 0.2 ng h^{-1} mg^{-1} protein; $P = 0.0015$), whereas insulin expression was the same in both types of cells. Tolbutamide-induced insulin secretion was also suppressed in the AP-2β-overexpressing cells, but KCl-induced insulin secretion was not affected by AP-2β overexpression. In addition, Kir6.2 and glucokinase expression was significantly decreased in the AP-2β-overexpressing cells.
Conclusion: We identified for the first time that AP-2β expressed and functioned in insulin-secreting cell-line HIT-T15. These results suggest that AP-2β contributes to susceptibility to type 2 diabetes by inhibiting glucose-induced insulin secretion in pancreatic β cells.

1. Introduction

Type 2 diabetes affects more than 240 million people worldwide [1]. Although the precise mechanism underlying the pathogenesis of type 2 diabetes remains unclear, this disease is thought to be caused due to insulin resistance in peripheral tissues together with dysfunction of β cells in the pancreatic islets [2,3].

We have identified the human AP-2β transcription factor gene (*TFAP2B*) located on chromosome 6p12 as a candidate susceptibility gene for type 2 diabetes [4]. Variations in the first intron of this gene have been shown to be significantly associated with type 2 diabetes in Japanese and British populations [4] and to directly affect the transcriptional activity of the gene [5]. The AP2 transcription factor family comprises 4 members – AP-2α, AP-2β, AP-2γ, and AP-2δ – each encoded by a separate gene. AP-2 proteins homo- and heterodimerize through a unique C-terminal helix–span–helix motif and bind palindromic DNA recognition sequences (consensus, 5′-GCCN3GGC-3′) through the basic domain that lies immediately at the N-terminal of the dimerization motif. The dimerization/DNA-binding region is highly conserved among the AP-2 isoforms. AP-2 factors appear to execute crucial, overlapping, and yet distinct functions during embryonic development and malignant transformation. In humans, mutation of AP-2β gene causes Char syndrome, a condition characterized by patent ductus arteriosus and variable degrees of facial dysmorphism and

* *Corresponding author*. Tel.: +81 45 503 9595; fax: +81 45 503 9567.
E-mail address: smaeda@src.riken.jp (S. Maeda).
0168-8227/$ – see front matter
doi:10.1016/j.diabres.2009.06.018

hand abnormalities [6]. However, any abnormality in the glucose metabolisms has not been reported in subjects with Char syndrome or in mice lacking *tfap2b*, and thus underlined mechanisms how AP-2β contributes to the pathogenesis of type 2 diabetes have not been elucidated yet.

AP-2β expression has been preferentially observed in human adipose tissues and found to be increased during adipocyte differentiation in mouse 3T3-L1 adipocytes [4]. Further, we found that AP-2β overexpression leads to lipid accumulation by enhancing glucose transport, thereby inducing insulin resistance in 3T3-L1 adipocytes [7]. AP-2β overexpression in 3T3-L1 adipocytes decreases the expression and secretion of adiponectin and increases those of interleukin-6 (IL-6) [8]. Therefore, AP-2β might play significant roles in regulating adipocyte functions; however, the functional roles of AP-2β in pancreatic β cells, which are another important type of cells associated with the pathogenesis of type 2 diabetes, have not been investigated thus far.

In this study, we identified for the first time that AP-2β expressed and functioned in insulin-secreting cell-line HIT-T15, and proved that AP-2β could inhibit glucose-induced insulin secretion.

2. Materials and methods

2.1. Materials

HIT-T15 cell-line, derived from hamster islet cells transformed with SV40 T-antigen [9], was obtained from Dainippon Pharmaceutical (Osaka, Japan). EX Taq HS DNA polymerase were purchased from Takara Bio Inc. (Otsu, Shiga, Japan), and SYBR Green I was purchased from Cambrian Chemicals Inc. (Oakville, Ontario, Canada).

2.2. Preparation of adenovirus vector encoding hamster AP-2β

Hamster AP-2β cDNA was generated by amplification of HIT-T15 cDNA, using the following primers: sense, 5′-CCT CGC AGG AAT GCA CTC ACC TCC T-3′; antisense, 5′-TCA TTT CCT GTG TTT CTC CTC CTT G-3′. The amplified products were separated on a 0.8% agarose gel, and the desired band was purified using the MinElute Gel Extraction Kit (QIAGEN, Valencia, CA). The purified hamster AP-2β cDNA was subcloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) and verified by direct sequencing to confirm that the obtained fragment corresponded to

Table 1 – Human, rat, and hamster gene specific primers and probes used in this study.

Gene		Sequence
Human AP-2β	Sense primer	5′-AACCTATTGGACCAGTCAGTCATTAA-3′
	Antisense primer	5′-AAAATACCTCGCCGGTGTTG-3′
	TaqMan probe	5′(FAM)-CCCTCCCAAATCTGTGACTTCTCTAATGATGA-(TAMRA)3′
Rat and HIT-T15 AP-2β	Sense primer	5′-GCTCTGGAAACTCGTGGAGAA-3′
	Antisense primer	5′-CAGAGCCCAGCTGAGAGAGTCT-3′
	TaqMan probe	5′(FAM)-CACGATGGCGTCCCAAGCCATAG-(TAMRA)3′
HIT-T15 β-actin	Sense primer	5′-CGTGCGTGACATTAAAGAGAA-3′
	Antisense primer	5′-TGGATGCCACAGGATTCCAT-3′
	TaqMan probe	5′(FAM)-CCACTGCCGCATCCTCTTCCTCC-(TAMRA)3′
HIT-T15 Insulin	Sense primer	5′-AGAAGCCATCAGCAAGCAGG-3′
	Antisense primer	5′-AGAGTGCCTCCACAAGGTGG-3′
HIT-T15 GLUT2	Sense primer	5′-TCTGCTTCCAGTACATTGCGGACT-3′
	Antisense primer	5′-CTGTACAAATGGAATTCCTGG-3′
HIT-T15 SUR1	Sense primer	5′-TGTCATCATTCTGCTGGCTCCTGT-3′
	Antisense primer	5′-TTTCGTTCCTGCGTGTCTTCTCCA-3′
HIT-T15 Kir6.2	Sense primer	5′-AAGGCCCGCACCTCCTATCT-3′
	Antisense primer	5′-TGGAGTAGTCCACAGAATAG-3′
HIT-T15 Glucokinase	Sense primer	5′-CATCACTGTGGGCGTGGAT-3′
	Antisense primer	5′-TGATTTCGCAGTTGGGTGTC-3′
HIT-T15 HNF-4α	Sense primer	5′-CTGGCAGATGATCGAGCA-3′
	Antisense primer	5′-GTCACTGGCAGACCCTCCAA-3′
HIT-T15 HNF-1α	Sense primer	5′-ACACCTGGTACGTCCGCAAG-3′
	Antisense primer	5′-CGTGGGTGAATTGCTGAGC-3′
HIT-T15 IPF-1	Sense primer	5′-TGAAATCCACCAAAGCTCACG-3′
	Antisense primer	5′-CCGAGTGTAGGCTGTACGGG-3′
HIT-T15 HNF-1β	Sense primer	5′-CAAGCTCCTCTCCACCCAAC-3′
	Antisense primer	5′-GACTGGCTGGTCACCATGG-3′
HIT-T15 NeuroD	Sense primer	5′-TTCGATAGCCATTCGCATCA-3′
	Antisense primer	5′-CGGGAATGGTGAAACTGACG-3′