Establishment of a Tet-On Gene Expression System in Glucose-Responsive and -Unresponsive MIN6 Cells

Yasushige Kashima, Takashi Miki, Kohtaro Minami, and Susumu Seino

The tetracycline (or doxycycline)-inducible gene expression (Tet-On) system developed by Gossen and Bujard (1) is useful for controlling the expression of targeted genes and for determining the roles of the gene products in cellular functions. The Tet-On system has recently been applied to the rat insulinoma cell line INS-1 to study the roles of wild-type and mutant genes in insulin secretion (2,3). We have recently established two cell lines, MIN6-m9, and MIN6-m14, derived from the mouse insulinoma-derived cell line MIN6. MIN6-m9 and MIN6-m14 cells are glucose-responsive and glucose-unresponsive, respectively. To study the roles of newly identified genes in pancreatic β-cell functions, we have established a Tet-On system in these MIN6 sublines.

RESEARCH DESIGN AND METHODS

To establish stable cell lines that express the reverse tetracycline/doxycycline-responsive transcriptional activator (rtTA) and are capable of accessing transcription, MIN6-m9 cells and MIN6-m14 cells were transfected by electroporation with 40 μg pTet-On encoding rtTA. After selection with 400 μg/ml G418 for 28 days, resistant clones were selected by polymerase chain reaction (PCR) of genomic DNA and were tested for expression of rtTA and luciferase activity. Insulin-secretory responses to glucose and glibenclamide in MIN6-m9 Tet-On and MIN6-m14 Tet-On cells were determined in batch incubation experiments.

RESULTS

Of the five G418-resistant MIN6-m9 Tet-On cell lines selected by the PCR method, one subline, m9Tet-On 35, showed the highest increment (126-fold) in luciferase activity by transient transfection of pTRE-Luc, a reporter plasmid with the luciferase gene (maximal induction with 2 μg doxycycline). Of the 24 G418-resistant MIN6-m14 Tet-On cell lines selected, one subline, m14Tet-On 33, showed the highest increment (133-fold) in luciferase activity. Glucose-stimulated insulin secretion in m9Tet-On 35 occurred in a dose-dependent manner. Glibenclamide (100 nmol/l) also stimulated insulin secretion in m9Tet-On 35 cells. By contrast, there was almost no insulin response to either glucose or glibenclamide in m14Tet-On 33 cells. Thus, the features of m9Tet-On 35 cells and m14Tet-On 33 cells are similar to those of their parental glucose-responsive and -unresponsive MIN6 cells, respectively.

DISCUSSION

In the study of the role of ATP-dependent K^+ (K_{ATP}) channels in pancreatic β-cell function at the cell level, m9Tet-On cells were secondarily transfected with the expression plasmid encoding a mouse Kir6.2 dominant-negative mutant (mKir6.2G132S). m9Tet-On cells expressing mKir6.2G132S are currently under selection with hygromycin. We will investigate the roles of the mutant K_{ATP} channel in cellular functions, including cell proliferation and death.

REFERENCES

1. Gossen M, Bujard H: Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci U S A* 89:5547–5551, 1992
2. Wang H, Iynedjian PB: Modulation of glucose responsiveness of insulinoma β-cells by graded overexpression of glucokinase. *Proc Natl Acad Sci U S A* 99:4372–4377, 1997
3. Wang H, Maechler P, Hagenfeldt KA, Wollheim CB: Dominant-negative suppression of HNF-1α function results in defective insulin gene transcription and impaired metabolism-secretion coupling in a pancreatic β-cell line. *EMBO J* 17:6701–6713, 1998

From the Department of Molecular Medicine, Chiba University Graduate School of Medicine, Chiba, Japan.

Address correspondence and reprint requests to Susumu Seino, Department of Molecular Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. E-mail: seino@molmed.m.chiba-u.ac.jp.

Received for publication 11 May 2000 and accepted 19 June 2000.

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.

K_{ATP}, ATP-dependent K^+ channel; PCR, polymerase chain reaction; rtTA, reverse tetracycline/doxycycline-responsive transcriptional activator.