Identification of Genes Involved in Glucose-Stimulated Insulin Secretion

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Type 1 and type 2 diabetes are widespread diseases, and the numbers of afflicted individuals are increasing. It is now well recognized that it is very difficult to control blood glucose adequately by insulin injection therapy. Our laboratory has been investigating the potential utility of genetically engineered cell lines for insulin replacement therapy. One aspect of this initiative is to define all of the genes that control glucose-stimulated insulin secretion (GSIS). Although a variety of rodent insulinoma cell lines have been described, these lines are often not suitable for genetic studies because they exhibit either very small or left-shifted glucose responses relative to normal islets. Other lines exhibit appropriate GSIS, but this property vanishes over time in culture. We have recently begun work involving INS-1–derived clones. INS-1 cells respond to glucose at a concentration threshold of 4–5 mmol/l, although with maximal responses of 2- to 3-fold compared with the 15- to 20-fold increase in insulin secretion seen in perfused pancreas or freshly isolated islets. We stably transfected INS-1 cells with the human insulin gene under control of the cytomegalovirus promoter, resulting in isolation of new clonal cell lines with poor, intermediate, and strong responses to glucose. The strongly responsive clones have a 10- to 15-fold elevation in insulin secretion when the glucose level in the medium is raised from 3 to 15 mmol/l. One of the strongly responsive clones, 832/13, has been studied intensively, and the phenotype has been stable for more than a year. We have recently embarked on a genetic screen of strongly responsive and poorly responsive INS-1–derived clones to identify genes involved in GSIS. Two methods are being used: candidate gene analysis using multiplex polymerase chain reaction (PCR) and representational difference analysis, a PCR-based subtractive-cloning strategy. Using multiplex PCR we have been able to subdivide the poorly responsive clones into two subsets, one expressing glucagon. These two subsets are also clearly distinguished in appearance. The expression of glucagon in some of the poorly responsive clones suggests that this subset has dedifferentiated from the mature β-cell to an earlier stage, a process likely accompanied by loss of strong GSIS. By comparing gene expression in strongly and poorly responsive clones, we hope to identify genes involved in regulating or mediating GSIS. Such identification could lead to better understanding of type 2 diabetes and be a useful tool in generating insulin-secreting cell lines. Furthermore, we could potentially identify genes involved in the process of dedifferentiation. If so, suppression of such genes by knockout or antisense strategies could help to maintain desired phenotypes in cell lines for use in diabetes therapy.

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