

TITLE

**Novel Method for
Detection of Diabetes
by Methylation-
Specific Polymerase
Chain Reaction**

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Methylation of DNA is a mechanism by which cells control gene expression, and cell-specific genes often exhibit unique patterns of DNA methylation. We recently investigated the methylation pattern of the mouse insulin-2 gene (INS2) and found that there are 14 potential methylation (CpG) sites, all of which are unmethylated in insulin-producing cells but methylated in other tissues. Based on the hypothesis that damaged beta cells release their DNA into circulation, we aim to develop a sensitive and quantitative assay for circulating beta cell DNA for the detection of diabetes by methylation-specific polymerase chain reaction (MSP).

Methylation-specific primers were designed based on the methylation of particular CpG sites in the gene using an approach to interrogate three or more CpG sites in the same assay. The cloned INS2 gene was unmethylated or methylated in vitro, representing insulin-producing and non-insulin-producing cells respectively, and then bisulfite-treated and used in qPCR. We found that the production of PCR products depended on matching the 3'-terminus of the primers with unmethylated bisulfite-treated template, while mismatches produced no product. The MSP method is sensitive and specific enough to differentiate between insulin-producing cells and other tissues as the MSP can detect as little as 2 copies per reaction and can detect 10 copies in the presence of genomic DNA background.

These results suggest that this MSP assay can be used for quantification of circulating DNA from insulin-producing cells, which will provide the basis for developing assays to detect beta cell destruction in early diagnosis of type-1 diabetes mellitus.

Biography

Mohamed I. Husseiny El Sayed has completed his Ph.D at the age of 35 years from Friedrich-Alexander University, Erlangen-Nürnberg, Germany and postdoctoral studies from LA Biomed at Harbor-UCLA Medical Center, California, USA and Childrens Hospital Los Angeles, USC School of Medicine, California, USA. He is a postdoctoral fellow at the City of Hope, California, USA. He has published 14 papers in several reputed journals and serving as a reviewer for several peer-reviewed journals.