Novel Pathways for Stimulation of Adult Islet β-cell Proliferation with Retention of Function

Hans E. Hohmeier, Jonathan C. Schisler, Patrick T. Fueger and Christopher B. Newgard

Sarah W. Stedman Nutrition and Metabolism Center
Departments of Pharmacology and Cancer Biology, Medicine, and Biochemistry
Duke University Medical Center, Durham, NC 27704

Type 1 and type 2 diabetes are characterized by a loss of pancreatic islet β-cell mass and function. Therapeutic strategies for cell-based insulin replacement will therefore require methods to expand βcell mass while retaining a well differentiated β-cell phenotype. Microarray and genetic engineering studies in our laboratory have led to the identification of several genes that control the function and growth of adult β-cells. Two examples of such genes are the homeodomain transcription factor Nkx6.1 and trefoil factor 3 (TFF3). Adenovirus mediated overexpression of Nkx6.1 in adult rat islets causes a robust increase in islet cell proliferation as measured by ^{3}H-methyl-thymidine and 5-bromo-2'-deoxyuridine (BrdU) incorporation, accompanied by an approximate 30% increase in islet cell numbers over a period of 4 days in culture. Immunolocalization studies reveal that >80% of BrdU positive cells in AdCMV-Nkx6.1 treated islets are positive for insulin. This increase in islet β-cells is associated with a clear enhancement of glucose-stimulated insulin secretion (GSIS). Microarray, real-time PCR, and immunoblot analysis reveal that overexpression of Nkx6.1 in rat islets causes concerted up-regulation of a cadre of cell cycle control genes, including cyclins A, B, and E, and several regulatory kinases. Moreover, chromatin immunoprecipitation assays demonstrate direct interaction of Nkx6.1 with the cyclins A2 and B1 genes. In human islets, the overexpression of Nkx6.1 caused a doubling in ^{3}H-methyl-thymidine incorporation with retention of GSIS.

Similar to the effects of Nkx6.1, overexpression of TFF3 in adult rat islets causes a 5-fold increase in ^{3}H-methyl-thymidine and 5-bromo-2'-deoxyuridine (BrdU) incorporation, which is again predominantly due to replication of insulin positive cells. The ability of TFF3 to stimulate proliferation requires the presence of serum or epidermal growth factor and can be blocked by inhibiting the Akt pathway by overexpression of dominant negative Akt protein or an Akt inhibitor. TFF3 overexpression enhances β-cell replication with no impairment in GSIS.

We conclude that Nkx6.1 and TFF3 are capable of stimulating β-cell replication by two distinct pathways, while retaining or enhancing β-cell function. These properties may be exploitable for the expansion of β-cell mass in treatment of both major forms of diabetes.