Making More And Better Insulin-Producing Cells With Cell Regeneration

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This DRC sponsored project is aimed to develop a model of expanding functional beta cell mass for replenishment therapy. We have identified a key molecular regulator of islet cell proliferation, Cdc7 (cell division cycle 7), which marks rapidly expanding, functionally immature beta cells in the fetal life and is absent in functionally mature, non-proliferating beta cells after birth. Cdc7 is a critical replication initiation factor and is only expressed in replicating cells. We therefore proposed to transiently re-express this regulator in beta-cells to drive a rapid burst of proliferation, followed by withdrawing this factor to stop proliferation and restore beta cell function.

To achieve the transient expression of Cdc7 in cells, we generated a mammalian conditional expression construct contaning full length human *Cdc7* cDNA (tagged on its amino-terminus with a myc epitope) flanked by a multimerized Tetracycline Responsive Element (TRE) in a synthetic promoter (TRE-mycCdc7). The construct was validated in the HeLa Tet-On cell line, which constitutively expresses the reverse tetracycline TransActivator (rtTA), such that the expression of Cdc7 can be switched on or off by adding or withdrawing doxycycline (Dox). Next, we used the TRE-mycCdc7 construct along with adenoviral rtTA, in cultured mouse beta cell line Min6, as well as cultured mouse islets and examined islet proliferation. Cdc7 expression induced by Dox treatment in beta-cells resulted in increased proliferation, accompanied by increased expression of metabolic genes characteristic of fast replicating, neonatal beta-cells. This was accompanied by moderate impairment of glucose stimulated insulin secretion. Removal of Dox to stop Cdc7 expression, followed by culture in Dox free medium for 4-5 days led to restoration of glucose stimulated insulin secretion. Thus, transient Cdc7 expression allows for beta cell proliferation and expansion *ex vivo*, while stopping the Cdc7 expression by Dox withdrawl drives re-establishment of function.

Beta-cells acquire glucose stimulated insulin secretion in postnatal life, concurrent with reduced proliferation and quiescence, a process called beta-cell maturation. Therefore, we carried out comparative gene expression analysis of islets from neonatal and adult mice to identify candidates that can be harnessed to promote islet function. We have identified several other transcription regulators and signaling molecules that are expressed transiently during beta-cell maturation process.

Future studies will focus on understanding how Cdc7 regulates islet growth and function, and on further developing our beta cell expansion model. This will be a big step towards expanding adult functional insulin-producing cell mass for replacement therapies, and will also provide a way to develop methods for expansion of these cells inside the body. This project will ultimately lead to development of protocols for deriving functional beta cells in abundant quantities for Type 1 diabetes therapy.