CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
Sanjana Marpadga

Project Number
S0418

Project Title
Beclin I: A Novel Marker to Evaluate Human Islet Quality

Abstract

Objectives/Goals
The objective of the current study is to develop novel assays based on autophagic and apoptotic cell death markers to evaluate the function of transplanted islets in Type 1 Diabetic (T1D) mouse models.

Methods/Materials
Islet cell death from apoptosis and autophagy was determined by using DNA fragmentation (TUNEL Assay) and Beclin I staining, respectively. Insulin producing beta-cells were detected by staining with insulin specific antibodies. Briefly, human islet sections on glass slides were incubated with primary antibodies followed by appropriate secondary antibodies conjugated to fluorescent dyes. Fluorescence intensity was quantified by using a fluorescence microscope equipped with an iCys laser scanning cytometer (LSC). Islet function was evaluated by monitoring blood glucose levels in streptozotocin induced T1D mice models up to one month post islet transplantation.
Protein levels in cell lysates were determined by immunoblotting with antibodies specific to cell death markers and proteins on blots visualized using chemiluminescence methods. Statistical analysis was performed using Microsoft Excel software.

Results
Treatment of human islets with various stress conditions, such as hypoxia, oxidant stress (hydrogen peroxide) and inflammatory cytokines, ƒnincreased levels of proteins involved in both apoptosis (phospho-H2AX, activated caspases) and autophagy (Beclin 1). However, immunostaining of islets showed only an increase in TUNEL and Beclin I positive staining in insulin producing beta-cells, suggesting only the TUNEL assay and Beclin I are useful biomarkers to detect islet cell death. Screening of human islets from different batches displayed varying levels of both Beclin I and TUNEL staining. Furthermore, Beclin 1 and TUNEL positive staining significantly correlated with islet dysfunction, i.e., elevated blood glucose levels in islet transplanted T1D mice.

Conclusions/Discussion
These results show that both autophagy and apoptosis play an important role in islet cell damage during isolation. The data with T1D mice clearly demonstrated that islet damage from autophagy or apoptosis during isolation can reduce the ability of beta-cells to produce sufficient insulin to reduce blood glucose levels. Therefore, Beclin 1 can be used effectively in parallel with the apoptosis specific TUNEL assay for the evaluation of islet quality prior to transplantation.

Summary Statement
Beclin I was identified as a novel biomarker to evaluate islet quality prior to transplantation into Type 1 Diabetic patients.

Help Received
Performed research at Department of Diabetes, Beckman Research Institute of City of Hope, Duarte, CA, under the guidance of Dr. Ivan Todorov.