CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

S0524

Project Title

Effects of Diabetes Mellitus on Vasculogenesis Capacities of Mesenchymal Stem Cells

Abstract

Objectives/Goals

Underlying severe complications of diabetes mellitus (DM) is impaired neovasculogenesis. Vasculogenesis (wound healing) involves recruitment of progenitor cell populations, including mesenchymal stem cells (MSCs), to assemble neovessels. The goal of the project is to elucidate the biological mechanisms of diabetic MSC dysfunction within the context of vasculogenesis. Functional aspects of MSCs in vasculogenesis were identified: (1) robust proliferation rates and (2) adequate stem-like capacity. The hypothesis was that both proliferation and stemness were compromised by DM.

Methods/Materials

Bone marrow MSCs were harvested from wild type and db/db (Type II diabetic mice), subcultured, and grown in high- and low-glucose conditions to assess cell type and culture condition as testing variables. Robust proliferation rates were evaluated using immunostaining for presence of ki67, a nuclear protein whose expression is elevated during mitosis. The TUNEL stain was conducted to evaluate apoptosis as a potential mechanism to explain proliferation trends.

MSC stemness was assessed using western blotting for protein encoded by the embryonic stem cell marker KLF4, an indicator of stem-like capacity. Microfluidics-based quantitative RT-PCR will be used to corroborate results of immunoblotting.

Results

Results indicated that diabetic MSC proliferation is reduced compared to a wild type standard in low- (-41.8%, $p<0.009$) and high-glucose conditions (-29.6%, $p<10^{-6}$). Data also indicated that apoptosis is likely not responsible for decreased diabetic MSC proliferation: diabetic apoptosis was actually decreased in low- (N.S.) and high-glucose conditions (-72.8%, $p<10^{-8}$).

With reference to assessment of MSC stemness, blots consistently indicated that KLF4 expression (and therefore stem-like capacity) was compromised in diabetic MSCs in high- and low-glucose conditions. Results for qPCR procedures are expected to validate this observation and will be discussed.

Conclusions/Discussion

The major conclusion of this study, that diabetic MSCs exhibit lower rates of proliferation and lower capacity for differentiation, is an important step in further understanding the physiological mechanisms of MSC derangement in diabetics. Results also corroborate a proposal for a Reactive Oxygen Species (ROS)-mediated mechanism for diabetic MSC dysfunction. Further research could entail validating this model.

Summary Statement

The project indentifies unprecedented physiological implications of diabetes mellitus, particularly with reference to MSC derangement in vasculogenesis.

Help Received

Used lab equipment at Stanford University under the supervision of Dr. Jason Glotzbach.