Mitochondrial Antioxidant Manganese Superoxide Dismutase (MnSOD) Up-regulated Human Mesenchymal Stem Cells (MSCs) Reduce Inflammation in High Glucose (HG) Exposed Adipocytes

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Introduction

- In humans MSCs differentiate into mature adipocytes, osteocytes or chondrocytes. We examined how HG influences MSC differentiation, particularly Adipogenic differentiation which was confirmed by Oil Red O stain. We postulated that reactive oxygen species (ROS) production secondary to HG exposure may be the cause of increased adipogenesis in MSCs. Thus, intra-cellular antioxidants could counteract the adverse differentiation potential of HG.

Methods

We exposed hMSCs to normal glucose-NG, 5.5mM and HG, 25mM for 10 days. Using FACS, ROS generation was analyzed using DCF-DA dye and mitochondrial function by Mitosox Red Dye.

We also transduced hMSCs with Adenovirus containing SOD2(also known as MnSOD) or Catalase (CAT) at 100 MOI before exposing to HG. Ad-CMV-Null was the control vector. We interrogated mitochondrial respiration (Seahorse) and complexes 1 and 2 protein by BN-PAGE and SDS-PAGE.

Results

- DCFDA Dye detected ROS presence in Cytosol in Mesenchymal Cells in High Glucose using FACS Analyzer (not shown).

- Mitosox Red was used to detect ROS

- Shows increased Mitochondrial swelling, possibly secondary to ROS in Hi Glucose state (Red vs Green) at Day 7

- There is increased inflammation (TNF, IL6) adipogenesis (PPARG, CREBP) and decreased osteogenesis (ALPL) in the MSCs in HG

- Anti-oxidant Gene Up-regulation particularly MnSOD appears To Reduce MSC inflammation and increase Adipocyte marker genes

- SEAHORSE: MSCs Seeded In 25mM glucose compared to 5.5mM Glucose showed reduced respiration

Summary

Presence of HG appears to promote ROS accumulation, inflammation, increased fat formation and leads to mitochondrial Complex-1 dysfunction.

MnSOD helps to reduce HG induced inflammation, fat accumulation and improves oxygen consumption rate (OCR). CAT or SOD1 or SOD3 up-regulation does not appear to have these effects in human MSCs.

We are studying effect of MnSOD up-regulated MSC delivery in obese diabetic mouse models.

Conclusion

Our findings emphasize the role of intracellular mitochondrial anti-oxidant up-regulation, in preventing HG associated ROS accumulation, super-oxide mediated inflammation, fat formation and poor cellular respiration with therapeutic implications in diabetes and obesity.