Diabetic Complications Consortium

Application Title:
A Pilot Study to Develop New Therapy Approach for Diabetic Foot Ulcers

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1. Project Accomplishments:

We developed proof-of-concept for one-step reprogramming of adipose progenitor cells (ASCs) derived from either old or diabetic individuals to improve their healing capacity. Specifically, when catalase expression is increased in either ASCs obtained from diabetic or old individuals their capacity to promote wound healing is restored to the level comparable to that of ASCs obtained from young individuals. The results are published in Molecular Therapy and the data generated served to support R01 application R01DK129039.

2. Specific Aims:

Specific aim 1: Determine whether catalase replete allogenic diabetic ASCs achieve reprogramming of the delayed healing.

Results: We demonstrated that topical application of ASCs derived from young persons, Y-ASC, (<40 y.o.) promoted wound healing, whereas derived from old persons, O-ASCs, (>60 y.o.) did not, when compared to untreated controls. When we used catalase activator (CRISPR Activation plasmid) plasmid to transfect O-ASC it resulted in increase of catalase activity. When tested in ex vivo wound healing assay O-ASC-CAT+ cells promoted wound healing similar to Y-ASCs, confirming that repletion of catalase restores healing capacity in O-ASCs. Conversely, when we performed knockdown experiments using CRISPR derived plasmids to inhibit catalase expression in Y-ASC it resulted in 50% decreased catalase expression and activity. Y-ASC-CATKO show reduced wound healing capacity that is similar to O-ASC, confirming that Y-ASCs acquired the non-healing phenotype of the O-ASCs after knockout of catalase. Next, we isolated ASCs from diabetic patients and performed similar experiments. We found that ASCs

derived from diabetic patients, D-ASC, show decreased healing capacity, similar to O-ASCs. When transfected with catalase D-ASC-CAT+ promoted wound healing similar to Y-ASCs.

Specific aim 2. Evaluate therapeutic potential of exosomes derived from catalase replete ASCs.

Results: We evaluated the therapeutic potential of ASC-derived exosomes on wound healing. We have successfully isolated exosomes from ASCs; exosome size from cells was 30-150nm confirmed by Nanosight. Electron microscopy was performed on isolated exosomes and CD63, an exosome marker, was confirmed by Western analysis. First, we compared if ASC-derived exosomes promote healing compared to whole cell ASCs which they originate from. Indeed, ASC-exosomes have similar efficacy as whole cell ASCs in healing wounds. Next, we isolated exosomes from O-ASC-CAT+ cells and confirmed that they promote healing similar to O-ASC-CAT+, suggesting that exosomes from re-programmed old ASCs can also be utilized to promote would healing.

Taken together, we have shown that reversal of oxidant stress in diabetic and/or old ASCs by the repletion of catalase recovers their healing properties and promotes wound healing. Such properties are also extended to exosomes derived from these cells. This one-step reprogramming opens up many possibilities and expands potential use of ASCs.

3. Publications:

Sharon J. Elliot1, Paola Catanuto, Simone Pereira-Simon, Xiaomei Xia, Irena Pastar, Rivka Stone, Seth Thaller, Cheyanne R. Head, Olivera Stojadinovic, Marjana Tomic-Canic and Marilyn K Glassberg: Catalase, a therapeutic target in the reversal of estrogen-mediated aging. (2021) Molecular Therapy, accepted for publication