Inducing Resistance to Senescence in Adult Stem Cells Using Transcriptional Reprogramming

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INTRODUCTION:
In this study our aim was to develop an understanding of how partial reprogramming can most effectively be induced in adult stem cells to renovate the potency, proliferative capacity, and regenerative utility of aged adult stem cells. Stem or progenitor cells are highly promising candidate cells for regenerative medicine applications because they possess a high proliferative capacity and the potential to differentiate into other cell types. Mesenchymal stem cells (MSCs) in particular exhibit several properties that make them an excellent cell source for regenerative medicine strategies. They can be easily harvested from a patient’s own tissues, eliminating the need for immunosuppressive therapy. They possess innate immunomodulatory properties, home to sites of injury or inflammation, and secrete bioactive factors and signaling molecules. They are currently in clinical use as gene delivery agents to enhance tissue regeneration, to destroy cancer cells, and to regenerate cardiac or neuronal tissue. However, MSCs do not maintain their proliferative and multi-lineage differentiable capacities after long ex vivo propagation. Modifying MSCs to enhance their ability to differentiate into many cell types and also to resist senescence during ex vivo propagation would yield a very favorable starting cell source for tissue engineering and cell therapy projects. The recent development of transcriptional reprogramming technologies, pioneered by Yamanaka, Thomson and others, has opened a new chapter in the epigenetic manipulation of cell fate [1, 2]. Partial reprogramming is the process of moving an adult cell along the pathway from limited multipotency toward pluripotency, without returning it to the completely pluripotent state of an embryonic stem cell. In this study, we developed a method to induce partial reprogramming in adult stem cells. We hypothesize that controlled partial reprogramming of aged MSCs will restore the proliferative and differentiative functions lost during in vitro ageing.

METHODS:
MSCs from hip arthroplasty patients were harvested from bone marrow according to published protocols and cryopreserved for a minimum of one week with approval of the Institutional Review Boards of the University of Pittsburgh and the University of Washington. A portion of the cryopreserved MSCs were plated on tissue culture plastic at a density of 1000 cells/cm² and passed at 80% confluency for no less than eight total passages. MSCs were maintained in passage number P8 or greater and within two passages of senescence were considered aged MSCs. The remaining portion of cryopreserved MSCs were thawed and passed for no more than three total passages. MSCs with a passage number P3 or less were considered non-aged MSCs (Figure 1). Size and morphology of aged MSCs were documented at each passage number over the course of their expansion and compared to non-aged MSCs.

Non-aged MSCs were partially reprogrammed with transient transfection of Oct4, Sox2, C-Myc, and Klf4. Non-aged, aged, and reprogrammed MSCs were stained for markers associated with MSCs and ESCs, as well as other non-mesenchymal stem cell types to generate an immunophenotype for each population. Proliferation of the MSC population was monitored with PicoGreen (Invitrogen) to quantify dsDNA and Alamar Blue (Invitrogen) to quantify metabolic activity. CFU-F (colony forming unit-fibroblast) assays were used to measure the ability of aged, non-aged, and reprogrammed single cells to form colonies using the methods of Baksh et al. [3]. Non-aged, aged, and reprogrammed MSCs were induced to undergo (1) osteogenic differentiation and (2) chondrogenic differentiation. Differentiated cultures were examined histologically and also analyzed for lineage-specific gene expression using real time RT-PCR. The rate and extent of differentiation were compared between the three cell populations and significant differences were determined using mixed-design multivariate analyses of variance.

RESULTS:
We developed a protocol for reprogramming gene transfer that produced the optimal amount of partial reprogramming in MSCs (Figure 2). We compared different vectors and methods of gene transfer, including lentiviral and plasmid vectors and plasmid electroporation platforms. (Amaxa, BTX)

Following in vitro aging of MSCs, reprogramming transcription factor genes were transferred to aged MSCs and colony counts were performed to determine the most efficacious gene transfer protocol in terms of iPS colony formation (Figure 2). Colonies were identified by morphology and cell size and confirmed using immuno-cytochemistry (Figure 3). We then assessed partially reprogrammed aged MSCs to determine if their phenotype had been changed to that of non-aged MSCs in terms of the following characteristics: morphology, immunophenotype, proliferative index, differentiation potential (Figure 4), and resistance to senescence of restored MSCs alongside aged and non-aged MSCs.

Restored (partially reprogrammed) MSCs demonstrated significantly improved differentiation potential and other properties relative to aged MSCs.

DISCUSSION:
We were able to restore significant osteogenic and chondrogenic (data not shown) differentiation potential to near-senescence MSCs using partial transcriptional reprogramming. We were also able to enhance proliferative function in partially reprogrammed MSCs without inducing teratoma-forming capability (data not shown).

This study has far-reaching implications for regenerative medicine. The art of reprogramming by over-expressing transcription factors has just been studied in the last five years, and almost no work has been done in the area of partial reprogramming. Thus, our development of techniques for partial reprogramming is highly unique, and our approach to enhancing the properties of the adult stem cells that have been considered a primary candidate cell type for tissue engineering by partially reprogramming them has never been attempted before. Further refinement may yield a cell type with superior qualities for tissue engineering.

REFERENCES: