**Introduction:** Studies in our hybrid rat model of transplantation/ischemic reperfusion injury (IRI) have demonstrated improvement in kidney function post injection of adipose-derived regenerative cells (ADRCs) into the renal artery. This technique has translational value in human transplant surgery as ADRCs provide a robust supply of cells from accessible tissue, do not require culturing, and can be generated/delivered at point of care during the time of transplant. The mechanism on how these cells establish regenerative conditions during IRI remains elusive.
**Objectives:** Initial research in our model indicates multiple active subsets of ADRCs contribute to pro-angiogenic and anti-oxidant conditions with the kidney. We performed further studies on the interplay of these effects within the injured kidney microenvironment and its relationship with inflammation at early timepoints post ADRC treatment.

**Methods:** Flow cytometric analysis was performed on rat ADRCs extracted from inguinal tissue (n=14). Cells were surveyed for markers that identify viability, immune cells (CD45+) immune cell subsets (CD3+, CD3+CD4+, CD3+CD8+, CD11b+ cells), epithelial cells (CD45-CD31+), pericytes (CD45-CD146+), and mesenchymal stem cells (CD45-CD90+CD34). Rat ADRC RNA expression for angiogenic, anti-oxidant, and anti-/inflammatory markers were measured by real-time PCR. ADRCs were extracted from inguinal fat of the Fisher 344 rat, labelled with a near infrared lipophilic dye, DiR, and injected via the renal artery of the IRI rat model. Whole body imaging was performed. IRI model kidneys were processed for histological analysis, and gene expression measured at 1 hour 24, and 48 hours post renal injection. Protein isolated from the kidney at 1 hour 24, and 48 hours post injection were surveyed with a proteome profile array and through western blotting.

**Results:** 61-91% fraction of injected inguinal ADRCs were viable. Cell subsets consisted of high and varying levels of immune cells (47-92%) and low levels of pericyte cells (1-3%). Stem cell-like markers identified a median of 25% of the total ADRC cell population. RNA expression from whole rat ADRCs indicate production of growth and repair factors, and notably- angiogenin and matrix metallopeptidase-2; and chemokine, CXCL1.

A large accumulation of ADRCs appeared clustered in the corpuscle of the kidney 1-hour post injection of ADRCs. Cells remain visible within the kidney at 24 hours, however by 48 hours appear to dissipate. Histological analysis of the injected kidney identified labelled cells predominantly within the cortex (likely within the renal glomeruli) of the injected kidneys at 1 hour and 24 hours post injection.

Gene expression studies performed on the kidneys at 1, 24, and 48 hours post injection identified an upregulation of angiogenic factors VEGFa and angiogenin; anti-oxidant HO-1; and inflammatory factors IFN-ɤ and IL-6 when compared to sham-injected control. Protein profiling indicated an upregulation of TIMP-1 and of ligands important in leukocyte trafficking.

**Discussion**: ADRCs appear to be a pleomorphic cell suspension with multiple potential active subsets including T-cells, macrophages and mesenchymal stem cells. Data in the context of our model, suggest they serve as a vehicle for discrete administration of angiogenic and anti-oxidant factors. Counterintuitively, data also suggests that concomitant to secreting repair factors, at early timepoints, factors are secreted which attract inflammatory-related immune cells. Collectively, changes in RNA and protein levels of leukocyte trafficking-related factors suggest a major role for leukocytes in early IRI repair.

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