

Introduction: Therapeutically bioactive cell populations may promote regenerative outcomes *in vivo* by leveraging mechanisms of action including secretion of growth factors, site specific engraftment and directed differentiation. Constitutive cellular populations also participate in regenerative processes. Adipose tissue is a source of therapeutically bioactive cell populations. The potential of these cells to participate in regenerative processes is broadly demonstrated¹. However, organ association of regenerative markers to specific peri-organ adipose depots has not been investigated. To characterize this topographical association, we explored the potential of cells isolated from the stromal vascular fraction (SVF) of kidney and non-kidney sourced adipose to express key renal associated factors. We report that adipose tissue is a novel reservoir for EPO expressing cells. Kidney adipose-derived SVF cells show hypoxia regulated expression of EPO transcript/protein and VEGF transcripts. Using iso-electric focusing, we demonstrate that kidney and non-kidney adipose derived cells present uniquely different patterns of EPO post-translational modification, consistent with the idea that kidney and non-kidney sources are functionally distinct adipose depots. In addition, kidney adipose-SVF cells specifically express the key kidney developmental transcription factor WT1 while non-kidney adipose does not express WT1. Taken together, these data are consistent with the notion that kidney adipose sourced stromal cells could be used to recreate a regenerative micro-environment within kidney. These findings open the possibility of isolating solid organ associated adipose cell populations for therapeutic application in organ-specific regenerative medicine products.

Materials and Methods: Human non-renal adipose was sourced subcutaneously or through visceral liposuction (zen-bio.com). Human kidneys were sourced through NDR1. Canine kidneys were a gift of Dr. Tim Nichols, UNC Chapel Hill. Rat kidneys were from Charles River Labs. Adipose was washed with PBS/0.1% gentamicin and digested for 1 hour with 0.3% collagenase/1% BSA/DMEM-HG, prior to centrifugation at 600g, 20 min. SVF was resuspended in α -MEM/10% FBS for 24-48hr. For studies with hypoxia inductions, cells were maintained in O₂-enriched (2%) incubator. IEF gels (pH3-7) were from Invitrogen and were transferred using iBlot transfer system (Invitrogen), probed with α -EPO monoclonal (R&D Systems). Primary kidney cells were made as described^{2,3}.

Results:

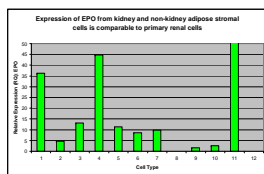


Figure 1. TaqMan qRT-PCR comparative analysis of EPO expression from kidney adipose-derived cells, non-kidney adipose derived cells and primary kidney cells. Primary kidney cell populations display considerable variability in expression of EPO (Lanes 1,2). Kidney or non-kidney adipose-derived cells express EPO at levels comparable to primary kidney cells.

1. Primary kidney cells
2. Primary kidney cells
3. Primary kidney cells, hypoxia
4. Adipose-SVF cells, P0 (visceral)
5. Adipose-SVF cells, P0(subQ)
6. Adipose-SVF cells, P1(visceral)
7. Adipose-SVF cells, P1(subQ)
8. Kidney adipose-SVF cells, P1 (calyx)
9. Kidney adipose-SVF cells, P1 (pedicle)
10. Kidney adipose-SVF cells, P3 (pedicle)
11. Fetal hepatocyte
12. H₂O

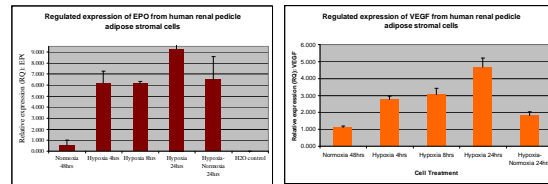


Figure 2. Regulated expression of EPO and VEGF from human renal pedicle adipose sourced SVF cells (n=3).

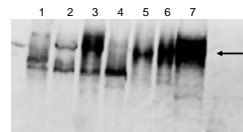


Figure 3. Expression of EPO from human kidney and non-kidney sourced adipose SVF cells is comparable with primary kidney cells, hepatocytes and keratinocytes. pH 3-7 IEF gel of EPO (Western blot): (1) human keratinocyte (2) hepatocyte (3) kidney SVF cells (4) non-kidney SVF cells (5) primary kidney cells-normoxia (6) primary kidney cells-hypoxia (7) HepG2. All lanes normalized by mass protein (10 μ g)

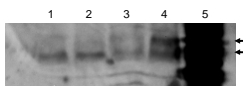


Figure 4. Expression of EPO from canine kidney adipose SVF cells is comparable to canine primary kidney cells. Canine kidney adipose sourced SVF cells express distinct isoforms of EPO relative to canine primary kidney cells. pH 3-10 IEF gel of EPO (Western blot): (1) primary kidney cells-normoxia (2) primary kidney cells-hypoxia (3) kidney adipose-SVF cells-calyx (4) kidney adipose-SVF cells-pedicle (5) recombinant EPO. All lanes normalized by mass protein (10 μ g).

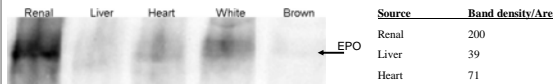


Figure 5: Adipose tissue is a reservoir for EPO expressing cells. IEF gel (Western blot) showing EPO expression from differently sourced rat adipose tissues. White and brown adiposes were derived from visceral depot. All lanes normalized by mass protein (10 μ g).



Figure 6. Semi-quantitative RT-PCR analysis of WT1 splice variants (KTS+/KTS-) in human kidney and non-kidney adipose SVF cells

LA: visceral adipose SVF cells (lipospirate)
SQ: visceral adipose SVF cells (subcutaneous)
MC: kidney adipose SVF cells (major calyx)
RP: kidney adipose SVF cells (renal pedicle)

Expression of WT1 mRNA is only detected from kidney sourced adipose. Note that ratio of KTS+/KTS- splice variants⁴ differs between major calyx and renal pedicle sourced adipose SVF cells derived from the same individual. All lanes normalized by mass cDNA.

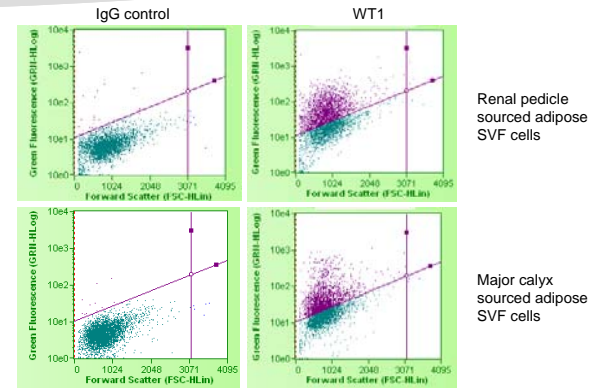


Figure 7. Distribution of WT1+ cells within human kidney adipose-derived SVF cells. FACS analysis of distribution of WT1+ cells in major calyx and renal pedicle associated adipose SVF cells. 45.6% of renal pedicle sourced adipose SVF cells was WT1+. 52.4% of major calyx sourced adipose SVF cells was WT1+

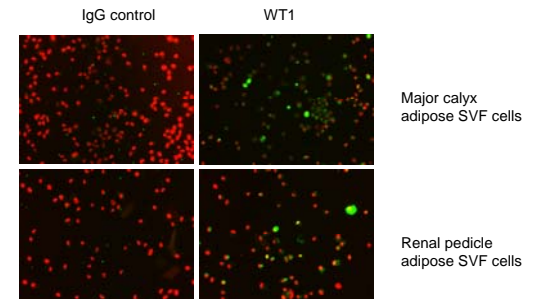


Figure 8. Cellular localization of WT1 in kidney adipose-derived SVF cells. Immunofluorescence analysis of distribution of WT1+ cells in kidney sourced adipose SVF cells. Note localization of WT1 (green) is both cytoplasmic and nuclear⁵. DNA (red)

Summary:

- Expression of EPO mRNA from kidney adipose-derived SVF cells is regulated by hypoxia
- Adipose-derived SVF sourced from the same or distinct organs can be distinguished by EPO expression or IEF iso-forms
- WT1 expression is specific to kidney sourced adipose tissue
- Distinct renal depots of adipose (major calyx and renal pedicle) may be defined by WT1 transcriptional splice variation

Conclusions:

- Adipose tissue is a novel reservoir for EPO expressing cells
- Kidney and non-kidney sourced adipose-derived SVF represent functionally unique adipose depots

REFERENCES: (1) Casteilla et al. (2006). Adipose tissue-derived cells: from physiology to regenerative medicine. *Diabetes Metab* 32, 393. (2) Basu et al., (2011). Functional evaluation of primary renal cell/biomaterial Neo-Kidney Augment prototypes for renal tissue engineering. *Cell Transplant.* In Press (3) Presnell et al., (2011). Isolation, characterization and expansion methods for defined primary renal cell populations from rodent, canine and human normal and diseased kidneys. *Tissue Eng. Part C* 17, 261 (4) Hammes et al. (2001). Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. *Cell* 106, 319. (5) Niksic et al., (2004). The Wilms' tumor protein (WT1) shuttles between nucleus and cytoplasm and is present in functional polysomes. *Hum. Mol. Genet.* 13, 463.