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PHENOTYPIC AND FUNCTIONAL DISTINCTION BETWEEN MESENCHYMAL STEM CELLS AND THE STROMAL VASCULAR CELL FRACTION OF HUMAN ADIPOSE TISSUE

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ABSTRACT

The heterogeneous cell population comprising the stromal vascular fraction (SVF) of human adipose includes endothelial, smooth muscle cell (SMC), and mesenchymal stem cell (MSC)-like cells (as defined by the ISCT criteria). We investigated the cellular composition of the primary "passage zero" (P0) adherent human SVF-derived cell population using quantitative real-time PCR (TaqMan). Although the SVF-derived P0 population expressed genetic markers associated with endothelial, SMC, and adipocyte cells; expansion of SVF-derived P0 cells under defined media conditions that select against the growth of MSC yielded a cell population with markedly distinctive biological properties when compared to MSC cultures. The differentiation potential and marker expression profile of this expanded SVF-derived cell population (X-SVF) partially overlapped that historically associated with MSC; however, X-SVF cells have a more pronounced smooth muscle cell phenotype relative to MSC based on FACS and RT-PCR (reverse transcription PCR) analysis of key nuclear and cell surface marker expression. X-SVF cells also expressed noticeably fewer endothelial-specific genes relative to MSC. These observations suggested that the predominant phenotype of the X-SVF cells was that of SMC. Manifestation of SMC phenotype was independent of passage number or individual adipose donor (n=4) and directed differentiation of X-SVF with recombinant cytokines and growth factors was not required. Additionally, X-SVF cells expressed a distinctive SMC-like proteomic signature that unambiguously distinguished it from MSC. Finally, X-SVF cells and MSC had opposite responses to the thromboxane A2 mimetic U46619, demonstrating an unambiguous functional distinction between the two cell types. Taken together, these data support the conclusion that X-SVF cells are more accurately described as adipose-derived smooth muscle cells (Ad-SMC), and represent a separate and distinctive cellular species compared to other classes of adipose-derived cells; including adipocytes, endothelial cells, and MSC.

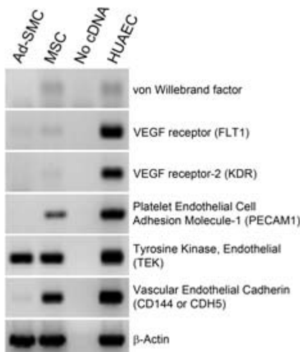
RESULTS

Figure 1: Expansion of smooth muscle cells from the stromal vascular fraction of human abdominal adipose is influenced by growth media formulation

SMC markers	RQ value relative to Bladder SMC as a function of cell culture media formulation			
	DMEM-HG 10% FBS	α-MEM 10%FBS	L15	SMCM
SMAA	85.35	1.98	1.43	1.45
SM22	61.19	1.95	1.55	0.038
Myocardin	13.17	0.37	0.20	0.009
SMMHC	192.88	1.74	2.17	0.10
Calponin	2960.0	2.44	1.46	0.00

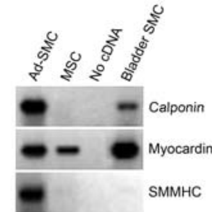
- Expression of SMC-specific markers is closely associated with growth and expansion in DMEM-HG plus10 % FBS

Figure 2: Ad-SMC are less endothelial-like than MSC



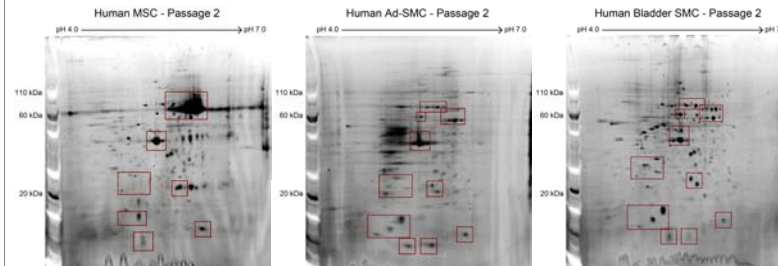
- Endothelial cell marker expression was assayed semi-quantitatively by RT-PCR with cDNA prepared from Adipose-derived SMC (Ad-SMC) and mesenchymal stem cells (MSC). Samples to which no cDNA was added (No cDNA) and HUVEC cDNA were control comparators.
- Von Willebrand factor, FLT1, KDR, PECAM1, and CD144 expression was greater in MSC compared to Ad-SMC

Figure 3: Increased SMC marker expression in Ad-SMC vs. MSC



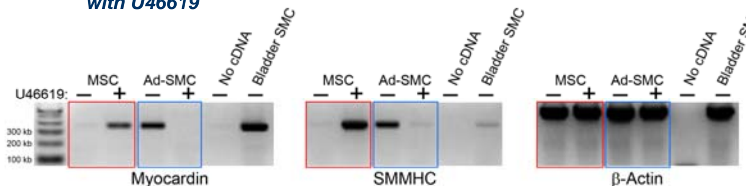
- Smooth muscle cell marker expression was assayed semi-quantitatively by RT-PCR with cDNA prepared from Adipose-derived SMC (Ad-SMC) and mesenchmal stem cells (MSC). Samples to which no cDNA was added (No cDNA) and Bladder-derived SMC cDNA were control comparators.
- Calponin, myocardin, and SMMHC expression was greater in Ad-SMC compared to MSC

Figure 4: Proteomic signatures of Ad-SMC and MSC are dissimilar



- Proteomic signatures were compared by two-dimensional gel electrophoresis. Protein lysates (40 μg) were isolated from bone marrow-derived MSC (A), Adipose-derived SMC (B), and bladder SMC (C) cultures at the end of passage two, subjected to 2D-electrophoresis (Invitrogen), and stained with SYPRO Ruby stain (Invitrogen) according to the manufacturer's instructions
- Comparative analysis of the distribution of proteins from each cell type (red boxes) revealed that the proteomic signatures of Ad-SMC and MSC are distinct; in contrast, the proteomic signatures of Ad-SMC and Bladder SMC are similar

Figure 5: Ad-SMC and MSC have opposite responses to treatment with U46619



- U46619 is a thromboxane A2 mimetic whose effects include increasing intracellular Ca2+ levels and activating multiple signaling cascades (e.g., RhoA, cAMP, and myosin light chain kinase). MSC and Ad-SMC cultures were exposed to 1 μM U46619 at 37°C for 72 hours (+ lanes) prior to preparing cDNA for semi-quantitative RT-PCR analysis. Samples to which no cDNA was added (No cDNA), Bladder SMC cDNA, and β-actin expression, which does not respond to U46619, were control comparators.
- Responses of mature smooth muscle markers myocardin and smooth muscle myosin heavy chain (SMMHC) to U46619 addition to MSC cultures (red boxes) were as previously reported.
- Myocardin and SMMHC responses to U46619 addition to Ad-SMC cultures (blue boxes) was opposite that observed in MSC cultures.

Figure 6: Comparative gene expression analysis discriminates Ad-SMC from MSC

Comparative Expression Category	Marker Symbol	Description of Marker	Fold expression MSC vs. Ad-SMC
MSC > Ad-SMC	CD44	CD44 molecule (Indian blood group)	347.8
	BMP6	Bone morphogenetic protein 6	35.6
	IL1B	Interleukin 1-beta	32.3
MSC ~ Ad-SMC	NT5E	5'-nucleotidase, ecto (CD73)	1.4
	ENG	Endoglin (CD105)	1.1
	ALCAM	Activated leukocyte cell adhesion molecule (CD166)	1.1
	THY1	Thy-1 cell surface antigen (CD90)	-1.4
MSC < Ad-SMC	RUNX2	Runt-related transcription factor 2	-12.1
	HGF	Hepatocyte growth factor	-36.5
	LIF	Leukemia inhibitory factor	-63.9
	MCAM	Melanoma cell adhesion molecule	-66.2
	GDF5	Growth differentiation factor 5	-120.9
	VCAM1	Vascular cell adhesion molecule 1	-1103.6

- At passage four, nine out of thirteen markers exhibited between >10 and >1000-fold differences in expression between MSC and Ad-SMC cultures as measured by quantitative PCR.
- A marker panel of CD44, BMP6, IL1B, RUNX2, HGF, LIF, MCAM, GDF5, and VCAM1 consistently discriminates between MSC and Ad-SMC.
- These results are consistent across multiple donor samples (n=3), suggesting that the ability to discriminate between MSC and Ad-SMC is not a consequence of donor variability or random fluctuations in gene expression levels.

CONCLUSIONS:

- ***Primary isolation of adipose-derived smooth muscle cells (Ad-SMC) from the adherent stromal vascular fraction of abdominal adipose is tightly dependent on media formulation, with DMEM-HG plus 10% fetal bovine serum yielding the highest levels of smooth muscle cell marker expression at initial passage (P0).***
- ***Ad-SMCs consistently express smooth muscle cell markers, independent of donor source and across multiple passages (P0-P4).***
- ***Ad-SMCs are phenotypically distinct from MSC as demonstrated by gene expression, proteomic signature, and surface marker analysis***
- ***Ad-SMCs are functionally distinct from MSC as evaluated by their response to U46619, a pharmacologic agent that targets smooth muscle cell-associated signaling pathways***
- ***Isolation of Ad-SMCs does not require directed differentiation during expansion***
- ***Ad-SMC are a separate and distinct cell population compared to other classes of adipose-derived cells, including MSC.***

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