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Whole Transcriptome Comparison between Multipotent Adult Stem Cells (MASCs) and Two Families of Mesenchymal Stem Cells (MSCs)

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BACKGROUND

Stem cells are defined as undifferentiated cells with the capacity for both self-renewal and future differentiation. Importantly, stem cells can proliferate while maintaining an undifferentiated state, and can differentiate into at least one phenotype. Adult, or somatic, stem cells are maintained throughout life to renew and repair the local tissues. There are several different varieties of adult stem cells, which include mesenchymal stem cells (MSCs), hematopoietic stem cells, and neural stem cells. Multipotent adult stem cells (MASCs) are distinct from MSCs as undifferentiated cells found in several tissues in post-natal animals. They are able to generate progeny of several distinct cell types of all three dermal lineages, and have an apparent unlimited proliferation potential. Their differentiation is determined by local cues. To date, very little is known about the gene expression profile of MASCs. Since bone marrow-derived MSCs were first experimentally expanded and differentiated through 4 mesodermal lineages in the 1990's[1], MSCs have been the subject of investigation due to their relative accessibility and supposed regenerative potential. Despite the wealth of knowledge these studies have provided, the exact nature of the relationship between MSCs and MASCs remains to be elucidated.

PURPOSE & HYPOTHESIS

Given the differences in differentiation and reproductive potential, **we hypothesize that MASCs have a unique gene expression profile in comparison to other adult stem cells, particularly MSCs.**

Table 1:Minimal Criteria of Mesenchymal Stem Cells

ISCT Criteria	BM-MSC	PL-MSC	HfSC
Plastic Adherent	+	+	+
CD73+	+	+	+
CD90+	+	+	+
CD105+	+	+	+
CD11b-	-	-	-
CD14-	+	-	-
CD19-	-	-	-
CD34-	-	-	-
CD45-	-	-	-
HLA-DR-	+	-	-
Other Criteria	**BM-MSC**	**PL-MSC**	**HfSC**
CD106 (VCAM1)+	+	-	-
CD117 (KIT)-	-	+	+
CD271 (NGFR)+	-	+	-
CD56 (NCAM1)-	-	+	+

Minimal criteria set by the International Society for Cellular Therapy

Dominici M, Le Blanc K, Horwitz E, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy (Taylor & Francis Ltd)*. August 2006;8 (4):315-317.

Other criteria:

Ravenska Wagey. Mini-Review Mesenchymal Cells. STEMCELL Technologies Inc. www.STEMCELL.com;(2011).

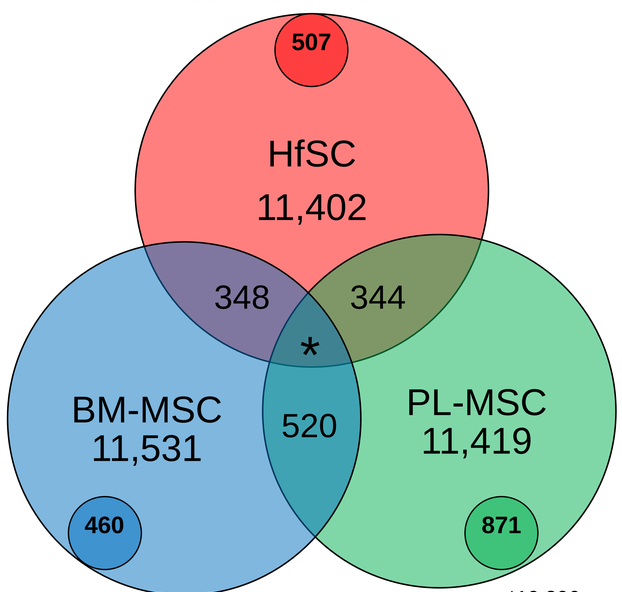
MATERIALS & METHODS

RNA-seq was performed on MASCs cultured in vitro in the laboratory. MASCs were isolated from the dermis layer of human foreskin (HfSC-2) and were tested at Passage (P) 20. We then compared the gene expression of the MASCs to databases of genes expressed by bone marrow-derived mesenchymal stem cells (BM-MSCs) and placenta-derived mesenchymal stem cells (PL-MSCs)[2]. Significant differences were found in the relative gene expressions of the PL-MSCs, BM-MSCs, and HfSCs. Previously published data have shown that differences in gene expression exist even between two families of MSCs[2]. The NIH Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to perform gene functional annotation clustering, with default options and annotation categories. Specific studies of pathway enrichment were performed utilizing DAVID's GO-BG annotation (Gene Ontology- Biological Groupings)[3].

RESULTS

Both HfSC and PL-MSC met the minimum criteria for MSCs (Table 1). Ironically, BM-MSC did not. However, we have identified 507 genes that are expressed in HfSCs but not in BM-MSCs or PL-MSCs, and 520 genes that were expressed in the MSC lineages but not the HfSCs. The most notable differences among HfSCs, BM- and PL-MSCs were observed in genes involved with neural development. All three stem cells do not express Oct-4, Sox-2, and Nanog – genes considered critical genes for embryonic stem cells (ESCs). Those uniquely expressed by the HfSCs included epiregulin, a gene required for sustained cell growth without senescence; several genes for early neural development (ROBO2, FRMPD4, PCDH9); genes associated with development of germline cells (LHX9 and IGF2BP3); genes for early neuroectodermal development (RSPO2 and ZIC1); genes for development of endodermal phenotypes (NKX6-1 and ONECUT1); and genes associated with cardiac development (TBX5).

Number of Genes Significantly Expressed in each Stem Cell Line



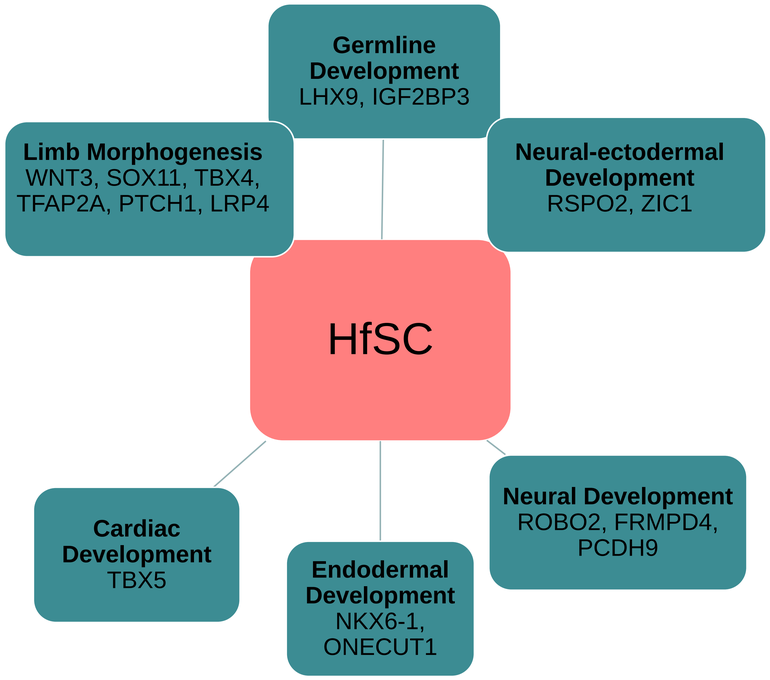
*10,206 genes shared by all three lineages

CONCLUSIONS

The 6 developmental pathways listed below provide clues to explain the wide differentiation potential of MASCs; they uniquely express genes important for the early progression of these pathways. In addition to epiregulin, the genes involved in germline development may provide insight into the apparent unlimited proliferation potential of MASCs. Future studies will include a comparison of gene expression profiles between HfSCs and human ESCs, and between HfSCs and human differentiated cells, specifically smooth muscle cells.

The BM-MSC mRNA expression does not conform to the ISCT standards, or other common MSC cellular markers. That the PL-MSCs do not conform to certain MSC cellular markers either, indicates that current protein and gene expression criteria for defining adult stem cells may be inadequate as they currently stand.

Developmental Pathways



BIBLIOGRAPHY

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal stem cells. Science 1999, 284:143–147
2. Roson-Burgo, Beatriz, Fermin Sanchez-Guijo, Consuelo Del CaÃ±izo, and Javier De Las Rivas. "Transcriptomic Portrait of Human Mesenchymal Stromal/Stem Cells Isolated from Bone Marrow and Placenta." *BMC Genomics* 15.1 (2014): 910
3. Huang, Da, Brad T. Sherman, Qina Tan, Jack R. Collins, W. Gregory Alvord, Jean Roayaei, Robert Stephens, Michael W. Baseler, H. Clifford Lane, and Richard A. Lempicki. "The DAVID Gene Functional Classification Tool: A Novel Biological Module-centric Algorithm to Functionally Analyze Large Gene Lists." *Genome Biology*8.9 (2007)