ISCT Perspective on Characterization of MSCs for Clinical Trials

Workshop on Improved Characterization of MSCs for Clinical Trials

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Criteria to Consider

- Tissue source
  - Are all MSCs created equal?
- Surface phenotype
  - Do phenotypic markers adequately reflect the composition and biological activity of MSCs.
- Species-specific differences.
  - Do animal cells/models accurately predict human MSC biology?
- Complexity of function
  - Can we achieve efficacious therapies by pursuing a reductionist approach to studying MSCs?

Tissue Specific Differences in MSC Biology

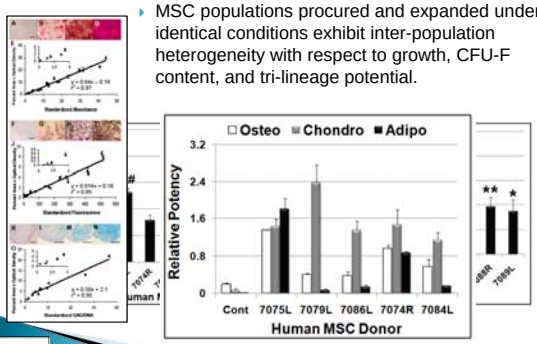
- Ubiquitous presence of MSCs in tissues attributed to similarity to pericytes.
  - Transcriptome profiling clusters MSCs from different tissues together.
  - Provides static representation of culture adapted cells.
- Functional differences in biology evident using stringently graded assays.
  - Accessibility and amenability to expansion not appropriate criteria for predicting efficacy.
- Lineage tracing studies identifies tissue-specific differences.
  - CFU-Fs in teeth, thymus and BM are entirely neural crest derived, entirely mesoderm-derived, and mostly NC-derived cells, respectively. (Komada et al. PLoS One 2012;7(11):e46436).

Phenotype vs. Function

- Most scientific and clinical laboratories characterize MSCs based on expression of several surface antigens
  - CD73, CD105, & CD90.
- Many other markers yield CFU-F activity of bone marrow but not unique to MSCs.
  - nestin, CD271, STRO1, CD146, etc
- Do these markers reflect inter and intra-population heterogeneity of MSCs and predict differences in biological function?

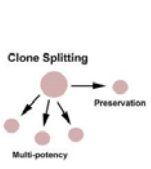
Inter-Population Heterogeneity

- MSC populations procured and expanded under identical conditions exhibit inter-population heterogeneity with respect to growth, CFU-F content, and tri-lineage potential.

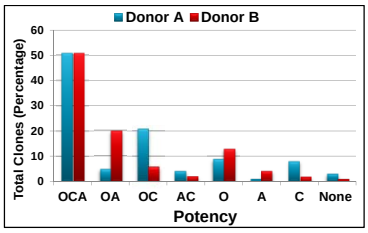


Intra-Population Heterogeneity

- Parental populations plated at clonal density.
- First generation clones (~100) split to evaluate multi-potency.
- Identified clones of every possible potency (OCA, OA, OC, AC, A, C, O).



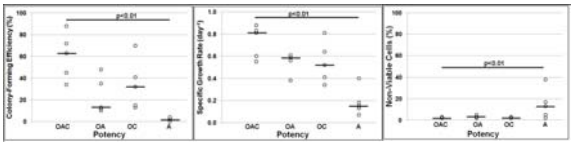
A= adipogenic,
C = chondrogenic,
O = osteogenic



Russell et al. Stem Cells 2010; 28:788-798

Intrinsic Differences Among Clonal Populations

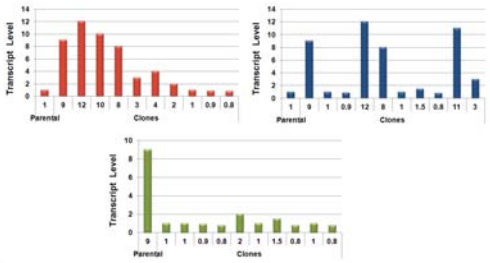
- Colony-forming efficiency and growth rate were significantly greater for tri-potent vs. uni-potent human MSC clones.
- Overall cell death rates were significantly greater in uni-potent clones as compared to bi- and tri-potent clones.



Russell et al., Biotechnol. Bioeng. 2011; 108:2716-2726.

Paracrine Functions Are Specified Hierarchically

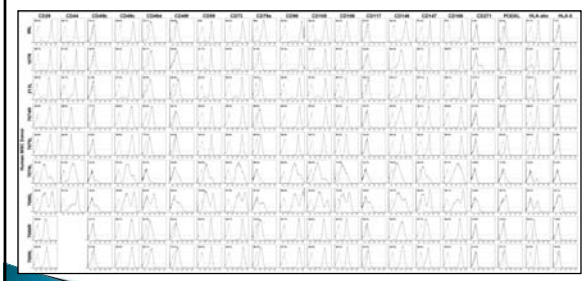
- Expressed levels of paracrine-acting factors show distinct patterns of clonal restriction within populations.



Phinney DG. J Cell Biochem. 2012;113:2806-2812.

MSC Surface Phenotype

- Flow cytometric analysis of 20 surface antigens in human MSC donors.

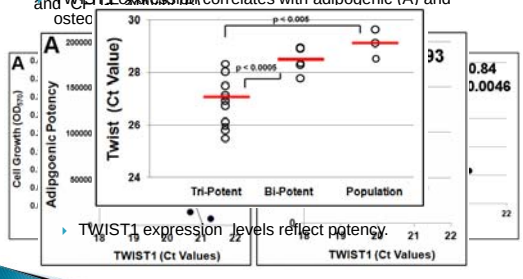


Correlation of Surface Marker Expression with MSC Growth & CFU-F

	Growth (Cell #)		Growth (OD_{570})		CFU-F	
Antigen	r-value	p-value	r-value)	p-value	r-value	p-value
CD29	0.496	0.175	0.484	0.187	0.308	0.42
CD44	0.379	0.402	0.348	0.443	0.563	0.188
CD49b	-0.309	0.419	-0.354	0.35	-0.564	0.114
CD49c	0.273	0.477	0.258	0.503	0.177	0.649
CD49d	0.309	0.419	0.362	0.338	0.336	0.333
CD49f	0.064	0.871	0.269	0.484	0.267	0.487
CD59	0.444	0.231	0.470	0.202	0.408	0.276
CD73	0.664	0.051	0.698	0.036	0.631	0.068
CD79a	0.454	0.22	0.499	0.172	0.565	0.113
CD90	0.475	0.196	0.527	0.15	0.391	0.298
CD105	0.568	0.111	0.657	0.055	0.554	0.122
CD106	0.431	0.247	0.423	0.257	0.357	0.346
CD117	-0.034	0.931	0.161	0.679	0.026	0.947
CD146	0.405	0.28	0.479	0.192	0.482	0.189
CD147	0.581	0.101	0.628	0.07	0.536	0.137
CD166	0.431	0.247	0.519	0.152	0.329	0.387
CD271	0.513	0.158	0.468	0.204	0.179	0.645
PODXL	0.692	0.039	0.489	0.182	0.599	0.088
HLA-abc	0.396	0.291	0.489	0.182	0.528	0.144
HLA-II	-0.524	0.148	-0.562	0.115	-0.572	0.108

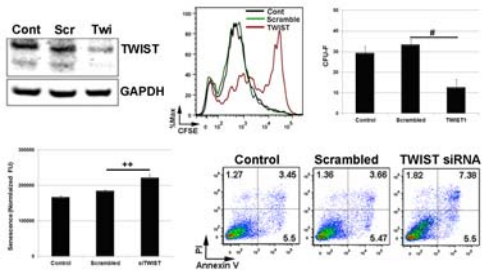
TWIST1 Predicts Inter-Population Differences Among hMSC Donors

- TWIST1 expression significantly correlates with MSC growth (A) and CFU-F efficiency (B)
- TWIST1 expression correlates with adipogenic (A) and osteo
- TWIST1 expression levels reflect potency.



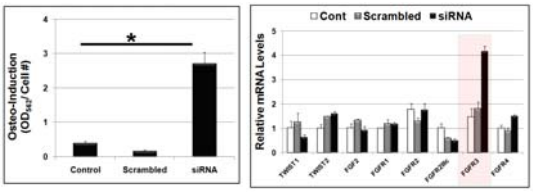
TWIST1 Loss-of-Function

- Knockdown of TWIST1 impedes growth and CFU-F activity and induces senescence and apoptosis in human MSCs.

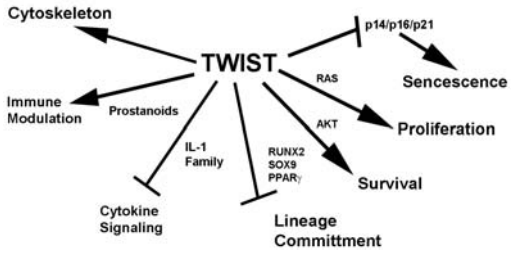


TWIST1 Represses Lineage Commitment

- TWIST1 knockdown induces spontaneous adipogenic, chondrogenic, and osteogenic differentiation.
- Concomitant de-repression of PPAR-γ, RUNX2, SOX9, and FGFR3.

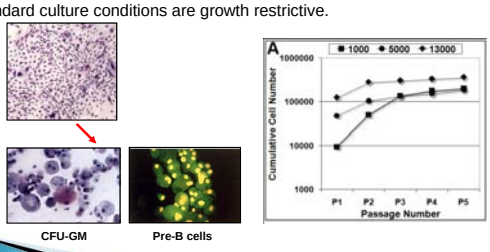


TWIST Confers MSC Phenotype



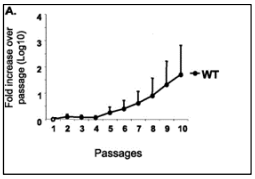
Species-Specific Differences in MSC Biology

- Plastic adherent cultures from mouse bone marrow support granulopoiesis and lymphopoiesis and reconstitute the hematopoietic system of lethally irradiated mice.
- Standard culture conditions are growth restrictive.

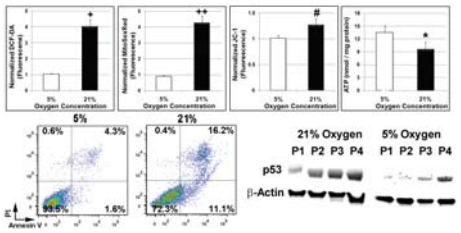


MSCs from Mouse Bone Marrow

- Various methods reported to isolate mouse MSCs
  - Positive/negative selection, sieving, compact bone, etc.
- Most laboratories employ long-term propagation (months) to remove hematopoietic lineages and select for rapidly dividing cells.
  - Some publications expand cells for up to **80** days prior to use.
  - Emergent cells are clonally restricted, immortalized cells.



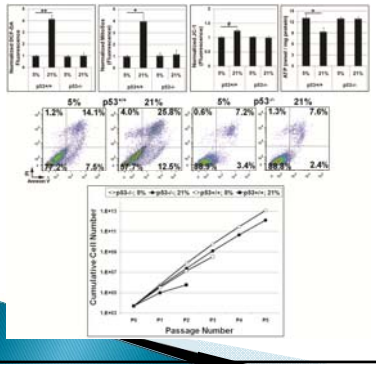
Oxygen Induces Intra-cellular Oxidative Stress



- Exposure to atmospheric oxygen (21%) results in mitochondrial ROS production and mitochondrial dysfunction (membrane depolarization, reduced ATP production).
- Atmospheric oxygen induced apoptosis and increased p53 expression.

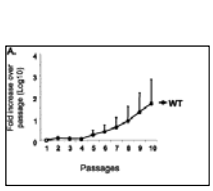
Boregowda et al. Stem Cells 2012; 30:975-987

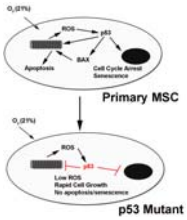
P53-Dependence (Cont)



- Oxygen does not induce ROS generation in p53 null MSCs.
- P53 null MSCs are protected from oxygen-induced apoptosis.
- P53 null MSCs exhibited sustained growth irrespective of oxygen levels.

Limitations of Rodent MSCs





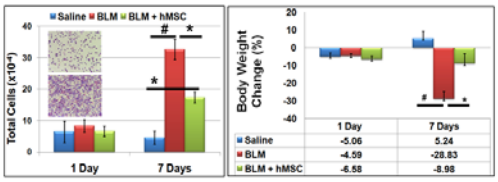
- Growth restrictive conditions (21% oxygen) selects for clones that lack functional p53 that escape oxygen-induced growth arrest.
  - Clonally selected lines that lack primary checkpoint controls and are insensitive to oxidative stress.
- Oxygen also limits lifespan and induces chromosomal abnormalities in human MSCs (Estrada et al., Cell Death Diff. 2012;19:743-755).

Reductionist Approach

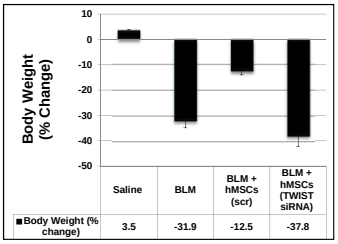
- Lee et al. showed that knockdown of TSG6 abrogates cardio-protective effect of MSCs.
  - Secrete other TNF antagonists (sTNFR), anti-inflammatory factors (PGE2, NOS, IL1RN, galectin-1) and cardio-protective factors (IGF1, VEGF, angiogenin1, IL-6).
- Knockdown of galectin1 or 3 abolishes T cell suppressive functions in MLR assays *in vitro*.
  - Neutralization of B7-H1, PGE2, HLA-G5 and other proteins also antagonize MSC immuno-suppressive functions.
- How is expression of these proteins and their function interrelated?

Boregowda SV, Phinney DG. BioDrugs 2012; 26:201-8

MSCs Suppress Bleomycin-Induced Lung Inflammation

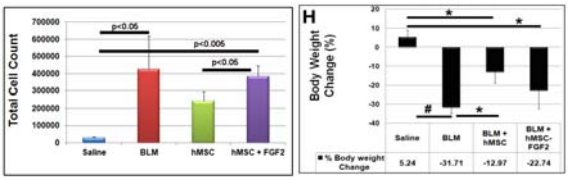


MSCs Lacking TWIST Exacerbates Bleomycin-Induced Lung Inflammation



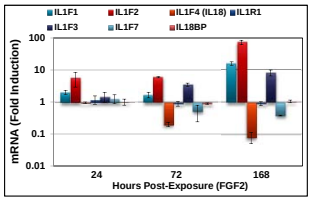
FGF2 Supplementation Diminishes the Anti-inflammatory Activity of MSCs

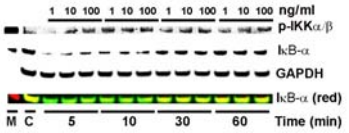
- FGF2 induces TWIST expression in hMSCs.
- FGF2 treatment should augment protective effect of hMSCs in vivo.



FGF2 Induces Cytokine Expression via NF-kB Activation

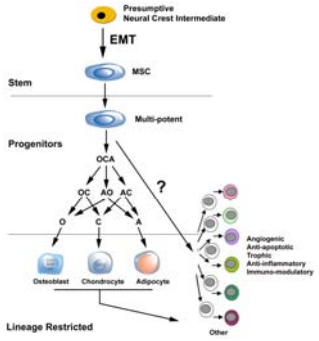
- FGF2 induces IL1F1 and IL1F2 to higher levels as compared to IL1F2.
- FGF2 treatment results in a sustained increase in pro-inflammatory cytokine expression.





Conclusions

- MSC populations are functionally complex.
- Lack of potency assays makes it difficult to assess how culture expansion alters the composition and function of populations.
- Few phenotypic markers predict functional differences - at least not the most widely used.
- Animal models limited in predictive value.
  - Impact of species-specific differences in biology not well documented.
  - Animal models evaluate efficacy over short intervals.
  - Lacking systemic evaluation of dose responsiveness, route of administration, MSC origin, etc.
- Reductionist approach is overly simplistic.



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Acknowledgements

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