Strategies to Improve Characterization of Stem-Cell Based Cellular Products

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Product Testing

Chemistry, Manufacturing, and Controls (CMC)

• Should ensure product safety
• Should ensure consistency of process and product
• Should (ideally) predict in vivo activity
• Is guided by detailed understanding of the manufacturing process and the product

Characterization
Multipotent Stromal Cells (MSCs) in Clinical Research

- MSCs are diverse
  - Characterization
  - Manufacturing
  - Source

- Quality attributes?

- Relation to performance in clinical trials?

MSCs and Product Characterization

Mesenchymal Stem Cell
- Immunomodulatory/Anti-Inflammatory
- Adipocytes
- Chondrocytes
- Osteoblasts

Manufactured MSC
- Adherent in culture
- CD73+, 90+, 105+
- Lymphoid lineage negative
CBER/FDA MSC Consortium: Identification and correlation of MSC attributes with *in vivo* and *in vitro* assays of safety and efficacy

**MSC Characterization**

Product Characteristics

- **Moos Lab**: gene expression, qRT-PCR, single cell PCR, NGS
- **Alterman Lab**: proteomics
- **Puri Lab**: genomics
- **McCright Lab**: *in vivo, in vitro* models of wound repair
- **Bauer Lab**: *in vitro* quantitative differentiation
- **Wei/Bauer Labs**: *in vitro, in vivo* immunosuppression
- **Hursh lab**: epigenetics, karyotypes
- **Sung Lab**: *3D in vitro* tissue/organ microfluidic models

**CORRELATE CANDIDATE ATTRIBUTES WITH ASSAY OUTCOMES**

- **Cytoplasm**: 33%
- **Unknown**: 25%
- **Nucleus**: 24%
- **Membrane**: 13%

**5** Sung Lab: *3D in vitro* tissue/organ microfluidic models
Consensus MSC Surface Markers Do Not Reveal Significant Differences

Lo Surdo JL et al., Cytotherapy, 2013.
Adipogenic Potential Varies Between Cell Lines and Decreases with Passaging

Automated microscopy-based quantification

Lo Surdo JL et al., Cytotherapy, 2013.
Are Morphological Differences Associated With Different Biological Functions?

Lo Surdo JL et al., Cytotherapy, 2013.

Stimulus

Undifferentiated MSC

\[ \text{Cell Signaling - Transcription - Translation} \]

Early Morphological Response

Different Biological Functions
MSC Morphology Predicts Osteogenic Activity

CULTURE-EXPANDED hMSCs

Long-Term Osteogenic Induction (35 Days)

Mineralization

Principal Component Analysis (PCA)

Extract Cell Shape and Nuclear Shape Features (96 total)

Morphological ‘signature’ at day 3 predicts mineralization at day 35

Marklein et al, Stem Cells 2016
Immunosuppressive Capacity Varies Between Cell Lines and with Culture Duration

Klinker, et al. PNAS 2017
IFNγ-Stimulated MSC Morphology Predicts Immunosuppressive Activity
MSC Consortium Outcomes

• Demonstrated that consensus markers do not predict functional biological heterogeneity of MSCs

• Identified cellular characteristics that predict relevant biological properties of MSC preparations
  – Functionally relevant morphology
  – Gene expression

• Published findings
  • Sector Overview
  • Quantitation of Differentiation
  • Proteomics
  • Immunomodulation
  • Genomics
  • Genetic and Epigenetic Stability
Potential Applications (1)

– Cell Source/Donor
  • Screen samples for desired biological activity

– Manufacturing
  • Evaluate impact of manufacturing process
    – Tissue culture conditions and duration

– Cell Characterization

– Identify Quality Attributes
  • Activity/Potency
    – Quantitative Bioassays
    – Molecular markers correlated with bioassay outcomes

– Guide cell enrichment techniques
Potential Applications (2)

— Standards Development
  • Quantitative bioassays
    — Osteogenesis
    — Adipogenesis
    — Immunosuppressive Capacity
    — Others?
      ➤ Angiogenesis
      ➤ Wound repair
MSC Consortium

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