Advancing Regulatory Science

Expanded and improved approach to identifying surface protein markers could support development of therapies with human bone marrow multipotent stromal cells

An FDA proteomic analysis of cell surface markers discovered expression of 14 proteins previously not seen on bone marrow multipotent stromal cells . The findings could provide new insights into how to assess differentiation and maturation of these cells into safe and effective therapies.

"Improved proteomic profiling of the cell surface of culture-expanded human bone marrow multipotent stromal cells"

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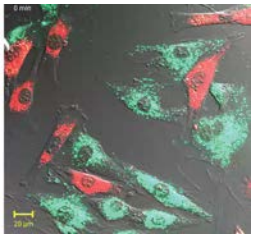


Mesenchymal stem cells (red)
interacting with cardiomyocytes (green)
Credit: Wikimedia Commons

Human bone marrow multipotent stromal cells (hBM-MSC): Key players in growth and regeneration of organs & tissues

- Repairing or regenerating organs
- Assisting in growth of blood vessels
- Preventing cell death
- Inhibiting unwanted immune rejection

Protein surface markers: Getting a handle on differentiation & maturation

- MSCs are heterogenous cell populations; their heterogeneity must be better understood to determine their ability to undergo useful differentiation and maturation.
- Protein markers often play critical roles in passage of maturation and differentiation signals into and out of hBM-MSC.
- Knowledge of hBM-MSC surface markers would provide valuable tools for:
    - assessing capacity for cell differentiation and maturation
    - facilitating manipulation of hBM-MSC with biological molecules to guide differentiation into safe and effective cell therapies

Multipotent stem cells/Wikimedia Commons

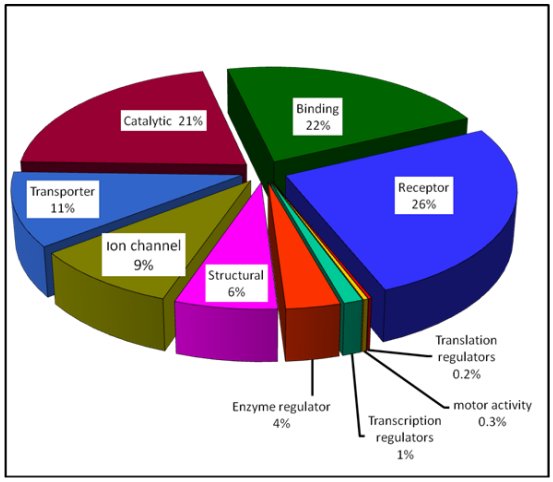
The challenge: Obtaining enough hBM-MSC for therapy and verifying they are appropriate for clinical use

- Cells harvested from bone marrow of patients must be grown to numbers large enough for use as therapies: repeated harvesting is not practical.
- Limited information on hBM-MSC protein markers makes it difficult to determine their ability to undergo appropriate differentiation and maturation, thus slowing their development as safe and effective therapies.
    - Some protein markers with critical function are difficult to extract for analysis.
    - Some cell markers undergo post-translation modification that makes them so complex they are difficult to identify.

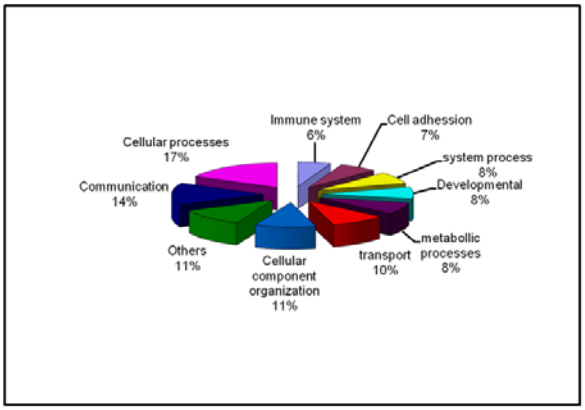
Meeting the challenge: FDA scientists demonstrate expanded and improved approach to identifying hBM-MSC markers

- Analyzed markers on hBM-MSC from 4 human donors aged 22-24 years old.
- Completed expanded, improved analysis of membrane markers from hBM-MSC that multiplied in a culture dish but were not yet differentiated or matured.
- Combined several existing techniques to extract and identify markers from cells that had been cultured in the laboratory to produce a large population—similar to what would have to be done to produce cells for actual therapy.
- Used novel technique involving cycling of cells between low and high pressure to improve extraction of the protein markers.

➢ **Identified twice as many membrane proteins than reported previously:**
  - o **84 possible markers identified**
  - o **14 of the 84 markers were identified for the first time**



Molecular functions of protein markers identified on hBM-MSCs



Biological process distribution of protein markers identified on hBM-MSCs

The large number of hBM-MSC membrane proteins identified in this study will contribute to further exploration and understanding of the self-renewal, differentiation, and characterization of these cells.