Comparative proteomic analysis of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) of different origin

Natalia Pripuzova¹, Melkamu Getie-Kebiete¹, Christopher Grunseich², Colin Sweeney², Harry Malech³ and Michail Alterman¹

¹Tumor Vaccine and Biotechnology Branch, Division of Cellular and Gene Therapies, Office of Cellular, Tissue and Gene Therapies, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD; ²Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD; ³Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

Background and Objective

Induced pluripotent stem cells (iPSC) can be obtained from somatic cells using ectopic expression of 4 basic reprogramming factors: OCT3/4, SOX2, KLF4, and c-MYC. Somatic cells pass through the early, mitotic stages of reprogramming towards iPSC. The generation of human iPSCs (hPSCs) from adult somatic cells holds important scientific and clinical implications. While many studies have characterized and compared gene expression profiles of iPSCs and human embryonic stem cells (hESCs), only few have dealt with their proteomes. iPSCs have been found to be highly similar to hESCs, but their gene expression profiles differ from hESCs: iPSCs may not completely silence the expression of ESC genes. There are 19 clinical trials now registered using iPSC technology. There is a strong interest on iPSCs therapeutic potential ranging from autism and bipolar disorder to traumatic spinal cord injury. The current proteomic study aims to answer several questions: Does the somatic origin, reprogramming method, culture condition, passage number affect different iPSC lines? What are the differences between hPSCs and hESCs on the molecular and functional levels?

Materials and Methods

Study design:

<table>
<thead>
<tr>
<th>hESC</th>
<th>iPSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood of healthy adults</td>
<td>Peripheral blood of healthy adults</td>
</tr>
<tr>
<td>MNC</td>
<td>MNC</td>
</tr>
<tr>
<td>reprogrammed with lentiviral vector</td>
<td>reprogrammed with lentiviral vector</td>
</tr>
<tr>
<td>lentiviral vector (STEMCCA-loxP)</td>
<td>lentiviral vector (STEMCCA-loxP)</td>
</tr>
</tbody>
</table>

Quality control of both iPSC lines has been performed.

Differently expressed proteins

General Comparison by Biological Process

Protein Analysis Through Evolutionary Relationships (PANTHER)

Comparison of quantification by ESI MS-MS and Western blot results

IPA (Ingenuity Pathway Analysis) of differentially expressed proteins

Conclusions

1) Considerable differences in protein expression profiles were observed between the iPSCs and parental somatic cells. The major differences were revealed in DNA replication, transcription, translation, and cell death.

2) The somatic origin, reprogramming method, culture condition, and passage number affect different iPSC lines.

3) The biological functions affected were very similar to those affected in comparison with primary cells.

4) The following pluripotency markers were quantified by ESI MS-MS in both ESCs and iPSCs: SALL4; Lin28; FUBP3; Oct3/4; Nestin; TUBB3; DNMT3B; PODXL; SERPINB9; TPM1 and SFN (14-3-3 protein Sigma).

IPA of differentially expressed proteins

12 novel potential markers for iPSC were proposed and some were confirmed by Western blot analyses.

Contact information:

3421 Research Pkwy, WFM-310, Rockville, MD 20852.