Identifying Epigenetic Factors in Hepatocyte Differentiation to Improve Liver Therapy

Manraj S. Gill, Manveer Garcha, Tia Hackett, Ben Tschudy-Seney, Yu-yyu Duan Ph.D, Mark A. Zern M.D.
Stem Cell Clinical Research, Institute for Regenerative Cures, UCD Health System, Sacramento, CA

Introduction to Hepatology

- Hepatology is the study of the liver and involves means of administering its malfunctions and damages. The liver's greatest internal organ is composed largely of various types of hepatic cells. 

Hepatocytes, cells arising from the endoderm that are responsible for metabolism control, protein synthesis, maintenance of blood osmolarity and excretion, comprise ~80% of all hepatic cells.

Disorders of the liver include hepatitis, an inflammation of the liver caused by hepatocyte specific viruses, and hepatocellular carcinoma, liver cancer. (Chances of cancer are increased in patients with hepatitis as the natural regenerative processes can activate oncogenes, thereby triggering tumorigenesis).

Whole organ transplantation in cases of severe liver failure serves as an option but the life-long usage of immunosuppression and organ rejection along with the shortage in availability of organ donations express the need for restoring a patient's liver through more efficient methods such as insertion of cells with capacities to repair damage through regeneration.

Such types of cells are called stem cells, unspecialized cells present in our bodies with self-renewing and proliferative capacities and the multipotential to differentiate, become more specialized, into all tissue lineages.

Culturing hESCs

- Only specific lines (e.g. Line 17, 119) of stem cells are allowed and regulated by the FDA for use in research.
- These lines are grown on artificial media that allow intercellular connections and communications and the differentiation process due to the presence of growth factors. The extra-cellular matrix is essential for cell attachment. MEFs are grown on MEFs (because Embryonic Feeder). MEFs send proliferation signals to hESCs. MEFs are plated onto gelatin after 24 hours to prevent die-offs as cells usually attach to the MEF only on the sides and untreated areas would permit cells to attach to the plastic and prevent them from maintaining pluripotency.
- Cells need to be split if they are too confluent in the culture of colonies, this is essential for maintaining equal contact for each cell with the media and the required nutrients for survival and expression of proper characteristics. Each split is termed a passage.
- Confluency and pasaging ratios are determined by cell counts using a trypan blue assay: tests for cell viability and proliferating cells.
- Cell counting results:

<table>
<thead>
<tr>
<th>Passage</th>
<th>Cell Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6 x 10^6</td>
</tr>
<tr>
<td>2</td>
<td>1.2 x 10^6</td>
</tr>
<tr>
<td>3</td>
<td>1.0 x 10^6</td>
</tr>
</tbody>
</table>

- Average adjusted with dilutions concentrations and multiplied with 10,000 to determine number of cells in flask. (98 ± 8 x 10^6 = 7.84 million)
- Phase contrast of 119 cells grown on MEFs:

- DE induction through addition of Activin A growth factor media can be verified through test cell surface marker analysis. Cell surface markers are small molecules present on the surface of cells that act as unique identifiers of the cell type (FOX40, SOX17 and CXC4R4 are identifiers of Definitive Endoderm)

- Therefore, since methylated histones can either activate or repress activity based on specific patterns of regulation, knowledge of these patterns in DE cells would allow identification of conditions to efficiently derive hepatocytes from hESCs.

- DE cells present specific demethylases will be tested for relative activity

Epigenetics and Demethylases

- Genetic and epigenetic modification of stem cells would help in accelerating recovery through use of hESCs. Understanding of the epigenetic modifications currently involved in hepatocyte differentiation would allow us to up-regulate or down-regulate characteristics in the environment that expedite the process or those that hinder it, respectively. Various epigenetic modifications are achieved from methylation and demethylation of DNA to histone phosphorylation. Such factors are responsible for modulating chromatin structure and influence the presence of transcriptional factors.

- Note: histone proteins have an N-terminal amino-acid tail that undergoes epigenetic modifications. The N-terminal determines the state of the chromatin

- Figure 1.1 Primary Hepatocytes

Figure 1.1 Primary Hepatocytes

Hepatocytes derived from hESCs

- Albumin, essential for maintaining plasma osmolarity, is the most prevalent protein hepatocytes synthetize and its expression is highly regulated. 

Figure 4.1 e47 AT immuno-fluorescence to determine expression level of hepatocyte protein

Immunohistochemistry

- Immunofluorescence staining is the use of an anti-body based method to detect a specific protein. Gene expression analysis using Oct4, SSEA3 in hESC.

Figure 6.1 Markers for the 2 transcription factors are used to detect presence of proteins based on their cell surface markers by using a secondary anti-body stain

Figure 7.1 Integrity tests on extracted RNA to determine quality.