Pluripotent Stem Cells for Drug Discovery and Toxicity Assessment

December 5, 2017

Q) Which cell types can embryonic stem cells not differentiate into?
A) Most embryonic stem cell lines cannot differentiate spontaneously into trophectoderm

Q) Is it possible to bank differentiated iPSC cells in liquid nitrogen and then use them overtime for assays?
A) Yes, cells can be banked at progenitor stages. With this, large batches of cells can be generated and validated according to rigorous QC parameters. Same batches can then be used throughout screening campaigns in order to minimize variation.

Q) Did they stain for contaminating glia?
A) ICC images presented were obtained at early timepoints (day 22 in vitro). At this stage our cultures are 95-99% neuronal (according to beta III tubulin+ quantification). Glia cells take longer to differentiate in vitro. Best protocols published so far revealed iPSC-derived astrocytes within 30 days.

Q) The literature shows hyper then hypo, please explain?
A) We have performed long-term analyses of these motor neurons and could show that hyperexcitability is indeed followed again by hypoexcitability. So it is complex and long-term studies across consecutive time points are crucial to understand the disease-relevant phenotype better.

Q) Could you comment on the ability to derive differentiated blood cells (T-cells, monocytes, NK cells etc) from iPSEC cells?
A) Monocytes and NK cells work reasonably well. T-cells are a bit more complicated because the best way to make them requires feeder cells

Q) Do you have iPSC protocols optimized for DA neurons for α-syn mutation or neuron with pTau abnormalities? Thanks!
A)Yes, we also have protocols for DA neuron differentiation and we are testing different mutant backgrounds with it.

Q) What is the influence of patient genetic background on building iPSC bank?
A) The genetic background of a patient might affect the molecular and differentiation propensities of iPSC lines.

Q) How long have you kept your motoneurons in which ECM have the cells been cultured to generate patch clamp data?
A) We have kept the motor neurons for up to 70 days, either on PLO/Lam or astrocyte co-cultures for manual patch clamp.

Q) Other than neurons, what tissue type have you successfully differentiated into and scaled up?
A) We have made macrophages, keratinocytes, endothelial cells and cardiomyocytes at large scale.

Q) Have these iPSC-derived motoneurons been tested for modelization of NeuroMuscualr junction?
A) Not yet, but we are currently working towards such a co-culture system.

Q) How do you plan to use iPSc for drug discovery when it is very difficult to genetically manipulate them?
A) Actually, pluripotent stem cells are easily manipulated but the process is quite long and labor intensive!

Q) This a relatively new subject for me. However, specializing in serum free media I would like to know how these cells differentiate in vitro?
A) For all our protocols, we aim to use defined differentiation media that are serum-free and produced from pre-screened materials to ensure highest levels of batch-to-batch consistency and experimental reproducibility. This allows us to minimize the variation in our research. In addition we also aim to replace the Engelbreth-Holm-Swarm-mouse derived extracellular matrix, often marketed as Matrigel or Geltrex, which is a heterogeneous undefined mixture. Instead we use recombinant attachment factors, such as Laminins, Vitronectin etc. wherever possible. Serum free, defined media conditions allow for a controlled and reproducible cell culture environment that is amenable for industrial scales.

Q) Are there examples of using differentiated iPSC cells in drug potency assays in place of cell lines?
A) Yes, we are currently using sensory neurons derived from iPSC specific to patients suffering from neuropathic pain conditions to assess functions of analgesic drugs

Q) What are all the cell types we can make right now? How well are they characterized and what are ways to improve their performance?
A) Different cell types characterized by specific markers, the challenges is still to create specific cell types of any age. Lengthy differentiation protocols make studies with human pluripotent stem cells laborious, costly and difficult to standardize. That's why the most common strategy to induce age-like features in iPSC-derived lineages is the use of stress paradigms, i.e compounds that trigger mitochondrial stress or reactive oxygen species (ROS). Alternative strategies to manipulate cellular age in iPSC-derived lineages include insights from premature aging disorders, such as Hutchinson Guildford Progeria Syndrome (HGPS). Miller *et al*. developed a strategy to induce aging with progerin overexpression in human iPSC-derived lineages. The long-term goal is to reliably program and re-program cellular age independently of cell fate and thereby to create specific cell types of any age, e.g. 70-year old neurons, to model neurodegenerative disease.

Q) Proximal tubular kidney cells: have they been validated in regard to predicting kidney tox

A) We are currently doing this. At present, we can make cells very similar to PTEC from iPSC but the function of their transporter proteins are not identical to ex vivo derived PTEC. Nephrotoxic responses are predictable but again not completely identical so we have a bit more development work before we can reliably use the iPSC derived versions for drug testing

Q) May I have a little review of all the typical toxicity assays done during the screening process?
A) The development of high content imaging platforms with the capability to analyze multiple complex phenotypes in different cell types has a major impact on the way drug discovery is performed. This allows us to get a better understanding cellular responses and cytotoxicity in various cell types.

For Cytotoxicity and Cell viability assays on our High content imaging platform we use:
- Nuclei counting
- Quantification of cytoplasmic markers
- Spot analysis
- Nuclear shrinking
- Mitochondrial mass detection

For apoptosis assays we use:
- Nuclear fragmentation
- Quantification of cytoplasmic markers
- Quantification of nucleur markers

We also use a so called Cell Painting approach, which is a high-content imaging based assay for morphological profiling using multiplexed fluorescent dyes, with the aim to identify biologically relevant similarities and differences among samples based on these profiles. With this, profiles of different cell populations can be compared to identify the phenotypic impact of chemical or genetic pertubations (Knock out screens). We can then group compounds and/or genes into functional pathways and identifying signatures of disease. For details, please refer to Bray et al, Nature Protocols 2016 (Vol 11(9), 1757-1774).

Q) How do you ensure 100% differentiation of iPSCs into the target tissue of interest? and what is the prospect of using such cells in therapy in the near future?
A) One of the key advantages with HCA and sophisticated imaging software is that 100% purity of target cell type is not required. For those methods that take advantage from pure populations, i.e. for transcriptome analysis, we have FACS sorting established.

Q) What are all the different types of cells you can start with for iPSC? (Bone/Skin/etc?) How does the starting cell effect the drug discovery process?
A) iPSC can be generated from a number of cells types, such as Fibroblasts, PBMCs, or keratinocytes. Global DNA methylation and gene expression analyses revealed a tendency of iPSC lines to cluster according to the donor rather than cell type of origin. iPSCs derived from transcription factor mediated reprogramming may bear different types of genetic or epigenetic variations, that can be introduced form various sources either the original material (fibroblast), the reprogramming or the culture conditions.

Genetic variations are

- Aneuploidy
- Copy number variations (CNVs)
- Or Single nucleotide variations (SNVs)

Epigenetic variations are

- Source cell memory
- Aberrant DNA methylation
- X chromosome inactivation