

Standardisation of Pluripotent Stem Cell Cultures for Toxicity Testing

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INTRODUCTION

- Human pluripotent stem cells (PSCs) and their derivatives can support safety assessments in a mode of action framework, by making relevant human cellular models available.
- Stably culturing PSCs (hESCs & hiPSCs) and obtaining defined differentiated cell cultures are prerequisites for robust in-vitro tests.
- Since the quality of the initial undifferentiated stem cell cultures can affect the differentiation process, it is necessary to set up a panel of quality control (QC) assays to judge the suitability of a stem cell culture and their differentiated derivatives for toxicity testing.
- An agreement within the cluster on the functionality and differentiation status of stem cell derivatives used in toxicological experiments is of high relevance in order to interpret results deriving from different partner organisations.

OBJECTIVES

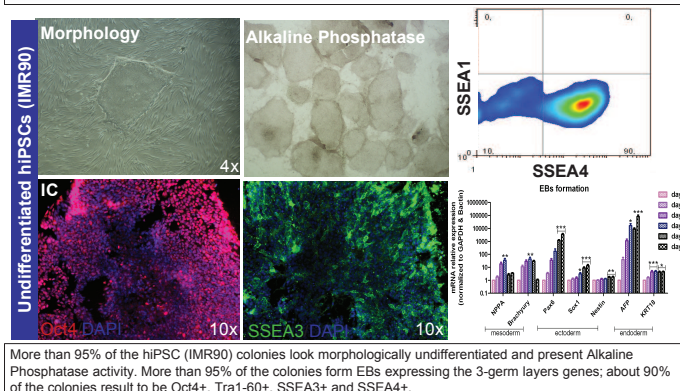
- Review of the proposed quality standards in the literature (Pistollato F, Bremer-Hoffmann S, Healy L, Young L, Stacey G. Standardization of pluripotent stem cell cultures for toxicity testing. Expert Opin Drug Metab Toxicol. 2012 Feb;8(2):239-57. Epub 2012 Jan 17.)
- Development of QCs for PSC based toxicological studies by: (i) defining final aims of the differentiation process (define genotype, phenotype and functionality of reference cells such as e.g. primary hepatocytes); (ii) identifying a minimum set of markers and functional assays characterising the cellular models used in various toxicity studies; (iii) making standard operating procedures for assessments of markers and functional assays available, allowing a comparison of data from different experimental groups.
- Agreement within the cluster on a minimum set of markers and functional assays.
- Defining QCs for the PSC-based toxicological studies.
- In house application of the main QCs for human induced pluripotent stem cells (hiPSC, IMR90, from I-Stem) characterization & neuronal differentiation.

LIST OF PROPOSED QCs

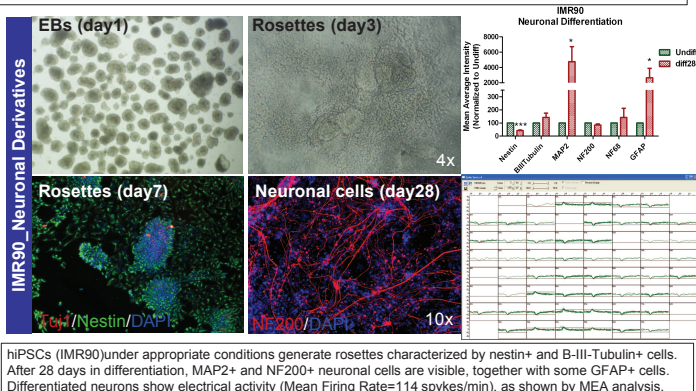
Undifferentiated PSCs & Principal QC analyses	Derivatives developed for Toxicology & Principal QC analyses	
Analysis of cell morphology	Hepatocytes	Analysis of markers/genes expression: CYP3A4, CYP2B6, CYP1A1/2, etc...
Analysis of colony morphology		General activities: urea synthesis, glycogen uptake, albumin secretion, etc...
Analysis of proliferation		phase II activities: Measurement of activities GST and UGT isoenzymes
Analysis of gene expression (Positivity for: Nanog, TDGF, Oct4, GABRB3, GDF3, DNMT3, PODXL, others...)		drug transporter capacity: analysis of ABC transporter expression and activity; analysis of solute carrier (SLC) transporter expression.
Analysis of cell surface markers (Positivity for: Oct-4, SSEA-3/-4, Tra-1-60/Tra-1-81, Sox 2, CD30, CD9)	Cardiomyocytes	Number of contracting clusters
Analysis of Telomerase activity		Analysis of markers expression: Tropomyosin, Troponin I, Actinin, etc...
Analysis of Alkaline Phosphatase activity		Analysis of gene expression: brachyury, Nkx2.5, alpha-cardiac actin, npa
Analysis of genetic stability		Generation of action potentials and sensitivity to channel blockers
Assessment of pluripotency by Embryoid Bodies formation & analysis of lineage specific markers (e.g. AFP, brachyury, Sox-1, others...)	Neurons	Analysis of markers expression: B-III-tubulin, MAP2, NF200, etc...
		Analysis of gene expression: Sox1, Pax6, NCAM, nestin Axons formation, Generation of action potentials, Presence of ion channels, Neurite outgrowth

RESULTS

In House QCs for iPSCs characterization



In House QCs for neuronal derivatives characterization



NEXT STEPS

- Agreement within the cluster on a minimum set of markers and functional assays for cells entering into hepatotoxicity and cardiotoxicity assessments.
- Define an easy-to-use reporting template allowing an evaluation on the maturation status of toxicological target cells (in collaboration with Toxbank). The data collection should support the identification of optimal differentiation protocols.
- Discuss the final aims of the differentiation protocols and identify suitable reference cells (e.g. human primary hepatocytes).

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