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In vitro toxicity testing using human pluripotent stem cell derivatives

Akademisk avhandling
Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Biotech-huset, Arvid Wallgrens Backe 20, torsdagen den 15 december, klockan 09.00

av Gustav Holmgren

Fakultetsopponent:
Professor Chris Denning
University of Nottingham, United Kingdom

Avhandlingen baseras på följande delarbeten

I. Holmgren, G., Sjögren A. K., Barragan I., Sabirsh A.,. Sartipy P, Synnergren J., Bjorquist P., Ingelman-Sundberg M., Andersson T. B., and Edsbagge J. *Long-term chronic toxicity testing using human pluripotent stem cell-derived hepatocytes* Drug Metab Dispos 42(9): 1401-1406. 2014

II. Holmgren, G., Synnergren J., Bogestal Y., Améen C., Akesson K., Holmgren S., Lindahl A., and Sartipy P. *Identification of novel biomarkers for doxorubicin-induced toxicity in human cardiomyocytes derived from pluripotent stem cells* Toxicology 328: 102-111. 2015

III. Holmgren, G., Synnergren, J., Andersson, C.X., Lindahl, A., and Sartipy, P. *MicroRNAs as potential biomarkers for doxorubicin-induced cardiotoxicity* Toxicol *In vitro* 34, 26-34. 2016

IV. Holmgren, G., Sartipy, P., Andersson, C.X., Lindahl, A., and Synnergren, J. *Expression profiling of human pluripotent stem cell-derived cardiomyocytes exposed to doxorubicin – integration and visualization of multi omics data* Manuscript

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In vitro toxicity testing using human pluripotent stem cell derivatives

Gustav Holmgren
Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, 2016.

Abstract

Toxicity testing of chemicals, drug candidates, and food additives is in need of a change. The present methods, mainly consisting of animal models with their associated ethical concerns, are expensive, time-consuming, and importantly they are often poor predictors of the human *in vivo* toxicity. With the rapid biotechnology development, a paradigm shift for toxicity testing is emerging, focusing on bioinformatics, computational toxicity, systems biology, and cell-based *in vitro* models.

The aim of this thesis was to investigate the utility of using cells, i.e. hepatocytes and cardiomyocytes, derived from human pluripotent stem cells (hPSC) as *in vitro* models for toxicity testing. The first part explored the feasibility of using hPSC-derived hepatocytes to study toxic drug exposure, and in addition investigated the relevancy of the cellular response. The second and major part of this thesis focused on hPSC-derived cardiomyocytes and the in-depth study of doxorubicin-induced toxicity.

The studies revealed that the differentiation processes and culturing of hPSC-derivatives are stable and reproducible to form the basis for *in vitro* models for toxicity testing, even for longer studies over two weeks. The hepatocytes and the cardiomyocytes showed sensitivity towards the toxic compounds and both cell models displayed a relevant cellular response to the toxic exposure. For example, the hepatocytes showed evidence of steatosis and phospholipidosis when incubated with hepatotoxic compounds over time. Besides an evident effect of doxorubicin on the cardiomyocyte function, the cells also proved to be useful for more in-depth mechanistic evaluations, as these studies gave insight, on multiple biological levels, in plausible mechanisms and identified potential biomarkers for doxorubicin-induced cardiotoxicity.

In conclusion, this thesis presents findings that supports the vision and strategy of using *in vitro* models based on hPSC-derivatives together with advanced omics technologies for toxicity testing and risk assessment of drugs, food additives, and chemicals.

Keywords: toxicity testing, human pluripotent stem cells, cardiomyocytes, hepatocytes, microarray, quantitative proteomics, bioinformatics, transcriptomics, microRNA

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