Human-induced pluripotent stem cell reporters for high-content screening of stress response activation identifying target organ-specific toxicities

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P16-17
Imaging-based 3D spheroid clearing method for prediction of cardiotoxicity

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Toxicity prediction and quantitative analysis using spheroids is an attractive research tool, but it is difficult to use for a wide range of screening because the experimental process of imaging and analysis is not easy. Moreover, image-based research is difficult to use in a toxicity prediction model because the interpretation of results may differ depending on the skill level of the researcher or image resolution. To overcome this problem, we applied clearing technology to spheroids to improve the overall image resolution and build an easy platform for image-based toxicity prediction. We previously produced tissue clearing kits through collaboration with BINAREE KOREA, a bio-venture company, and entered into a technology use agreement, and additionally established a technology for clearing in iPSC-derived spheroids that can be used to implement disease models. We proceeded to clear the iPSC-derived cardiospheroids with an established clearing method, use pro-BNP as a toxicity marker, implement a 3D bio-image of the entire cell tissue using a light sheet fluorescence microscope, and verify the improvement in image resolution. Cardiospheroids treated with levofloxacin showed weak expression of toxicity markers in the center of spheroids, and cardiospehroids treated with the cardiotoxic drug moxifloxacin showed toxicity in the entire spheroid. We previously verified the toxicity of cardiomyocytes through cardiac-spheroids containing only cardiomyocytes, and has now grafted clearing technology on more evolved cardiac-organoid models to evaluate toxicity and to evaluate cellular tissue within cardiac-organoid-derived disease models. We are continuously conducting research on toxicity evaluation by realizing the entire 3D bioimage. Through this research, it is expected that it will be used for the identification of the cause of the disease and the development of treatment by developing a disease model and predicting the efficacy and toxic response of new drugs more accurately and quickly by using the transparency technology for cardiac organoids. Furthermore, we also present the possibility that our protocol can also be utilized for patient-tailored toxicity prediction evaluation.

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P16-18
Human-induced pluripotent stem cell reporters for high-content screening of stress response activation identifying target organ-specific toxicities

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The development and validation of next generation *in vitro* test systems to improve the prediction of chemical-induced adversity for specific target organs is critical. Human-induced pluripotent stem cells (hiPSCs) are a valuable source for studying chemical-induced toxicities in various organ specific cell types while having the same genetic background. This enables to identify organ specific toxicities during chemical safety screening. The activation of stress response pathways is one of the early key events leading to organ injury. Monitoring critical genes within these pathways would both give insight in the mode-of-action as well as aid in the prediction for liabilities for adverse outcomes upon exposure. Here, using the CRISPR/Cas9 technology we have build a reporter panel in hiPSCs in which genes based on transcriptomic analysis for critical stress response pathways were endogenously tagged with eGFP. The hiPSC reporter panel covers inflammatory signalling, DNA damage, ER stress and oxidative stress response pathways. The activation of stress response pathways was monitored using live cell confocal imaging upon chemical exposure in multiple cell types after differentiation (e.g. hepatocyte-like cells, proximal tubular-like cells, cardiomyocytes). Differences in sensitivity towards chemical exposure between different organ specific cell types could be observed. To allow to study multi-cellular dependent responses and the utility of more advanced 3D systems, these reporters can be grown as organoids for both the liver and kidney. This allows to study cell specific responses within a multi-cellular environment more recapitulating the *in vivo* settings. We anticipate that these stress response hiPSC reporters will aid in improving chemical safety testing allowing the identification of the mode-of-action as well as specific target organ or cell specific toxicities.

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P16-19
The role of glucose homeostasis and glucocorticoid signalling for the development of relevant hepatic *in vitro* models for toxicology, drug metabolism and energy metabolism studies

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