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Oxidative Stress Disrupt Differentiation of Human Induced Pluripotent Stem Cells

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An array of substances mediate induction of reactive oxygen species (ROS) and it is thus important to understand its potential implications. One unexplored area is the process of early embryonal development.

We thus embarked on addressing this issue by exposing human induced pluripotent stem cells (hIPSC) during differentiation into the three germ cell layer specific cell types, cardiomyocytes, hepatocytes and neural rosettes in the Reprotracker assay. Model compounds inducing oxidative stress such as Potassium Bromate (KBrO3) and radiation (X-rays) were investigated along with benzo(a)pyrenediolepoxide (BPDE), and the cell cultures were exposed continuously (KBrO3 and BPDE) or intermittent (X-rays) to the compounds during the differentiation. Well-established human teratogen (thalidomide/retinoic acid) and non-teratogen (saccharin) were included as assay controls.

Cell viability was first broadly assessed to instruct selection of exposure concentrations/ doses of the agents. The status of differentiation of hiPSCs into the three germ layer specific cell types was assessed by detection of morphological abnormalities and decline in contraction of cardiomyocytes combined with expression patterns of biomarker genes using qRT-PCR. Extended gene expression analyses of genes involved in differentiation to all three germ cell layers, DNA damage response, DNA repair and epigenetic regulation is being conducted. Preliminary data will be presented.

The preliminary data suggest that ROS-producing compounds lead to disruption of differentiation into neural rosettes indicating developmental toxicity. The finding was supported by the gene expression patterns of the biomarker genes PAX6 and NESTIN.

Keywords:
Human induced pluripotent stem cells; reactive oxygen species; DNA damage; differentiation, germ cell layer.