# Letter to the editor:

# STEM CELL-BASED TEST METHODS

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*Dear Editor,*

Recently, Agapios Sachinidis and colleagues from the University of Cologne published a review about possibilities and limitations of stem cell-based test methods in pharmacology and toxicology (Sachinidis et al., 2019). In recent years, much progress has been achieved concerning *in vitro* techniques of liver (Godoy et al., 2013; Grinberg et al., 2014; Leist et al., 2017; Ghallab et al., 2016), kidney (Sjögren et al., 2018; Jiang et al., 2018; Su et al., 2016; Valente et al., 2012; Lee et al., 2017), neuronal (Keil et al., 2018; Yang et al., 2018; Colaianna et al., 2017; Sisnaiske et al., 2014) and developmental toxicity (Adam et al., 2019; Bridges et al., 2019; Abbott, 2019). Particularly, in developmental and reproductive toxicity testing, large numbers of animals are needed for analysis of a single compound (Krug et al., 2013). Therefore, stem cell-based test systems are currently developed (Shinde et al., 2014, 2015, 2016; Krug et al., 2013; Meganathan et al., 2012). They recapitulate differentiation into cells of the three germ layers (Meganathan et al., 2012; Shinde et al., 2016, 2017) or differentiation into neural ectodermal progenitor cells (Rempel et al., 2015; Waldmann et al., 2014). Stem cells are exposed to test compounds during the differentiation process and compound associated gene expression changes are monitored.

Indices have been developed to identify a possible hazard of developmental toxicity based on genome-wide expression data. A precondition is the definition of so-called developmental genes of a test system. Developmental genes are up- or down-regulated during the differentiation process in the absence of test compounds. Developmental potency describes the fraction of all developmental genes, whose expression is altered by test compounds (Shinde et al., 2017). Although large validation studies are still required, several developmental toxicants, e.g. thalidomide and valproic acid, have been successfully differentiated from negative control compounds (Meganathan et al., 2012; Waldmann et al., 2014).

Much progress has been achieved in analyzing disturbed developmental processes. However, it still remains challenging to differentiate stem cells to adult cell types that closely resemble the corresponding mature cell type *in vivo* (Godoy et al., 2015; Cameron et al., 2015). The authors of the present review describe why this is so difficult, using stem cell derived hepatocyte-like cells as an example (Sachinidis et al., 2019). Problems are due to incomplete endoderm patterning (Zorn, 2008; Gordillo et al., 2015) and to still suboptimal protocols to trigger the final differentiation of hepatoblasts to mature hepatocytes.

Stem cell-based alternative test methods offer powerful tools to analyze developmental toxicity *in vitro*, but there is still a long way to go until the techniques are ready for routine use and to replace animal studies in pharmacology and toxicology.

## *Conflict of interest*

The author declares no conflict of interest.

## REFERENCES

Abbott BD. Embryonic midfacial palatal organ culture methods in developmental toxicology. Meth Mol Biol. 2019;1965:93-105.

Adam AHB, Zhang M, de Haan LHJ, van Ravenzwaay B, Louisse J, Rietjens IMCM. The in vivo developmental toxicity of diethylstilbestrol (DES) in rat evaluated by an alternative testing strategy. Arch Toxicol. 2019 May 22. doi: 10.1007/s00204-019-02487-6. [Epub ahead of print].

Bridges KN, Magnuson JT, Curran TE, Barker A, Roberts AP, Venables BJ. Alterations to the vision-associated transcriptome of zebrafish (Danio rerio) following developmental norethindrone exposure. Environ Toxicol Pharmacol. 2019;69:137-142.

Cameron K, Tan R, Schmidt-Heck W, Campos G, Lyall MJ, Wang Y, et al. Recombinant laminins drive the differentiation and self-organization of hESC-derived hepatocytes. Stem Cell Reports. 2015;5:1250-62.

Colaianna M, Ilmjärv S, Peterson H, Kern I, Julien S, Baquié M, et al. Fingerprinting of neurotoxic compounds using a mouse embryonic stem cell dual luminescence reporter assay. Arch Toxicol. 2017;91:365-91.

Ghallab A, Cellière G, Henkel SG, Driesch D, Hoehme S, Hofmann U, et al. Model-guided identification of a therapeutic strategy to reduce hyperammonemia in liver diseases. J Hepatol. 2016;64:860-71.

Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S, et al. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicol. 2013;87:1315-530.

Godoy P, Schmidt-Heck W, Natarajan K, Lucendo-Villarin B, Szkolnicka D, Asplund A, et al. Gene networks and transcription factor motifs defining the differentiation of stem cells into hepatocyte-like cells. J Hepatol. 2015;63:934-42.

Gordillo M, Evans T, Gouon-Evans V. Orchestrating liver development. Development. 2015;142:2094-108.

Grinberg M, Stöber RM, Edlund K, Rempel E, Godoy P, Reif R, et al. Toxicogenomics directory of chemically exposed human hepatocytes. Arch Toxicol. 2014;88:2261-87.

Jiang S, Lin Y, Yao H, Yang C, Zhang L, Luo B, et al. The role of unfolded protein response and ER-phagy in quantum dots-induced nephrotoxicity: an in vitro and in vivo study. Arch Toxicol. 2018;92:1421-34.

Keil KP, Miller GW, Chen H, Sethi S, Schmuck MR, Dhakal K, et al. PCB 95 promotes dendritic growth in primary rat hippocampal neurons via mTOR-dependent mechanisms. Arch Toxicol. 2018;92:3163-73.

Krug AK, Kolde R, Gaspar JA, Rempel E, Balmer NV, Meganathan K, et al. Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach. Arch Toxicol. 2013;87:123-43.

Lee WK, Probst S, Santoyo-Sánchez MP, Al-Hamdani W, Diebels I, von Sivers JK, et al. Initial autophagic protection switches to disruption of autophagic flux by lysosomal instability during cadmium stress accrual in renal NRK-52E cells. Arch Toxicol. 2017;91:3225-45.

Leist M, Ghallab A, Graepel R, Marchan R, Hassan R, Bennekou SH, et al. Adverse outcome pathways: opportunities, limitations and open questions. Arch Toxicol. 2017;91:3477-505.

Meganathan K, Jagtap S, Wagh V, Winkler J, Gaspar JA, Hildebrand D, et al. Identification of thalidomide-specific transcriptomics and proteomics signatures during differentiation of human embryonic stem cells. PLoS One. 2012;7(8):e44228.

Rempel E, Hoelting L, Waldmann T, Balmer NV, Schildknecht S, Grinberg M, et al. A transcriptome-based classifier to identify developmental toxicants by stem cell testing: design, validation and optimization for histone deacetylase inhibitors. Arch Toxicol. 2015;89:1599-618.

Sisnaiske J, Hausherr V, Krug AK, Zimmer B, Hengstler JG, Leist M, et al. Acrylamide alters neurotransmitter induced calcium responses in murine ESC-derived and primary neurons. Neurotoxicology. 2014;43:117-26.

Sachinidis A, Albrecht W, Nell P, Cherianidou A, Hewitt N, Edlund K, et al. Road map for development of stem cell-based alternative test methods. Trends Mol Med. 2019, doi: 10.1016/j.molmed.2019.04.003. [Epub ahead of print].

Shinde V, Chaudhari U, Sotiriadou I, Hescheler J, Sachinidis A. In vitro methods for cardiotoxicity testing. In:Bal-Price A, Jennings P (eds): In vitro toxicology systems (pp 45-77). New York: Springer, 2014.

Shinde V, Klima S, Sureshkumar PS, Meganathan K, Jagtap S, Rempel E, et al. Human pluripotent stem cell based developmental toxicity assays for chemical

safety screening and systems biology data generation. J Vis Exp. 2015;100:e52333.

Shinde V, Sureshkumar P, Sotiriadou I, Hescheler J, Sachinidis A. Human embryonic and induced pluripotent stem cell based toxicity testing models: future applications in new drug discovery. Curr Med Chem. 2016;23:3495-509.

Shinde V, Hoelting L, Srinivasan SP, Meisig J, Meganathan K, Jagtap S, et al. Definition of transcriptome-based indices for quantitative characterization of chemically disturbed stem cell development: introduction of the STOP-Toxukn and STOP-Toxukk tests. Arch Toxicol. 2017;91:839-64.

Sjögren AK, Breitholtz K, Ahlberg E, Milton L, Forsgard M, Persson M, et al. A novel multi-parametric high content screening assay in ciPTEC-OAT1 to predict drug-induced nephrotoxicity during drug discovery. Arch Toxicol. 2018;92:3175-90.

Su R, Xiong S, Zink D, Loo LH. High-throughput imaging-based nephrotoxicity prediction for xenobiotics with diverse chemical structures. Arch Toxicol. 2016;90:2793-808.

Valente MJ, Henrique R, Vilas-Boas V, Silva R, Bastos Mde L, Carvalho F, et al. Cocaine-induced kidney toxicity: an in vitro study using primary cultured human proximal tubular epithelial cells. Arch Toxicol. 2012;86:249-61.

Waldmann T, Rempel E, Balmer, N, König, A, Kolde, R, Gaspar, JA, et al. Design principles of concentration-dependent transcriptome deviations in drug-exposed differentiating stem cells. Chem Res Toxicol. 2014;27:408-20.

Yang B, Bai Y, Yin C, Qian H, Xing G, Wang S, et al. Activation of autophagic flux and the Nrf2/ARE signaling pathway by hydrogen sulfide protects against acrylonitrile-induced neurotoxicity in primary rat astrocytes. Arch Toxicol. 2018;92:2093-108.

Zorn AM. Liver development (October 31, 2008). In: StemBook. Cambridge, MA: Harvard Stem Cell Institute, 2008.