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EDITORIAL

Highlight report: perspectives in stem cell research—unbiased quantification of the similarity between in vitro generated and primary hepatocytes

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In vitro systems represent a cutting-edge topic in toxicology (Grinberg et al. 2014; Frey et al. 2014; Godoy et al. 2013; Godoy 2011; Stewart and Marchan 2014). Particularly, in the fields of liver (Godoy et al. 2013; Zellmer et al. 2010; Godoy and Bolt 2012), kidney (Faiz et al. 2015; Yang et al. 2014), and neurotoxicity (Zimmer et al. 2014; Waldmann et al. 2014; Krug et al. 2013), in vitro systems are frequently used. Primary human cells still represent a gold standard in toxicological research (Heise et al. 2012; Ghallab 2013, 2014a, b). However, their use is hampered by difficult availability (Hewitt et al. 2007; Hengstler et al. 2000).

Theoretically, stem cells offer a possibility to overcome this limitation. However, it is discussed controversially to which degree stem cell-derived cells resemble primary cells. In the field of stem cell-derived hepatocyte-like cells, some authors reported that generation of 'functional,' 'high fidelity' hepatocytes from stem and precursor cells is already possible (Medine et al. 2013; Szkolnicka et al. 2014; Huang et al. 2014), while others present a more critical point of view (Morris et al. 2014; Hengstler et al. 2005; Brulport et al. 2007).

In this controversial situation, it is helpful that Godoy et al. (2015) have recently published a technique how the cellular nature of stem cell-derived hepatocytes can be quantitatively determined in an unbiased manner. The authors studied hepatocyte-like cells that have been differentiated from human embryonic stem cells as well as induced pluripotent stem cells in three different research centers. Genome-wide expression analysis was performed to be able to determine the nature of the stem cell-derived cells in an unbiased manner. The authors used CellNet, a recently established software to quantify the similarity of the stem cell-derived cells with human cell types, such as hepatocytes, colon, and kidney cells (Cahan et al. 2014; Morris et al. 2014). The results obtained with the genome-wide data of the cells from all three research centers were remarkably similar, although each center used an independently optimized differentiation protocol. As expected, the score for embryonic stem cells strongly decreased, while the score for hepatocytes strongly increased but not to values of 1.0, the result of primary human hepatocytes but to (depending on the different protocols) values ranging between 0.5 and 0.6. Therefore, the stem cell-derived cells covered only a bit more than half of the 'distance' between stem cells and real human hepatocytes. One reason for incomplete differentiation is a cluster of genes representing mature liver functions that are expressed at too low values. This 'unsuccessful cluster' is controlled by the transcription factors HNF1, CAR, FXR, and PXR, showing too low activities in the stem cell-derived hepatocytes. Genome-wide analysis of gene regulatory networks also revealed two 'unwanted' features of the stem cell-derived hepatocyte-like cells. The scores of colon- as well as fibroblast-associated genes clearly increased. The study of Godoy and colleagues leads to some generally relevant key messages for the stem cell community:

- Characterizing stem cell-derived cells by a limited number of selected markers is no longer state of the art. The risk of a bias and misleading conclusions is high.
- Today, genome-wide analyses, either by RNAseq or gene array, are fast and relatively cheap. They allow an unbiased analysis, and the nature of the analyzed cells

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can be quantitatively described. This also allows identification of unwanted features, such as induction of colon genes. Moreover, transcription factors responsible for 'unwanted side effects' of current hepatocyte differentiation protocols will be identified. Although numerous studies about hepatocyte differentiation have been published, the feature of unwanted colon differentiation remained unnoticed until recently. Software for analysis of genome-wide data, such as CellNet, or clustering as well as overrepresentation analysis tools is free and publicly available, further facilitating the application of unbiased genome-wide techniques.

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