The dilemma of cultivated hepatocytes

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Isolated and cultivated primary hepatocytes represent a frequently used in vitro system for studies of drug metabolism, enzyme induction and hepatotoxicity (Hewitt et al. 2007; Sinha et al. 2007; Lee et al. 2007; Ullrich et al. 2007; Carmo et al. 2005; Parody et al. 2007; Magdalan et al. 2009). An additional relatively new field of application is analysis of gene expression patterns in cultured hepatocytes (Muguruma et al. 2008; Thum and Borlak 2008; Schug et al. 2008; Tuschl and Mueller 2006). Major limitations are limited availability for human hepatocytes and the relatively fast dedifferentiation of cultured rodent hepatocytes. Establishment of cryopreservation techniques has improved the availability of human hepatocytes but still the situation is not fully satisfactory, because some hepatocellular functions decrease due to the freezing and thawing procedure (Hengstler et al. 2000; Kafert-Kasting et al. 2006; Gebhardt et al. 2003). Well-known strategies to reduce dedifferentiation of rodent hepatocytes are the cultivation in three-dimensional systems, such as collagen sandwiches or alginate beads (Hewitt et al. 2007; Ringel et al. 2005; Schug et al. 2008), hormonal additives as well as epigenetic modulators (Ringel et al. 2002; Snykers et al. 2007). However, to which degree all in vivo like functions of hepatocytes can be maintained by these techniques remains to be demonstrated by genome wide analyses in combination with functional tests. Unfortunately, one of the most promising approaches, differentiation of an unlimited supply of human hepatocytes from stem and precursor cells has not yet succeeded in generating fully differentiated hepatocytes

(Hengstler et al. 2005; Nussler et al. 2006; Brulport et al. 2007; Aurich et al. 2007; Ruhnke et al. 2005a, b). One of the most amazing limitations is the failure to achieve an efficient proliferation (and re-differentiation) of human hepatocytes in vitro, although hepatocytes have an excellent proliferation capacity in vivo after hepatectomy or regeneration from toxic liver damage (Höhme et al. 2007; Klingmüller et al. 2006). These limitations led to a situation where despite of their known dedifferentiation human hepatoma cell lines are still used in toxicity testing (O'Brien et al. 2006; Westerink et al. 2008; Bauer et al. 2006; Nagashima et al. 2007; Skupinska et al. 2007) instead of the better but limited primary human hepatocytes. Because rodent hepatocytes dedifferentiate in vitro still many scientists prefer in vivo hepatotoxicity testing to in vitro systems (Spicker et al. 2007; Franc et al. 2008; Abe et al. 2008; Chou et al. 2008; Nishimura et al. 2008; Chan et al. 2008). What may be a way of overcoming this dilemma? In brief: more thorough basic research and less alchemy. Research in the field of hepatocyte in vitro systems has suffered from “trial and error” approaches aimed at improving hepatocellular functions without knowing the responsible mechanisms. We first need to understand which signalling pathways or signalling network constellations promote hepatocyte dedifferentiation. Which are the critical transcription factors? Which signalling pathways and mechanisms are active in proliferating hepatocytes in vivo but can not sufficiently be activated in vitro to achieve cell cycle progression? We should realize that it represents a “mission impossible” to manipulate stem cells in vitro to form a cell type as complex as a fully differentiated hepatocyte, when we do not even know the critical factors to maintain the differentiated state of cultured primary hepatocytes. Based on this still missing knowledge the responsible mechanisms could specifically be modified by numerous strategies ranging

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from small molecules and siRNA to support by specific components of the extracellular matrix and 3D culture systems. An important aspect will be genome wide characterization of gene expression patterns and comparison to tissues *ex vivo*. Selection of just a few markers may lead to biased conclusions. Since the mechanisms switching hepatocytes between a differentiated, a dedifferentiated as well as a proliferating state are not yet fully known, a fast and easy way out of the dilemma is unlikely. However, understanding the mechanisms is the only promising strategy.

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