Generation of proliferating human liver sinusoidal endothelial cells (upcyte® LSECs)
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INTRODUCTION
Isolated liver cells, such as hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer Cells and hepatic stellate cells, are frequently used to study hepatic metabolism, toxicity and diseases. The current in vitro culture models, however, have several disadvantages e.g. short culture longevity and artificial culture conditions that focus mainly on a single cell type in 2D culture. The use of primary cells in vitro is compromised by the limited quantity of cells that can be isolated from one donor, a lack of or very restricted proliferation capacity (hepatocytes and LSECs) and/or the change from a quiescent to an activated state (hepatic stellate cells).

Therefore, we investigated whether the transduction of proliferation-inducing genes could extend the lifespan of primary LSECs without losing their primary characteristics (so-called “upcyte® technology”) as previously demonstrated with hepatocytes (upcyte® hepatocytes).

RESULTS

Function of LSECs in the liver

LSECs constitute the sinusoidal wall and can be regarded as unique capillaries, which differ from other capillaries in the body, as they possess open pores or fenestrae lacking a diaphragm and a basal lamina underneath the endothelium. Fenestrae, arranged in so-called selective “sieve plates”, filter fluids, solutes and particles that are exchanged between the sinusoidal lumen and the space of Disse. Among the various substances that are known to be endocyotically by LSECs are proteins, glycoproteins, lipoproteins, and glycosaminoglycans.

Foreign soluble macromolecules and colloids are eliminated from the circulation mainly by receptor-mediated pinocytosis. There are only three different receptors, which have been functionally observed in LSECs and are responsible for uptake of a large number of different ligands:

1. The Mannose receptor (MR) eliminates collagen waste molecules, preventing accumulation of intravascular clotting and recruits lysosomal enzymes for degradation.
2. The hyaluronan/scavenger receptor (HA/S-R) plays an important role in the physiological mechanism contributing to maintaining homeostasis and preventing atherosclerosis by binding to type I and III pro-collagen andatherogenic molecules such as oxidized LDL and advanced glycation end products.
3. The Fcγ-receptor (FcγR2bIII, CD32b) takes up IgG coated particles and soluble IgG immune complexes (taken up almost exclusively in the liver). Other functions include interaction with viruses (e.g. HIV-1). (Smedbro et al., 2004).

Characterization of upcyte® LSECs: the cells express...

...primary endothelial cell markers

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<th>Morphology</th>
<th>CD31</th>
<th>VWF</th>
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Morphology was comparable to primary cell LSECs. CD31 was present in all cells investigated and showed a typical membrane localization. VWF (von-Willebrand-factor) was evident as punctate structures that were more intense around the nucleus, indicating its presence in the endoplasmic reticulum.

The ability to form tubes in Matrigel® was analyzed in upcyte® LSECs at different densities. A seeding density of 12,500 cells/cm² was found to be optimal, since these upcyte® LSECs formed tubes from single cells. Uptake of ligand Ac-LDL (Alexa488-Ac-LDL), indicating the presence of the SR-A (Scavenger receptor A), a common endothelial cell receptor, was evident. Lectin (Ulex Europaeus Lectin1) was strongly expressed.

...LSECs specific markers

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<th>MR – FITC Ovalbumin</th>
<th>HA/S – FITC-AGG</th>
<th>FcyR – FITC-AGG</th>
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Immunofluorescence staining of LSEC-specific receptors

The cells express the three major uptake receptors: there was a high expression of MR and FcyR in upcyte® LSECs. The staining of HA/S-receptor was visible, but weak and not in all cells. A possible approach for better marker expression is elongation of culture time and medium optimization.

Gene expression profile

Expression profiles of upcyte® and primary LSECs were generated using Affymetrix whole genome BeadChip® Sentrix arrays HumanHT-12 v4. Preliminary analysis of upcyte® LSECs and primary LSECs that have been in culture for 3 days revealed very few changes in the expression profile. Only 0.45% (218 genes) of a total of 48,107 genes analyzed were found to be up- or downregulated more than 2-fold.

SUMMARY & CONCLUSION

In conclusion, we developed a novel technique which enables primary human LSECs to proliferate additional population doublings whilst retaining an adult phenotype when cultured at confluence. The resulting cells called “upcyte® LSECs” retained important endothelial cell markers, such as CD31 and VWF, and exhibited functional uptake of LDL, as well as the ability to form tubes in Matrigel®, LSEC-specific uptake of ligands or the expression of the corresponding uptake receptors (MR, FcyR2bIII and HA/S-R) could be detected. These data support that upcyte® LSECs are very uniquely and applicable to cell based assays as co-culture (e.g. with hepatocytes) and toxicity studies. Moreover, this technology allows for the generation of large batches of upcyte® LSECs enabling a reproducible and standardized experimental setting.