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

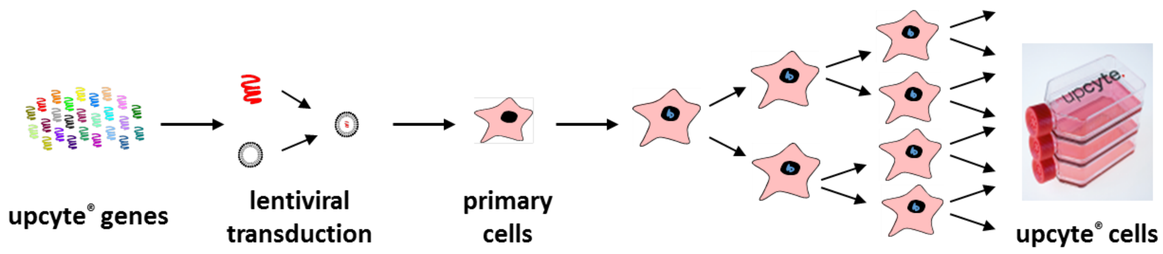
Function of LSECs in the liver

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 endocytosed 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 and atherogenic molecules such as oxidized LDL and advanced glycation end products.
(3) The **Fc-ɣ-receptor (FcɣR2BII, 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). [Smedsrod *et al*., 2004].

Generation of upcyte® LSECs

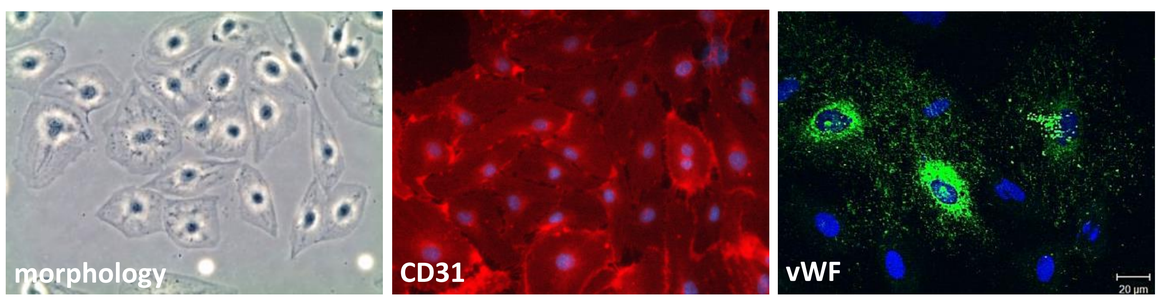


Transduction of primary cells with a defined cocktail of lentiviral vectors

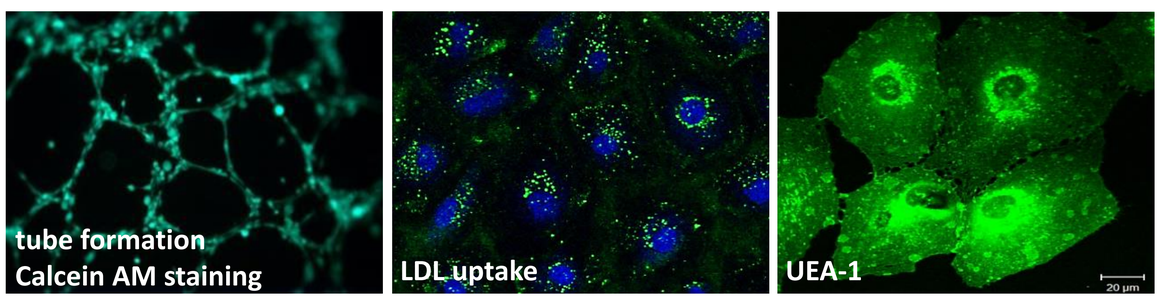
Previously, a novel technique was reported which enabled the generation of non-transformed proliferating liver cells from primary human hepatocytes with maintained adult phenotype, so-called "upcyte® cells". upcyte® cells start to grow from primary cells after transduction with a defined cocktail of lentiviral vectors carrying proliferation inducing genes. upcyte® cells have the ability to proliferate for additional cell doublings, depending on the cell type, without losing functional and phenotypic characteristics of mature cells.

Characterization of upcyte® LSECs: the cells express…

…primary endothelial cell markers

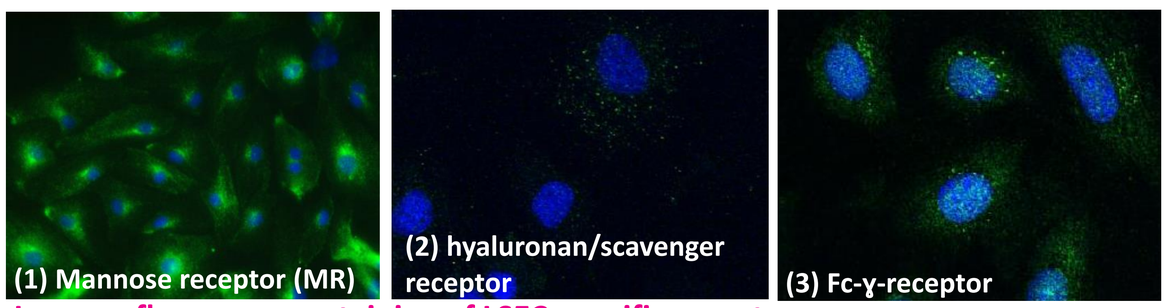


Morphology was comparable to primary cell LSECs. CD31 was present in all cells investigated and showed a typical membrane localization. vWF (von-Willebrand-factor) was evident as punctate structures that were more intense around the nucleus, indicating its presence in the endoplasmic reticulum.



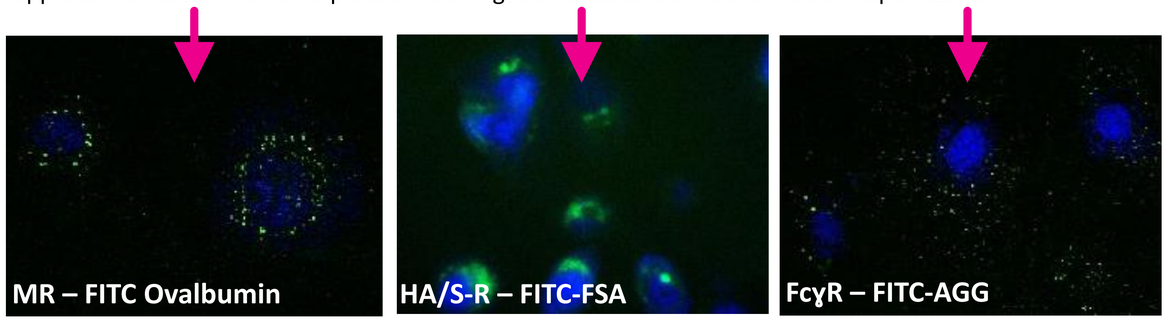
The ability to form tubes in Matrigel™ was analyzed in upcyte® LSECs at different densities. A seeding density of 12,500 cells/cm² was found to be optimal, since these upcyte® LSECs formed tubes from single cells. Uptake of ligand Ac-LDL (Alexa483-AcLDL), indicating the presence of the SR-A (Scavenger receptor A), a common endothelial cell receptor, was evident. Lectin (Ulex Europaeus Lectin1) was strongly expressed.

…LSECs specific markers



Immunofluorescence staining of LSEC-specific receptors

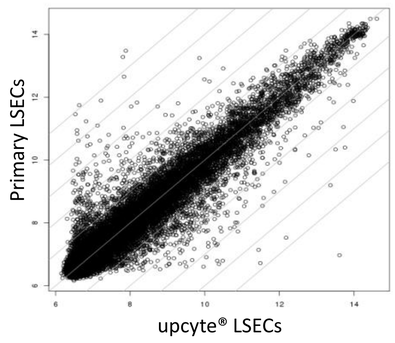
The cells express the three major uptake receptors: there was a high expression of MR and Fcɣ-R in upcyte® LSECs. The staining of HA/S-receptor was visible, but weak and not in all cells. A possible approach for better marker expression is elongation of culture time and medium optimization.



Scavenger receptor-mediated endocytosis (uptake function)

The functional test of receptor-mediated endocytosis was performed by adding fluorescent-labeled ligands to the medium in order to visualize how much of the label had been taken up. The following ligands were used: FITC-FSA (HA/S-R), FITC-AGG (FcɣR2BII) and FITC-mannan, DTAF-collagen-α-chains and FITC-ovalbumin (all three for MR, just one shown). Uptake of ligands could be shown for the MR and the FcɣR with the ligands FITC Ovalbumin and FITC-AGG (aggregated gamma globulin). Uptake of FITC-FSA (formaldehyde-treated serum albumin) for the HA/S-R could not be detected in all donors (1/3).

Gene expression profile



Expression profiles of upcyte® and primary LSECs were generated using Illumina 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 causes primary human LSECs to proliferate additional population doublings whilst still 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, FcɣR2BII 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.