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

Why LSECs?

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. Fenestrations, arranged in so-called selective “sewe 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 recruiting lysosomal enzymes for degradation.
2. The hyaluronan/scavenger receptor (HA/S-R) plays an important role in the physiological mechanism contributing to maintaining homostasis and preventing atherosclerosis by binding to type I and III pro-collagen and atherosclerotic molecules such as oxidized LDL and advanced glycation end products.
3. The Fcγ-receptor (FcγR) up-takes 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].

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

...primary endothelial cell markers

The morphology was comparable to primary LSECs. CD31 and vWF were present in all cells and showed a typical membrane localization (both not shown). upcyte® LSECs can form tubes from single cells in Matrigel® (Calbind AM staining). Uptake of ligand Ac-LDL (Al ox483-Ac,LDL), indicating the presence of the SR-A (Scavenger receptor A), a common endothelial cell receptor, was evident. LIVE-1 (lymphatic vessel endothelial hyaluronan receptor 1) and Lectin (Ulex Europaeus Lectin1 not shown) were strongly expressed.

Application of upcyte® LSECs

The Dynamic42 biochip is made of polyethylene terephthalate and contains two cavities, both of which have an integrated membrane. Cross-presentation of viral antigens by LSECs derived from infected hepatocytes triggers local activation of effector CD8 T-cells and thereby assures hepatic immune surveillance. [doi:10.1088/056.2016.5].

Future applications

include antigen specific tolerance induction by using nanoparticles serving as carrier for antigen delivery to LSECs.

immunology

The liver plays an important role in the immune response. LSECs separate parasite leukocytes in the sinusoidal lumen from hepatocytes. They act as a platform for adhesion for Kupffer cells, innate lymphoid cells or liver dendritic cells. LSECs cross-prime naive CD8 T cells, causing their rapid differentiation into memory T cells and provide protection when they re-encounter the antigen during microbial infection. Cross-presentation of viral antigens by LSECs derived from infected hepatocytes triggers local activation of effector CD8 T-cells and thereby assures hepatic immune surveillance. [doi:10.1088/056.2016.5].

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co & 3D cultures

The Dynamic42 biochip is made of polyethylene terephthalate and contains two cavities, both of which have an integrated membrane. This enables cells to be cultured under independent (upper & lower) perfusion channels. upcyte® LSECs were seeded together with human monocyte-derived macrophages in the upper channel while upcyte® hepatocytes were seeded on the other side (hanging fashion).

upcyte® LSECs were treated with palmitate and oleates in order to mimic nonalcoholic steatohepatitis (NASH). The expression of CD32b and CD31 (capillarization marker) is significantly increased after 7 days (not shown), indicating the progression to hepatic inflammation as seen in NASH patients.

...liver specific markers

The cells express the three major uptake receptors which differ liver sinusoidal endothelial cells from other endothelial cells: MR, HA/S-receptor (strong) as well as the FcγR (CD32b). The functional test of receptor-mediated endocytosis (uptake function) was performed by adding fluorescent-labeled ligands to the medium in order to visualize how much of the label had been taken up. FITC-ovalbumin was used as ligand for the MR.

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.

toxicology

cytotoxicity assay

Further characterization with respect to different sensitivities of LSECs and hepatocytes to different toxic compounds are ongoing and will be published soon. Stay tuned!

uptake of ADCs

ADCs (antibody drug conjugates) are complex molecules composed of an antibody linked to a biologically active cytotoxic payload or drug. Off-target hepatic toxicities have been reported for several ADCs (e.g. interactions/antispecific uptake for CD33b [doi:10.1007/jhep.3022] and MR [doi:10.1007/jhep.3022]) and [MR. bio.00262-012.13693].

SUMMARY & 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. Taken together, our data suggest that upcyte LSECs combine many characteristics of primary LSECs with the advantage of an extended lifespan, facilitating their use in hepatotoxicity assays under reproducible and standardized conditions. Future applications include e.g. in vitro uptake assays of ADCs (antibody drug conjugates) or triggering the hepatic immune response via the inclusion of T-cells and their antigen presenting capabilities to the LSECs.

Moreover, this technology allows for the generation of large batches of upcyte® LSECs enabling a reproducible and standardized experimental setting.

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expanded primary human liver sinusoidal endothelial cells (upcyte® LSECs) as a tool to complement in vitro hepatic studies

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