Generation of expanded primary cells for cell-based toxicity and metabolism screenings

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INTRODUCTION

Cell-based assays are a valuable tool to predict in vivo effects of drug candidates during early steps of development. Cell-based assays are performed using either cell lines or primary cells. Most cell lines are easy to handle and offer the advantage of infinite proliferation, allowing the generation of large cell banks and a facilitated use in screenings or long-term experiments. However, due to their transformed phenotype, many cell lines usually exhibit a reduced physiological relevance. In contrast, primary cells are more representative of the in vivo state when compared to cell lines. However, their use in vitro is hampered by limited tissue availability, scarce cell yields and a restriction or even lack of proliferation. Taken together, these factors may significantly compromise the scope, length and reproducibility of experiments and often for their use extended for cell-based screenings.

Here, we describe the controlled expansion of human primary cells by lentiviral transduction with proliferation-inducing genes, enabling production volumes of up to 2500 vials containing 5·10^6 cells each. As a proof of principle, primary cells from several relevant target tissues (liver, skin, kidney) were transduced, subsequently demonstrating successful expansion to large master and working cell banks.

RESULTS

generation of upcyte® cells

transduction of primary cells with a defined cocktail of lentiviral vectors

We first generated a library of lentiviral vectors carrying proliferation-inducing genes, allowing primary cells to bypass unecessess. Different primary cells such as hepatocytes, liver sinusoidal endothelial cells, keratinocytes, proximal tubular epithelial cells and bronchial epithelial cells were transduced. Resulting upcyte® cells gained the ability to proliferate for up to 40 additional population doublings without losing functional and phenotypic characteristics of mature cells. All cells exhibited expected morphology patterns and were restricted by the presence of specific growth factors, contact inhibition and anchorage dependence.

upcyte® hepatocytes for......

...acute and repeated-dose toxicity using sub-cytotoxic concentrations

Exposure time length had dramatic effects on the toxicity profile of a compound. For APAP, no effect was observed after 24 h, whereas 3-week treatment significantly induced apoptosis, mitochondrial depolarization, ROS production and intracellular Ca^2+ levels. Other tested compounds caused some effects after 24 h, although a significant difference was detected between the two incubation periods at the lowest concentration. CFC (non-hepatotoxic control) did not produce any significant effects after 24 h or 1-week treatment.

upcyte® hepatocytes (100% pure) for acute liver toxicity

Based on the results obtained above, cell viability assays were performed in vitro and in vivo for toxicological screening.

Future work will include further characterization of induced cell lines and performing long-term toxicity assays.

generated upcyte® cell types

hepatocytes

liver sinusoidal endothelial cells (LSECs)

keratinocytes

microvascular endothelial cells (mVECs)

upcyte® Kupffer cells

examples of functional assays using upcyte® cells

LSECs: receptor-mediated endocytosis

We observed high expression of endocytic receptors, e.g. MR, FcγR and LDLR in upcyte® LSECs. Corresponding ligand uptake could be demonstrated for fluorescent lipoprotein-conjugated ligands (FITC-CVA, FITC-AGG and AF488-azido).

keratinocytes: wound healing

upcyte® keratinocytes exhibit a similar migration capacity when compared to primary cells. Migration was determined by wound healing assay. Confluent monolayers of upcyte® keratinocytes were scratched using a pipette tip. Wound closure was achieved after 8 h.

mVECs: angiogenesis

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. upcyte® mVECs perform tube formation in matrigel and uptake of LDL. Cell migration is another typical feature of mVECs, shown here by transmigration through a Fluoroseck® insert; only the cells that migrated through the membrane are detectable.

Do you want to expand your primary cells?

SUMMARY & CONCLUSION

In conclusion, we developed a comprehensive platform enabling the controlled expansion of primary cells derived from various tissues for up to 40 population doublings. Importantly, upcyte® cells maintained a mature and primary-like phenotype as demonstrated by expression of marker proteins and functional assays.

We thus conclude that upcyte® expanded primary cells represent a promising model for biomedical research and drug discovery, potentially facilitating throughput and reproducibility of cell-based assays.