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 often 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 circumvent their use for extended 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 5×10³ cells each. As a proof of principle, primary cells from several relevant target tissues (liver, skin, kidney, lung) 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 lentivectors carrying proliferation-inducing genes, allowing primary cells to bypass senescence. Different primary cell types 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 (PDs) doubling 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.

overview: generated upcyte® cell types

We next investigated the phenotype of expanded upcyte® cells. Importantly, generated cells maintained expression of characteristic marker proteins throughout the study. For example, hepatocytes expressed cytokeratin 8/18, human serum albumin and alpha-1 antitrypsin. Accordingly, upcyte® LSECs were characterized by expression of CD143 and von-Willebrand factor.

examples of functional assays using upcyte® cells

hepatocytes: metabolism

upcyte® hepatocytes expressed metabolizing enzymes of phase I (e.g. CYP 1A2, 2B6, 2C9 and 3A4) and further exhibited phase II activities (UGT, SULT & GST). upcyte® hepatocytes further produced urea and secreted albumin (not shown). Differences in performance could be detected between cells derived from different donors.

LSECs: receptor-mediated endocytosis

We observed high expression of endocytic receptors, e.g. MR, FasR and LDLR in upcyte® LSECs. Corresponding ligand uptake could be demonstrated for respective fluorescence-conjugated ligands (FITC-OVA, FITC-AGG and AFA/488-acLDL).

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.

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.

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