Maturation of retinal pigment epithelium cells *in vitro* enhances the endogenous antioxidant defense system

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Introduction

The aim of this study was to to evaluate the endogenous antioxidant defense system in human induced pluripotent stem cell (hiPSC)-derived retinal pigment epithelium (RPE) cells.

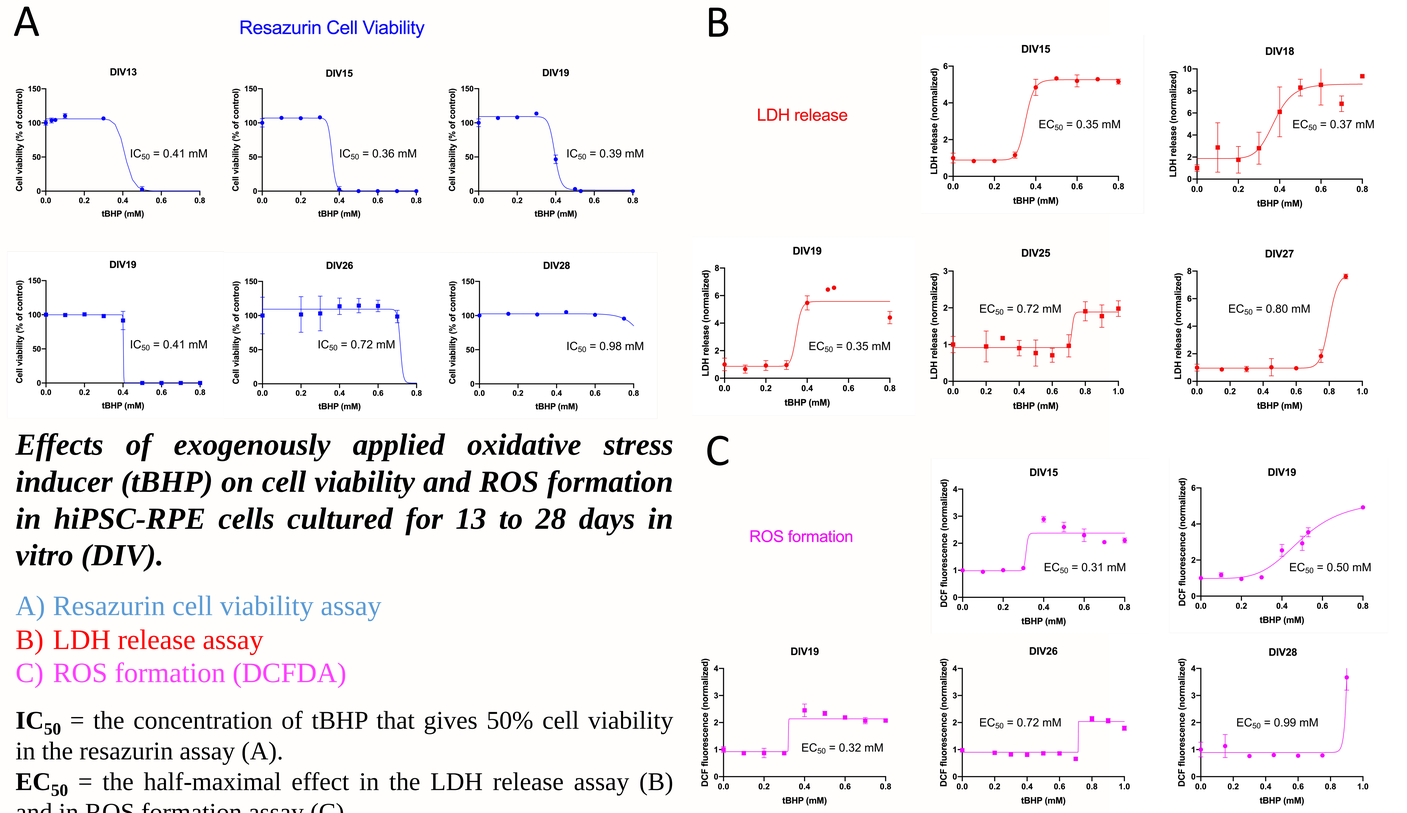
Materials and Methods

RPE cells derived from human induced pluripotent stem cells[1] (PCi-RPE, Phenocell, France) were cultured on Matrigel®-coated 96-well plates for 13 to 25 days (DIV) according to the instructions of manufacturer.

Oxidative stress was induced in RPE cells using *tert*-butyl hydroperoxide (tBHP) at 37°C for 22 hours. Cell viability of RPE cells was assessed by using general cell viability assays, resazurin assay and lactate dehydrogenase (LDH) release[2]. In addition, production of reactive oxygen species (ROS) in cells was quantified using the ROS indicator, chloromethyl 2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA)[3].

Resazurin cell viability was calculated ($RFU_{treated}/RFU_{control}$) × 100 %. LDH release and DCF data were normalized to the control condition (0 mM tBHP) and the four-parameter dose-response curves were fitted with GraphPad Prism 9 software (San Diego, CA, USA).

Results



Effects of exogenously applied oxidative stress inducer (tBHP) on cell viability and ROS formation in hiPSC-RPE cells cultured for 13 to 28 days in vitro (DIV).

A) Resazurin cell viability assay
B) LDH release assay
C) ROS formation (DCFDA)

IC_{50} = the concentration of tBHP that gives 50% cell viability in the resazurin assay (A).
EC_{50} = the half-maximal effect in the LDH release assay (B) and in ROS formation assay (C).
DCFDA trend matches the LDH trend.

References

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Conclusion

- Our data suggest that hiPSC-RPE cells have a strong endogenous antioxidant defense system against exogenous oxidative stress
- Maturation of hiPSC-RPE cells for longer than 26 days induce sudden increase in endogenous resistance
- Individual hiPSC-RPE cell pools need to be carefully and precisely characterized, as rapid changes in the endogenous antioxidant defense pose a significant confounding factor in experimental designs

Disclosures
JJH: Experimentica Ltd. (F,I,E,P,R,S)
OV: Experimentica Ltd. (E,R)
AKG: Experimentica Ltd. (F,R,S)
K&P Scientific LLC (C,F,R)
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