The silencing of multidrug resistance-associated protein 5 by siRNA complexes

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OVERALL OBJECTIVE

The purpose is to study the role of the multidrug resistance-associated protein 5, MRP5 in drug metabolism in human retinal pigment epithelium.

INTRODUCTION

Retinal pigment epithelium (RPE) forms the outer part of blood-retinal barrier (BRB) which restricts movements of drugs and other solutes from systemic bloodstream to the neural retina (Fig.1).

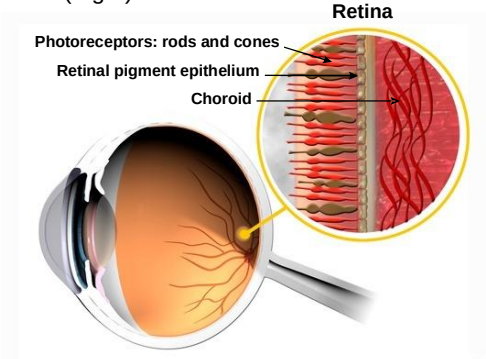

Figure 1. Eye Anatomy. The retina is situated at the back of the eye. The neural retina faces vitreous body and represent the largest part of retina. A monolayer of polarized cells form the outer layer of the retina, the retinal pigment epithelium (purple). Modified from (1)

The outer BRB functions similarly to the other more extensively characterized blood-neural barrier, the blood-brain barrier (BBB) (2).

BRB function may involve efflux proteins that transport a broad range of natural and xenobiotic compounds.

We investigated the expression of efflux protein genes in RPE cell lines.

METHODS

The messenger RNA levels of efflux protein genes (MDR1, MRP1, MRP2, MRP3, MRP4, MRP5, MRP6 and BCRP) in human RPE cell lines (ARPE-19 and D407) and primary human RPE cells (HRPEpiC) was measured by quantitative RT-PCR (Taqman®).

The expression of MRP1, MRP4 and MRP5 proteins was studied in ARPE-19, D407 and HRPEpiC by Western blotting.

RESULTS

MRP1, MRP4 and MRP5 were expressed in all studied cell lines both at mRNA and protein level (Fig.2 and Fig.3)

The expression of MRP4 and MRP5 was higher in ARPE-19 cultured on filter than in flask cultured cells (Fig.2)

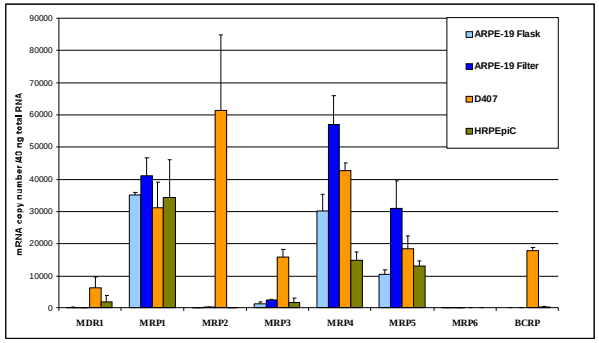

Figure 2. MRNA expression of efflux protein genes in RPE cell lines. ARPE-19 grown on flask and on filters, D407 and primary human HRPEpiC cells from flask cultures. Data is expressed as the mean±sd from two independent cell samples and each sample was measured in triplicate by quantitative RT-PCR.

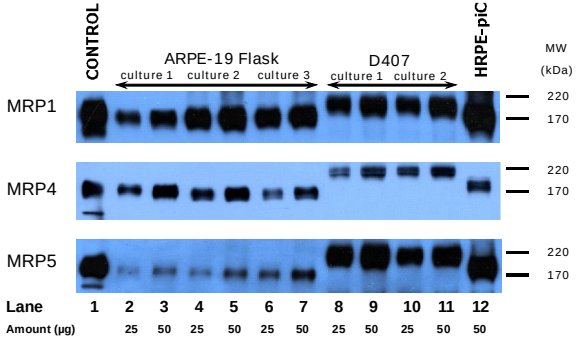

Figure 3. Western blot analysis of MRP1, MRP4 and MRP5 in RPE cells. Lane 1, positive control (stable MRP1-, MRP4- and MRP5-MDCKII cell lines); lanes 2-7, ARPE 19 cells from three different flask cultures; lanes 8-11, D407 cells from two different cultures; and lane 12, HRPEpi.

CONCLUSIONS AND FUTURE PLANS

The mRNA expression profile of investigated efflux proteins was similar in ARPE-19 and HRPEpiC cell lines.

Efflux protein expression profile of RPE differs from that of the blood-brain barrier (BBB).

MRP5 gene is expressed in ARPE-19 cell line.

The role of the MRP5 in permeation of drugs through BRB will be studied.

MRP5 siRNA will be tested as a tool to clarify the role of MRP5 in drug metabolism.

References
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2. Steuer H, Jaworski A, Elger B, et al: Functional characterization and comparison of the outer blood-retina barrier and the blood-brain barrier. Opth Vis Sci 46: 1047-53, 2005