Polarized Expression of Monocarboxylate Transporters in Human Retinal Pigment Epithelium and ARPE-19 Cells

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PURPOSE. To evaluate the expression and subcellular distribution of proton-coupled monocarboxylate transporters (MCTs) in human RPE in vivo and determine whether ARPE-19 cells retain the ability to express and differentially polarize these transporters.

METHODS. Total RNA was prepared from human donor eyes and from ARPE-19 cell cultures. Expression of MCT transcripts was evaluated by RT-PCR amplification. Expression of MCT proteins in human RPE and ARPE-19 cells was evaluated by immunolocalization and Western blot analysis with isoform-specific anti-peptide antibodies.

RESULTS. The expression of MCTs in human RPE was investigated by immunofluorescence analysis on frozen sections of human donor eyes. MCT1 antibody labeled the apical membrane of the RPE intensely, whereas MCT3 labeling was restricted to the basolateral membrane. MCT4 was detected in the neural retina but not in the RPE. ARPE-19 cells constitutively expressed MCT1 and MCT4 mRNAs. Expression of MCT3 mRNA increased over time as ARPE-19 cells established a differentiated phenotype. Western blot analysis revealed that ARPE-19 cells expressed high levels of MCT1 and MCT4 but very little MCT3 protein. Sections of differentiated ARPE-19 cells were labeled with MCT1, MCT4, and glucose transporter-1 antibodies. MCT1 was polarized to the apical membrane and MCT4 to the basolateral membrane, whereas GLUT1 was expressed in both membrane domains. CD147, which is necessary for targeting MCTs to the plasma membrane, was detected in the apical and basolateral membranes of human RPE in situ and ARPE-19 cells.

CONCLUSIONS. These studies demonstrate for the first time that human RPE expresses two proton-coupled monocarboxylate transporters: MCT1 in the apical membrane and MCT3 in the basolateral membrane. The coordinated activities of these two transporters could facilitate the flux of lactate from the retina to the choroid. ARPE-19 cells express two MCT isofoms, polarized to different membrane domains: MCT1 to the apical membrane and MCT4 to the basolateral membrane. The polarized expression of MCTs in ARPE-19 demonstrates that these cells retain the cellular machinery necessary for transepithelial transport of lactate. (Invest Ophtalmol Vis Sci. 2003;44:1716–1721) DOI:10.1167/iovs.02-0287

The retinal pigment epithelium (RPE) forms the outer blood–retinal barrier and actively regulates the volume and chemical composition of the subretinal space (SRS).1 Nutrients are transported from the blood to the retina, and metabolic waste and fluid are transported out of the retina. Vectorial transport of metabolites, ions, and fluids between the retina and the choroidal blood supply is dependent on tight junctional complexes and polarized distribution of transport proteins in the RPE.

Substances that are produced and used in large quantity, such as glucose, amino acids, and lactate are moved into and out of the retina by facilitated diffusion. Glucose, the preferred energy substrate for the neural retina is transported from the choroidal vessels to the outer retina through GLUT1, which is present in both apical and basolateral membranes of the RPE.2 Most of the glucose transported into the outer retina is metabolized through aerobic glycolysis, resulting in the production of quantities of lactate.3–6

Transport of lactate, pyruvate, and ketone bodies across the plasma membrane occurs in virtually all eukaryotic cells through a family of proton-coupled monocarboxylate transporters (MCTs).7 Eight members of this family have been cloned in humans and their homology, on the basis of primary structure, varies between 25% to 60% identity. Analysis of the derived amino acid sequences of MCTs has revealed that the cDNAs encode proteins with 12 membrane-spanning domains connected by short hydrophilic loops with a long cytoplasmic loop separating the sixth and seventh transmembrane (TM) segments. The amino and carboxyl termini are cytoplasmic. In accord with other transporter families, MCT isoforms differ in temporal and spatial patterns of expression. MCT1 is the most widely distributed isoform and is expressed in most mammalian tissues and in most cell lines.8 MCT3, originally cloned from a chicken RPE expression library is preferentially expressed in the RPE.9,10 MCT4 is a low-affinity lactate transporter that transports lactate out of glycolytic cells.11 Western blot and immunohistochemical analyses have shown that MCT4 is most abundant in fast-twitch muscle12 and neural retina.13

In previous studies we reported that two lactate transporters are expressed in both rat and mouse RPE. MCT1 in the apical membrane and MCT3 in the basolateral membrane.10,14 The coordinated activity of two transporters could provide a mechanism for transporting lactate out of the retina. In the present study, we examined the expression of MCTs in human RPE in situ and in ARPE-19 cells, a human RPE cell line. ARPE-19 cells have been used as a model system for studying differentiation, regulation of protein expression, and response of RPE to oxidative damage. We wanted to determine whether ARPE-19 cells properly express and polarize MCTs and their associated protein CD147.
MATERIALS AND METHODS

Cell Culture
ARPE-19 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with nutrient mixture F12 and 15 mM HEPES buffer (DMEM/F12; BioWhittaker, Walkersville, MD) supplemented with 10% (vol/vol) fetal bovine serum ( Gibco BRL, Grand Island, NY), 0.348% sodium bicarbonate, 2 mM l-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cell cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO2. The medium was changed every 2 to 3 days.

FRT cells, kindly provided by Enrique Rodriguez-Boulan (Dyson Institute of Ophthalmology, Cornell University Medical Center, New York, NY), were cultured in Coon’s modified Ham’s F-12 medium supplemented with 5% fetal bovine serum, 2 mM l-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were transfected with the full-coding sequence of human MCT3 cDNA cloned into the pTarget vector (Promega, Madison, WI) with a transfection reagent (FuGENE 6; Roche Molecular Biochemicals, Indianapolis, IN) used according to the manufacturer’s procedures. Stably transfected cell lines were selected by growth in the antibiotic G418 (Stratagene, La Jolla, CA), and resistant colonies were isolated and propagated. Expression of MCT3 protein was demonstrated by immunoblot analysis and immunohistochemistry.

Preparation of Antibodies against Human MCT1 and -3
Anti-peptide antibodies were raised in rabbits against the carboxyl terminal peptides of MCT1, -3, and -4. This domain of the MCTs is not conserved between isoforms ensuring the production of isoform-specific antibodies. An anti-human MCT1 antibody was raised in rabbits against the C terminus of MCT1, residues 483-500: (C)SPDQKDTEGGPK-EPEIEARPRLAAAESV-COOH. The peptide antibody was produced and affinity purified by Zymed Laboratories (San Francisco, CA). An anti-human MCT3 antibody was raised in rabbits to the C-terminal peptide of human MCT3 residues (C)GEPTEPIEAPRLAAAESV-COOH. The antibody was produced and affinity purified by Research Genetics (Huntsville, AL). MCT4 antibodies were raised against an 18-mer synthetic oligopeptide corresponding to the carboxyl terminal amino acids (KA-EPNKGEVYHTPETS) of mouse MCT4 (Zymed Laboratories). This sequence is conserved between mouse and human MCT4. All antibodies were evaluated for specificity in our laboratory by Western blot and immunohistochemical analyses in the presence and absence of the appropriate peptide antigen.

GLUT1 antibody was a gift from Ian Simpson (Pennsylvania State University, Hershey, PA) and CD147 antibody (RDI-CD147-M6) was purchased from Research Diagnostics, Inc. (Flanders, NJ).

PCR Analysis
Total RNA was prepared from adult human donor eyes obtained from the Lions Eye Bank of Delaware (Philadelphia, PA) by using extraction reagent (TRizol; Life Technologies Inc., Grand Island, NY), according to the manufacturer’s instructions. The protocol adhered to the provisions of the Declaration of Helsinki for the use of human tissue in research. After the retina was removed from the eyecup, 1 mL of extraction reagent was added to the eyecup to isolate RNA from the RPE-choroid. Total RNA was prepared from ARPE-19 cells by the same extraction reagent was added to the eyecup to isolate RNA from the RPE-choroid. Total RNA was prepared from adult human donor eyes obtained from Research Diagnostics, Inc. (Flanders, NJ).

RESULTS

Antibodies to Human MCT1 and -3
To examine expression and subcellular distribution of MCT isoforms in human tissue and established cell lines, antibodies
were raised in rabbits to the carboxyl terminal peptides of human MCT1, -3, and -4. The C-terminal domain of MCT is cytoplasmic and is not conserved among the MCT isoforms. Western blot analysis demonstrated that the affinity-purified MCT1 antibody cross-reacted with a single band in detergent soluble lysates prepared from ARPE-19 cells. The band had molecular weight of approximately 42 kDa on SDS gels and was not detected when the antibody was preabsorbed with the peptide antigen (Fig. 1). FRT cells were stably transfected with MCT3 cDNA to provide a standard for MCT3 protein. The MCT3 antibody labeled a protein that migrated with a molecular weight of approximately 50 kDa in the detergent lysates prepared from FRT-MCT3 cells. The band was not detected when the antibody was preabsorbed with the peptide antigen (Fig. 1). MCT3 did not label lysates prepared from nontransfected cells (data not shown). MCT4 antibody labeled a single protein with a mobility of approximately 42 kDa in detergent lysates prepared from ARPE-19 cells and antibody binding was inhibited by MCT4 peptide antigen.

Expression of MCT1 and -3 in Human RPE In Situ

Trans epithelial movement of lactate from the outer retina to the choroidal blood supply requires transport proteins in the apical and basolateral membranes of the RPE. Indirect immunofluorescence was used to assess cellular and subcellular distribution of MCTs in the RPE of human donor eyes. Figure 2 shows bright-field (Figs. 2A, 2C, 2E) and fluorescent (Figs. 2B, 2D, 2F) images of sections through a human retina labeled with antibodies to MCT1, MCT3, and the glucose transporter GLUT1. The MCT1 antibody labeled the apical membrane of the RPE (Fig. 2B). Antibody labeling was detected throughout the neural retina; however, the staining was far less intense than in the RPE. MCT3 expression was limited to the basolateral membrane of the RPE (Fig. 2D). The MCT3 antibody did not label the retina (Fig. 2D) or other human tissues examined, including liver, pancreas, lung, heart, and kidney (data not shown). MCT4 was not expressed in the RPE but was expressed in neural retina (not shown). Whereas MCT1 and -3 were polarized to discrete membrane domains, GLUT1 antibody staining was detected in both the apical and basolateral membranes of the RPE (Fig. 2F).

Expression of MCTs in ARPE-19 Cells

ARPE-19 is a human RPE cell line that retains many features of RPE cells in situ. When these cells are maintained in continuous culture, they acquire features characteristic of RPE: hexagonal packing of cells, pigment granules, and polarized distribution of membrane proteins. After 1 month in culture, islands of hexagonally packed cells are observed and after 2 to 3 months the cells form a monolayer of hexagonally packed cells. Differentiation of ARPE-19 cells proceeds in a similar manner, whether the cells are grown on tissue culture plastic or on porous filters.

ARPE-19 cells were cultured for 2 to 270 days to evaluate the expression of MCTs as a function of differentiation. RT-PCR was performed with a primer sets specific for MCT1, -3, and -4, as detailed in the Methods section. MCT1 and -4 mRNAs were constitutively expressed and were detected in cells cultured from 2 to 270 days (Fig. 3A). In contrast, MCT3 mRNA was upregulated as a function of cell differentiation. MCT3 mRNA was detected at low levels in cells cultured for 30 days and increased as the cells differentiated. After 60 days, the level of MCT3 mRNA remained relatively constant.

Western blot analysis was used to examine the expression of MCT proteins in ARPE-19 cells. Detergent lysates were prepared from ARPE-19 cells cultured for 24 and 120 days. Proteins were separated on SDS-polyacrylamide and transferred to membranes. Triplicate blots were probed with MCT1, -3, and -4 antibodies (Fig. 3B). MCT1 and -4 were detected in the lysates prepared from undifferentiated (24 days) and differentiated (120 days) ARPE-19 cells. MCT3 was not expressed in undifferentiated cells, and only a faint band was evident in cells cultured for 120 days.

The distribution of MCT1 and -4 in ARPE-19 cells was examined by immunofluorescence localization. ARPE-19 cells cultured for 120 days were sectioned and stained with antibodies to MCT1, -3, and -4 and GLUT1. MCT1 was primarily detected in the apical membrane of ARPE-19 cells (Fig. 4) similar to human RPE in situ (Fig. 2B). MCT4 antibody labeled the basolateral membranes of ARPE-19 cells. GLUT1 was detected in both membrane domains. ARPE-19 cells were not labeled with MCT3 antibody, suggesting that the expression was below the level of detection. This is consistent with the results from the Western blot analysis shown in Figure 5.

Colocalization of CD147 with MCTs

Targeting of MCTs to the plasma membrane requires association with CD147, a type I membrane glycoprotein. The expression and subcellular distribution of CD147 in ARPE-19 cells was examined by Western blot and immunofluorescence analyses. CD147 was abundantly expressed in both undifferentiated and differentiated ARPE-19 cells (Fig. 5A). There was an increase in the relative abundance of CD147 as ARPE-19 cells differentiated, which paralleled the increase in MCT1 and -4 (Fig. 3B). The CD147 antibody labeled both the apical and basolateral membranes of ARPE-19 cells (Fig. 5B), colocalizing with MCT1 in the apical membrane and MCT4 in the basolateral membrane, as shown in Figure 4.

Expression of MCT3 Splice Variant

MCT3 mRNA was expressed in differentiated ARPE-19 cells, but only a very low level of protein was detected. To determine whether the low level of protein was due to a mutation in the gene, the full coding sequence of MCT3 was amplified from ARPE-19 cells and RPE from human donor eyes. A single 1766-bp product was amplified from human donor eyes, whereas two products were amplified from ARPE-19 cells (Fig. 6). One product was the appropriate size for the full-length...
open reading frame and migrated with the same mobility as the product amplified from human RPE. Sequencing of the 1766-bp product revealed that it was identical with the published hMCT3 cDNA sequence. In comparison of the sequence of the 1544-bp product with the human MCT3 gene revealed that there was a deletion of exon 2, which contains the translation start site.

**DISCUSSION**

In this study, the expression and subcellular distribution of proton-coupled MCTs was examined in human RPE in situ and in ARPE-19 cells. We found that human RPE expressed two MCT isoforms polarized to different membrane domains: MCT1 in the apical and MCT3 in the basolateral membrane. The coordinated activities of these two transporters would provide a mechanism for transporting lactate from the retina to the choroid. In ARPE-19 cells MCT1 was polarized to the apical membrane and MCT4 to the basolateral membrane. MCT3 mRNA was detected in differentiated ARPE-19 cells, but only a very low level of protein was expressed.

MCT1 is the most widely expressed member of the MCT family. In human RPE, we found that MCT1 was abundantly expressed in the apical membrane of the RPE, in agreement with our previous studies in mouse and rat RPE. Whereas MCT1 was targeted to the apical membrane in RPE, in epithelia of the kidney, and small intestine, MCT1 was polarized to the basolateral membrane, adjacent to the capillary bed. Under normal physiological conditions, MCT1 transports lactate and ketone bodies into the cell to be metabolized through oxidative phosphorylation. However under hypoxic or ischemic conditions, MCT1 transports lactate out of the cells. The apical polarization of MCT1 is consistent with the observation that the RPE transports lactate from the subretinal space to the choroid, as discussed later.

Whereas MCT1 is widely expressed, MCT3 is preferentially expressed in the RPE. MCT3 labeling was restricted to the basolateral membrane of human RPE in situ. Labeling was not found in the neural retina or in other ocular tissues. Previously, we reported that MCT3 mRNA was expressed in human RPE but not in the neural retina. The MCT3 gene maps to chromosome 19p13.3.19
One of the critical functions of the RPE is to regulate the transport of nutrients into and out of the retina. The glucose transporter, GLUT1 is present in both the apical and basolateral membranes of the RPE facilitating the transepithelial movement of glucose from the choroid to the retina (Fig. 2F). In the retina, 80% of the glucose is metabolized through aerobic glycolysis, resulting in the production of substantial quantities of lactate. The lactate produced is used by photoreceptor cells to fuel oxidative phosphorylation and excess lactate produced is transported to the choroid by the RPE. The presence of MCT1 on the apical membrane and MCT3 on the basolateral membrane of the RPE identifies two proteins acting in concert that can mediate the transepithelial transfer of lactate to the choroid. 

As was observed in situ, GLUT1 was detected in the apical and basolateral membranes of ARPE-19 cells. This was not due to an absence of polarity but instead demonstrates that these cells retained the cellular machinery necessary to target the membrane proteins appropriately. In other polarized epithelial cells in culture, such as FRT cells, GLUT1 was polarized to the basolateral membrane (data not shown). CD147, which is required for targeting MCTs to the plasma membrane, colocalized with MCT1 in the apical membrane and MCT4 in the basolateral membrane of ARPE-19 cells. In summary, our study human RPE expressed two MCTs that were targeted to different membrane domains: MCT1 was primarily targeted to the apical membrane, as was observed in RPE in situ. MCT4 was targeted to the basolateral membrane. The coordinated activity of these transporters facilitates transepithelial movement of nutrients.

Figure 5: Expression of CD147 in ARPE-19 cells. Detergent-soluble lysates were prepared from 24- and 120-day cultures of ARPE-19 cells and proteins were separated on a 4% to 12% gradient SDS gel and transferred to a membrane. Membrane was probed with CD147 antibody. Section of 120-day culture of ARPE-19 cells labeled with CD147 antibody. Apical (arrow) and basolateral (arrowhead) surface.

Figure 6: RT-PCR analysis of MCT3 mRNA in human RPE and differentiated ARPE-19 cells. MYC1 was amplified with the forward primer 5’-ctc ggg gga tca act atg c-3’ and the reverse primer 5’-cag ttg tcc ttc aga gga tca act-3’. The expected size of the product was 1.7 kb. A 1.7 kb product was amplified from RNA prepared from RPE of human donor eyes (hRPE). Two bands were amplified from ARPE-19 cells: a 1.7 kb product and a 1.5 kb product. Sequencing of the 1.5 kb product revealed that exon 2 was deleted.
could regulate the transport of lactate from the retina to the choroid. ARPE-19 cells expressed the appropriate polarization of MCTs and GLUT1, demonstrating that these cells had the cellular machinery necessary for transepithelial transport of glucose and lactate.

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References


