Interaction of monocarboxylate transporter 4 with β₁-integrin and its role in cell migration

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Gallagher SM, Castorino JJ, Philp NJ. Interaction of monocarboxylate transporter 4 with β₁-integrin and its role in cell migration. Am J Physiol Cell Physiol 296: C414–C421, 2009. First published December 10, 2008; doi:10.1152/ajpcell.00430.2008.—Monocarboxylate transporter (MCT) 4 is a heteromeric proton-coupled lactate transporter that is noncovalently linked to the extracellular matrix metalloproteinase inducer CD147 and is typically expressed in glycolytic tissues. There is increasing evidence to suggest that ion transporters are part of macromolecular complexes involved in regulating β₁-integrin adhesion and cell movement. In the present study we examined whether MCTs play a role in cell migration through their interaction with β₁-integrin. Using reciprocal coimmunoprecipitation assays, we found that β₁-integrin selectively associated with MCT4 in ARPE-19 and MDCK cells, two epithelial cell lines that express both MCT1 and MCT4. In polarized monolayers of ARPE-19 cells, MCT4 and β₁-integrin colocalized to the basolateral membrane, while both proteins were found in the leading edge lamellapodia of migrating cells. In scratch-wound assays, MCT4 knockdown slowed migration and increased focal adhesion size. In contrast, silencing MCT1 did not alter the rate of cell migration or focal adhesion size. Taken together, our findings suggest that the specific interaction of MCT4 with β₁-integrin may regulate cell migration through modulation of focal adhesions.

MCT4; CD147

Monocarboxylate transporter 4 (MCT4) is a member of the SLC16 family of solute transporters and is primarily expressed in tissues that rely on glycolysis for their energy production (15, 17, 21, 57). MCT4 is a low-affinity, high-capacity symporter that catalyzes proton-coupled efflux of lactate across the cell membrane and is expressed at high levels in embryonic tissues, skeletal muscle, neural retina, and metastatic cancer cell lines (19, 25, 37, 52). Like other solute transporters, MCT4 has 12 membrane-spanning domains and forms a heteromeric complex with a highly glycosylated type I glycoprotein, CD147 (30). The catalytic (MCT4) and accessory (CD147) subunits of this heteromeric lactate transporter are assembled into a complex in the endoplasmic reticulum, and in the absence of either subunit, the other subunit is retained in the endoplasmic reticulum and is likely targeted for degradation (14, 19). CD147, which was first identified as an extracellular matrix metalloproteinase inducer, also interacts with CD98 (10, 55), CD44 (49), syndecan-1 (35), γ-secretase (58, 59), shrew-1 (41), and β₁-integrin (3, 10, 11). The association of CD147 with proteins involved in lactate and amino acid transport, cell attachment, motility, and proliferation suggest that the heteromeric lactate transporter is a component of a larger macromolecular complex at the plasma membrane that influences numerous cellular processes.

Recent work has demonstrated a role for both CD147 and β₁-integrin during cell migration in processes such as metas- tasis and wound healing. For example, increased expression of CD147 correlates with enhanced metastatic potential (4, 32, 40, 42, 56), while silencing CD147 in hepatic cancer, ovarian cancer, and lymphoma cell lines inhibited cell migration (8, 9, 60). CD147 plays a role in corneal epithelium wound healing as well, where increased expression and redistribution of CD147 and increased induction of matrix metalloproteinase 2 were observed following injury (18). Similarly, β₁-integrin is required for both cancer cell migration and migration of the retinal pigment epithelium (RPE) following injury (1, 20, 22, 51). Interestingly, work from our lab has demonstrated a role for MCT4 in cell migration in a metastatic breast cancer cell line. Specifically, silencing MCT4 in MDA-MB-231 cells resulted in a significant reduction of cell invasion (19). The functional similarity of CD147, MCT4, and β₁-integrin during cell migration, as well as the reports of CD147/MCT4 and CD147/β₁-integrin complexes, suggests that these proteins could be forming a “supercomplex” that modulates cell adhesion and motility.

The RPE forms the outer blood-retinal barrier and performs functions critical to maintaining the health and function of the photoreceptor cells. Under normal conditions, the RPE is nonproliferative and nonmigratory; however, following an ocular trauma, such as rhegmatogenous retinal detachment, RPE cells can engage in an inappropriate or uncontrolled healing response termed proliferative vitreoretinopathy (PVR) (7, 27, 29). Following injury, RPE and glial cells begin to migrate along both the front and back surfaces of the retina and proliferate, forming PVR membranes, which can lead to further retinal detachment and loss of vision (7, 28). The early formation of PVR membranes is largely due to RPE cell migration, which occurs after these cells undergo an epithelial-to-mesenchymal transformation (EMT). This cellular event is also seen in various cancers, where tumor cells undergo EMT as they become more metastatic (24, 31).

The purpose of these studies was to examine whether MCTs play a role in RPE cell migration during wound healing. Using the human RPE cell line ARPE-19, we found that MCT4 and β₁-integrin form a complex in the basolateral membrane of polarized cells and colocalized at the leading edge of migrating cells. Additionally, we found that silencing of MCT4 resulted in larger focal adhesions and slowed cell migration, indicating a role for the lactate transporter in migration of the RPE.

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MATERIALS AND METHODS

Reagents and cell culture. Cell culture reagents were purchased from Mediatech (Herndon, VA). Reagents used for SDS-PAGE were obtained from Invitrogen (Carlsbad, CA). All small interfering RNAs (siRNAs) used were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). ARPE-19 and MDCK cells were cultured as previously described (14, 37).

Antibodies. MCT1 and MCT4 antibodies were generated for our laboratory by Zymed as previously described (36). The α5-integrin antibody was generated as previously described (33). Additional antibodies used in these studies were α5-integrin (sc-10729; Santa Cruz Biotechnology), paxillin (610619; BD Biosciences, Franklin Lakes, NJ), and β-actin (A5441; Sigma). Additionally, anti-human β1-integrin (MAB20797Z, Chemicon, Temecula, CA) was used for immunoblotting. Anti-human β1-integrin antibody (AIIB2) [developed by Werb et al. (54) and obtained by Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242] was used for both immunoprecipitation and immunofluorescence labeling. Alexa-Fluor-tagged secondary antibodies were purchased from Molecular Probes (Eugene, OR). Horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Bio-Rad Laboratories (Hercules, CA) and Molecular Probes.

Immunoblot analysis. Cells were washed with PBS and lysed with ice-cold lysis buffer (25 mM HEPES buffer, pH 7.4, 150 mM NaCl, 5 mM MgCl2, and 1% Triton X-100) containing protease inhibitors (Complete Mini, Roche, Indianapolis, IN) for 30 min and then centrifuged at 14,000 × g for 30 min. The protein concentration of the cleared lysates was determined using BCA reagent (Pierce, Rockford, IL). Lysates were diluted in 2× LDS sample buffer (Invitrogen), and equal amounts of protein were loaded onto 4–12% NuPAGE Bis-Tris gels (Invitrogen). Separated proteins were transferred electrothermally from gels to Immobilon-P membranes (Millipore, Bedford, MA). Membranes were incubated for 1 h at room temperature in blocking buffer (20 mM Tris, 137 mM NaCl, pH 7.5, and 5% dry skim milk), followed by 1 h incubation with primary antibodies and 30 min incubation with HRP-conjugated secondary antibodies diluted 1:5,000. Blots were probed with the following antibodies: CD147 (goat, 1:500), MCT1 (rabbit, 1:1,000), MCT4 (rabbit, 1:1,000), α5-integrin (rabbit, 1:1,000), α3-integrin (goat, 1:500), and β1-integrin (mouse, 1:1,000). β-Actin (mouse, 1:50,000) was used as a loading control. Reactive bands were visualized with enhanced chemiluminescence labeling. Alexa-Fluor-tagged secondary antibodies were purchased from Molecular Probes (Eugene, OR). Horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Bio-Rad Laboratories (Hercules, CA) and Molecular Probes.

Immunoprecipitation. Cells were washed two times with PBS and lysed with ice-cold lysis buffer (25 mM HEPES buffer, pH 7.4, 150 mM NaCl, 5 mM MgCl2, and 1% CHAPS detergent) containing protease inhibitors (Complete Mini) for 30 min. The lysate was clarified by centrifugation at 14,000 × g for 30 min. An aliquot of the supernatant was used for normalized protein, and the remaining supernatant (300–500 μg in 400 μl) was incubated with 3–5 μl of MCT1, MCT4, or β1-integrin antibodies overnight with end-over rotation at 4°C. The following day, cells were washed with PBS, permeabilized with methanol for 3 min at –20°C, blocked using 5% BSA in PBS with 0.1% Tween 20 (PBST), and incubated with primary antibody overnight at 4°C. The next day, cells were washed with PBST and incubated in secondary antibody for 30 min, washed, and mounted with Gelvatol. Antibodies were used at the following dilutions: MCT1 (rabbit, 1:100); MCT4 (rabbit, 1:500); β1-integrin (DSHB, rat, 1:100-1:250); paxillin (mouse, 1:250-1:500); Alexa-Fluor 488 (rat and mouse, 1:500); Alexa-Fluor 555 (rabbit, 1:500). It should be noted that labeling with MCT1 antibody results in nonspecific nuclear staining. This staining was nonspecific in MCT1 silenced with siRNA, all cell-cell and plasma membrane staining was lost and nuclear staining persisted (Fig. 4, B and D).

Immunofluorescence. Preparation of ARPE-19 sections was performed as previously described (37). ARPE-19 cells used for wounding assays were cultured on two-well glass or Permanox slides or in 35-mm dishes. Cells were fixed in 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) in PBS for 5 min at room temperature, and then 25 min on ice. Cells were washed in PBS, permeabilized with methanol for 3 min at –20°C, blocked using 5% BSA in PBS with 0.1% Tween 20 (PBST), and incubated with primary antibody overnight at 4°C. The next day, cells were washed with PBST and incubated in secondary antibody for 30 min, washed, and mounted with Gelvatol. Antibodies were used at the following dilutions: MCT1 (rabbit, 1:100); MCT4 (rabbit, 1:500); β1-integrin (DSHB, rat, 1:100-1:250); paxillin (mouse, 1:250-1:500); Alexa-Fluor 488 (rat and mouse, 1:500); Alexa-Fluor 555 (rabbit, 1:500). Images were obtained on a laser scanning confocal microscope (Zeiss LSM510) with a ×63 oil objective. Images shown in Fig. 4 were prepared from projections (2 slices) of Z-Stack images taken at ×63 magnification (large panels) or ×63 magnification with a ×2 zoom (insets). Images shown in Fig. 5 are single slices taken at ×63 magnification (Fig. 5, A, C, and E) and ×63 magnification with a ×4 zoom (Fig. 5, B, D, and F). Images were processed with LSM510 software and Adobe Photoshop 7.0 (San Jose, CA). Adjustments were made to brightness and contrast only. For area measurements, 135–230 adhesions were counted over five to seven cells per treatment condition. Focal adhesion area was measured using ImageJ software and the polygon and measure tools. Focal adhesions were grouped by size and are expressed as a percentage of total adhesions. Data are representative of two individual experiments with duplicate samples in each experiment. Similar results were obtained in all experiments.

PCR reactions. Total RNA was extracted from cells using Qiagen RNeasy Mini kits according to the manufacturer’s protocol. cDNA was synthesized from 4 μg total RNA by SuperScript III reverse transcriptase (Invitrogen) with oligo(dT) primers. PCR was performed with 1 μl cDNA with the primers and conditions described in Table S1 (supplemental material for this article is available online at the American Journal of Physiology: Cell Physiology website) and Fisher Taq polymerase.

siRNA-mediated silencing of MCTs and CD147. Silencing of MCT1, MCT4, and CD147 expression was performed following the manufacturer’s protocols. Before transfection, cells were grown to 70–80% confluence in 12-well plates. Cells were transfected with siRNA pools specific for hCD147 [accession no. NM_001728], hMCT1 (accession no. NM_003051), or hMCT4 (accession no. NM_004207) using the DharmaFECT 2 transfection reagent (Dharmacon). As an additional control, cells were mock transfected using transfection reagent alone. All cells were harvested 48 h posttransfection for RNA and 72 h posttransfection for immunoblot analysis or immunofluorescence. For wounding assays, cells were scratch-wounded at 48 h posttransfection, and wounds were analyzed at 72 h posttransfection. All siRNA experiments were performed in triplicate and were repeated at least three times. Similar results were obtained in all experiments.

Wounding assays. Scratch-wounding assays were performed on ARPE-19 cells in a 12-well plate for protein and quantification, 35-mm dish, or two-well Permanox slides (for immunofluorescence). Two hours before wounding, cells were treated with the DNA polymerase inhibitor aphidicolin (2 μg/ml; Invitrogen) to block cell proliferation. After culture medium was removed, a wound was made in the center of ARPE-19 cultures by scratching with a 1-ml pipette.
tip. The cultures were washed twice with PBS to remove any debris resulting from wounding. Media were replaced and cells were incubated overnight at 37°C in a 5% CO2 atmosphere. For immunolocalization studies, cells were fixed 24 h postwounding as described. For analysis of wound closure, wounded cells were imaged on an inverted microscope with a ×4 objective at 0 h and 25 h postwounding. Following imaging at the 25-h time point, cells were harvested for protein as described. Wound area at each time point was measured using the polygon and measure tools in Zeiss LSM510 imaging software. Wound closure was expressed as percentage of the original wound area ± SE. Significance was determined using a one-way ANOVA test.

RESULTS

β1-Integrin preferentially interacts with MCT4. While studies have shown that CD147 associates with β1-integrin, none of these studies addressed whether MCTs were also present in the complex. In polarized monolayers of ARPE-19 cells, β1-integrin was restricted to the basolateral membrane where it colocalized with MCT4 (Fig. 1A). Since CD147 expression is not restricted to regions of β1-integrin expression, we hypothesized that the interaction between CD147 and β1-integrin is mediated by specific MCT isoforms. To test this hypothesis, reciprocal coimmunoprecipitation experiments were performed (Fig. 1A). β1-Integrin was immunoprecipitated from detergent-soluble lysates of ARPE-19 cells, and blots were probed with antibodies specific for MCT1, MCT4, α3-integrin, and α5-integrin. We found that MCT4 was more abundant in the immunoprecipitated pellet than MCT1 (Fig. 1B, top). Specifically, when expressed as a ratio of the input, ~80% of MCT4 coimmunoprecipitated with β1-integrin, while only ~8% of MCT1 was found to associate (Fig. 1B, bottom). As expected, both α3-integrin and α5-integrin coimmunoprecipitated with β1-integrin (Fig. 1B). In reciprocal coimmunoprecipitation experiments, more β1-integrin coimmunoprecipitated with MCT4 than with MCT1 (Fig. 1C). Similarly, in MDCK cells (where both MCT1 and MCT4 are expressed on the basolateral surface), β1-integrin preferentially coimmunoprecipitated with MCT4 (data not shown). These results, therefore, demonstrate that the interaction between MCTs and β1-integrin is isoform specific.

Silencing MCTs or CD147 does not impact integrin expression. Since β1-integrin associates with MCT4/CD147 heterodimers, we next wanted to examine whether silencing MCTs or CD147 altered β1-integrin expression. ARPE-19 cells were transfected with siRNAs specific for MCT1, MCT4, and CD147. Semiquantitative RT-PCR analysis showed that silencing with MCT1, MCT4, or CD147 specific siRNAs reduced the abundance of only the target RNA (Fig. 2A). Additionally, silencing MCTs or CD147 had no effect on the levels of β1-integrin mRNA (Fig. 2A). These results indicate that silencing of MCTs or CD147 does not affect the expression of each other or β1-integrin at the mRNA level.

Protein levels of β1-integrin were also examined in ARPE-19 cells silenced as described above with MCT1, MCT4, or CD147 siRNAs. Western blot analysis revealed that silencing either MCT1 or MCT4 in ARPE-19 cells resulted in decreased expression of their accessory subunit, CD147 (Fig. 2B). Likewise, silencing CD147 resulted in loss of both MCT1 and MCT4 protein expression. In contrast, silencing MCT1, MCT4, or CD147 had no effect on β1-integrin expression, indicating that expression of this integrin is independent of its association with the MCT4/CD147 lactate transporter (Fig. 2B).

Silencing MCT4 results in slowed cell migration. Previous studies with cancer cells demonstrated a role for MCT4 and CD147 in cell migration. In these studies, we examined whether MCT4 also plays a role in epithelial wound healing. Scratch-wound assays were performed on mock-transfected ARPE-19 cells and on cells transfected with MCT1, MCT4, or CD147 siRNAs. By 25-h postwounding, the wounds were 69% closed in mock-transfected ARPE-19 cells (Fig. 3). Similar results were observed when cells were transfected with MCT1-siRNA (72% closure), indicating that this transporter had no effect on the rate of wound closure. However, when ARPE-19 cells were transfected with MCT4 or CD147 siRNA, wound closure was reduced to 48% or 56%, respectively (Fig. 3).

MCT4 colocalizes with β1-integrin at the leading edge of migrating epithelial cells but is not required for its trafficking. It has been clearly demonstrated that integrin receptors are critical for cell migration and are often enriched in the leading edge of migrating cells. We have observed here that MCT4 and...
β1-integrin form a complex in ARPE-19 cells and that MCT4 appears to play a role in cell migration. Therefore, we next examined whether this complex was found at the leading edge of cells, where it could aid in cell migration. ARPE-19 cells were scratch-wounded as described, and protein localization to the leading edge was observed by immunofluorescence confocal microscopy. Following scratch-wounding, we found that MCT4 and β1-integrin colocalize at high levels in the lamellapodia of cells at the wound edge (Fig. 4A, arrowhead and inset) as well as at the lateral cell borders (Fig. 4A, arrows). MCT1 also colocalized with β1-integrin at moderate levels in both the lateral cell borders and the leading edge (Fig. 4B, arrows and arrowhead, respectively). However, since silencing MCT1 did not affect cell migration, we hypothesize that MCT1 does not impact β1-integrin-mediated attachment or motility.

Since MCTs and their accessory subunit CD147 are dependent on one another for their trafficking to the plasma membrane, we next wanted to determine whether β1-integrin also relies on MCT4 for its localization to the leading edge. ARPE-19 cells were transfected with siRNAs specific for MCT4 or MCT1 and were then fixed for immunofluorescence to visualize the distribution of β1-integrin. We found that when MCT4 was silenced, β1-integrin was still present in the lamellapodia of migrating cells (Fig. 4C, arrowhead and inset). Similar results were observed when cells were treated with MCT1 siRNA (Fig. 4D, arrowhead and inset).

Loss of MCT4 impacts focal adhesion size. It has recently been shown that intracellular pH can affect focal adhesion assembly and disassembly. Therefore, we next wanted to examine whether the slowed cell migration observed in MCT4 silenced cells was a result of alterations in focal adhesions at the leading edge. ARPE-19 cells were scratch-wounded after silencing of either MCT1 or MCT4 and were then labeled with paxillin to visualize focal adhesions. In mock-treated cells, we observed punctate paxillin labeling, indicative of focal adhesions at the leading edge (Fig. 5, A and B). These adhesions were small in size and persisted across the extent of the leading lamellapodium in migrating cells (Fig. 5, A and B, arrowheads). A few larger focal adhesions were also observed in the cell body (Fig. 5, A and B, arrow). Silencing of MCT1 yielded similar results to those seen in mock-treated cells (Fig. 5, C and D). However, when MCT4 was silenced, we noted larger focal adhesions across the leading edge, as well as in the cell body (Fig. 5, E and F, arrows). Overall, we found an increased proportion of large (>8 μm²) focal adhesions in MCT4-silenced cells (9.39%) compared with mock- or MCT1-silenced cells (0% and 1.48%, respectively; Table 1). This striking alteration of focal adhesion size could, therefore, provide an explanation for the decreased cell migration observed in cells in which MCT4 has been silenced.

DISCUSSION

Embryonic development, wound healing, and metastasis are characterized by increased glycolysis and cell migration. In the
In the present study, we found that cell metabolism and cell migration may be linked through the structural and functional interaction between β1-integrin and the heteromeric lactate transporter MCT4/CD147. Over the past decade, a number of studies reported an association between CD147 and β1-integrin, as well as other transmembrane proteins such as the amino acid transporter CD98 (3, 6, 10, 11, 55). It was suggested that this supercomplex could mediate a variety of cellular functions, including cell signaling, cell-cell adhesion, and cell spreading. One of the first studies demonstrating an interaction between CD147 and β1-integrin was performed in the HT1080 fibrosarcoma cell line, identifying CD147 as one of the proteins that coimmunoprecipitated with β1-integrin (3). The amino acid transporter, CD98, was also found in a complex with both β1-integrin and CD147 (6, 10, 55). Thus, it was suggested that CD98 binding to β1-integrin could enhance integrin affinity for its matrix substrates by activating the phosphatidylinositol 3-kinase signaling pathway (6). Other investigators have described the interactions among CD98/β1-integrin/CD147 as a “sensory complex” that activated signaling pathways to regulate cell physiology and function (10). Recent work examining the function of CD147 in the CD147/β1-integrin complex suggested that CD147 was able to impact cytoskeletal architecture (by inducing cell spreading) via an integrin-dependent mechanism (11).

One element lacking in all of these studies was consideration of the role of the obligate binding partner of CD147—MCT1, MCT3, or MCT4. As our lab has previously demonstrated, MCT1, MCT3, and MCT4 are codependent on CD147 for their maturation and trafficking to the plasma membrane (14, 19). The current studies sought to examine whether MCTs are also in a complex with CD147 and β1-integrin. Importantly, we found that β1-integrin preferentially binds to MCT4, even in cells in which MCT1 and MCT4 are polarized to the basolateral membrane. This is the first study to demonstrate an association between MCTs and β1-integrin, and it opens the door to new understanding of how components of this supercomplex could impact cellular function.

Previous work has shown that MCTs (specifically, MCT1, MCT3, or MCT4) and CD147 assemble as a heterodimer in the endoplasmic reticulum and are codependent on one another for their trafficking to the plasma membrane. However, it has not been determined whether this complex is also required for trafficking of β1-integrin. In these studies, we found that while silencing of subunits of the MCT/CD147 complex resulted in reduced expression of the other subunit at the protein level, mRNA levels were not affected. This indicates that association of MCTs and CD147 occurs either translationally or posttranslationally. In support of this hypothesis, previous work from our lab demonstrated that MCT1 and MCT3, the isoforms expressed in RPE in vivo, were not expressed in the RPE of CD147-knockout mice (36). Similar results were observed in MCT3-knockout mice (12). In vitro, we found that silencing MCT4 in the breast cancer cell line, MDA-MB-231, resulted in retention of CD147 in the endoplasmic reticulum (19). In contrast, we found that β1-integrin expression was unaffected after silencing either component of the MCT/CD147 transporter. Additionally, we found that β1-integrin remains present at the leading edge of migrating cells, even after silencing MCT1 or MCT4. These findings indicate that β1-integrin is not dependent on either of these components for its trafficking to the plasma membrane.

Fig. 4. MCT4 colocalizes with β1-integrin at the leading edge of migrating ARPE-19 cells. A: wounded ARPE-19 cell monolayers were fixed 24 h postwounding and immunolabeled with MCT4 and β1-integrin antibodies. Immunofocal micrographs show that MCT4 (red) and β1-integrin (green) colocalized (yellow, see merge) at the lateral cell borders and in the leading edge of migrating cells. B: moderate levels of MCT1 (red) colocalized with β1-integrin (green) at lateral cell borders (yellow, see merge) and at the leading edge of wounded cells (see merge and inset). C and D: silencing of MCT4 (C) or MCT1 (D) did not alter the distribution of β1-integrin (green) to the leading edge. Smaller panels in A–D denote higher magnification of boxed area in larger image. Arrows denote cell-cell borders and arrowheads denote the leading edge. Bar = 10 μm.
Extracellular lactate has been reported to upregulate the synthesis of two extracellular matrix proteins that enhance migration: hyaluronan and type I collagen (50, 51). Typically, a healing wound has a hyaluronan-rich stroma, which can lead to rearrangement of the extracellular matrix, making it more permissive for cell migration (16, 44, 53). It has been observed that addition of exogenous lactate to cells results in increased random motility (16). The presence of MCT4 at the leading edge could decrease such random motility by mediating polarized lactate and H+ efflux. Furthermore, silencing MCT4 could reduce lactate efflux at the leading edge of the cells, leading to decreased synthesis of collagen and hyaluronan needed for movement. Since CD147 is cotrafficked with MCT4 to the plasma membrane, silencing MCT4 could also result in a reduction of matrix metalloproteinase activity, thereby preventing the matrix remodeling needed for directed cell migration (34). While the effect of silencing MCT4 in ARPE-19 cells does not result in complete inhibition of migration, these results do suggest that this transporter has at least a partial role in mediating this process. It is likely that the MCT4/CD147/β1-integrin supercomplex acts in concert with other protein complexes to facilitate efficient, directed cell migration.

It has been well established that cells rely on ionic transporters and exchangers to modulate both intracellular and extracellular pH (23, 26, 38, 48). Much of this work has focused on a specific class of acid-base transporters, the sodium-proton exchangers (NHE). NHE-1 was shown to play a major role in cell migration by modulating intracellular and extracellular pH, as well as in the maintenance of cellular homeostasis and volume (5, 13, 45, 47). Interestingly, recent work has indicated a role for NHE1 in modulating integrin-mediated attachment to the matrix (45, 46, 47). Specifically, it was observed that the strength of cell attachment differed between cells cultured under either acidic (pH 6.6) or alkaline (pH 7.5) conditions. Acidic pH conditions resulted in stronger cell attachments and alkaline pH produced weaker attachments (45). Likewise, this group also showed that in melanoma cells, integrin-mediated motility was most efficient at pH 6.8 (46). An additional study published recently demonstrated a role for pH modulation in mediating intracellular attachments to the cytoskeleton. It was shown that an acidic intracellular environment resulted in stronger adhesion of talin to the actin cytoskeleton, whereas a more alkaline intracellular environment weakened the binding between talin and actin (43). These alterations in binding thus resulted in slowed cell migration, due to tighter adhesion of the cell to its matrix. We found that ARPE-19 cells express little NHE-1 (data not shown). Therefore, other acid-base transporters must be involved in regulating pH in these cells. The MCTs are prime candidates for this role since they are proton-coupled lactate symporters and are abundantly expressed in ARPE-19 cells.

The dynamic assembly and disassembly of focal adhesions is crucial for efficient cell migration. For example, actively migrating cells typically exhibit small, nascent focal adhesions (2, 39) that allow for their rapid assembly/disassembly, which is required for cell motility. Conversely, larger focal adhesions exert more resistive forces to migration by anchoring cells more strongly to their matrix substrate (2). Recently, a role for ion translocation in modulating focal adhesion assembly and disassembly has been reported. Specifically, mutation of the ion translocation domain of NHE-1 resulted in larger focal adhesions as compared with wild type (13, 43). These studies led us to examine whether silencing MCT4 could result in a similar phenotype, thus explaining the slowed cell migration observed following MCT4 knockdown in ARPE-19 cells. We did, in fact, observe larger focal adhesions at the leading edge of ARPE-19 cells following MCT4 silencing, as compared with mock- or MCT1 siRNA-transfected cells. Silencing

Table 1. Focal adhesion area after siRNA-mediated silencing of MCTs

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Values are % total adhesions; n, total number of focal adhesions measured. Mock, n = 233; monocarboxylate transporter (MCT) 1 small interfering (si)RNA, n = 135; MCT4 siRNA, n = 213.
MCT4 in ARPE-19 cells would be expected to disrupt H⁺ efflux at the leading edge required to enhance cell migration following wounding. Additionally, the alteration in intracellular pH in MCT4-silenced cells could result in tighter binding of actin-binding proteins to the cytoskeleton, thereby forming larger, more mature focal adhesions.

In summary, our results suggest that MCT4 plays a multifaceted role in ARPE-19 cell migration. During epithelial wound healing, the leading edge of migrating cells tends to be glycolytic, producing excess lactate in this region that, if not removed, could decrease intracellular pH. However, expression of MCT4 at the leading edge could relieve the cell of the intracellular acid load, allowing glycolysis to continue uninterrupted. Current discussion in the literature suggests that tight regulation of both extracellular and intracellular pH is necessary for cell migration. Thus, the efflux of lactate via MCT4 could serve to stabilize integrin-mediated attachment and associate assembly/disassembly of focal adhesions at the leading edge, thereby enhancing directed cell migration. Taken together, our findings support a role for MCT4 in pathologies characterized by aberrant cell migration, including PVR and metastatic cancer.

REFERENCES

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