Lactate Treatment Causes NF-κB Activation and CD44 Shedding in Cultured Trabecular Meshwork Cells

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Purpose. To challenge human trabecular meshwork (TM) cells using lactate to mimic cell stress and observe the effects on cell viability, NF-κB, and membrane type 1 matrix metalloproteinase (MT1-MMP) expression and the ectodomain shedding of soluble (s)CD44.

Methods. Human TM cells grown in 10% fetal calf serum (FCS) were incubated in 0.1% FCS with 1, 10, or 40 mM lactate or PBS for 5 and 30 minutes and 1, 3, and 6 hours. Cell viability was determined with trypan blue staining. NF-κB and MT1-MMP expression was evaluated through Western blot analysis of medium and the cytoplasmic and nuclear fractions. Media sCD44 concentration was determined by enzyme-linked immunosorbent assay and Western blot analysis.

Results. The TM cell viability was significantly decreased after incubation for 3 hours with 40 mM lactate ($P < 0.01$) and 6 hours with 10 and 40 mM lactate ($P < 0.001$). Western blot analysis showed an increased NF-κB p50 and MT1-MMP expression and activity by 5 minutes in lactate-treated TM cells compared with that of control cells. At 6 hours, NF-κB p65 was increased in nuclear fraction of lactate-treated compared with control cells. Treatment with 1 mM lactate caused an increase in the media concentration of both the 32 and 55 kDa sCD44 at 3 ($P < 0.05$) and 6 hours ($P < 0.01$).

Conclusions. Lactate treatment resulted in dose- and time-dependent effects on human TM cell viability, translocation of NF-κB, and activation of MT1-MMP. Increased shedding of sCD44 occurred with the l mM dose of lactate. Lactate treatment of human TM cells in culture offers a useful cell model to examine the stress responses that occur in glaucoma. (*Invest Ophthalmol Vis Sci.* 2007;48:1615–1621) DOI:10.1167/iovs.06-1086

Trabecular meshwork (TM) cells are a unique type of endothelium and cover the TM beams to form part of the filtration barrier to aqueous humor outflow. Human TM cells respond to a variety of stimuli. Disturbances in the vitality and functional status of human TM cells by genetic predisposition, oxidative stress, aging, or other factors may impair their response to cell insults and lead to elevated intraocular pressure and glaucomatous conditions.[1] In many cell types, the transcription factor NF-κB plays a pivotal role in inducing genes in response to a variety of biological stimuli (e.g., oxidative stress).[2] NF-κB is upregulated in the TM in glaucoma[3,4] and targets several genes, including those involved with extracellular matrix remodeling.[5]

One marker of cell stress is lactate concentration. Lactate accumulation has been implicated in the evolution of neuronal damage after ischemia[6] and in Alzheimer's disease.[7] In cell culture, lactate accumulation occurs with hypoxia.[8] Aging human platelets with mitochondrial dysfunction have enhanced lactate production under normal oxygen conditions (i.e., decreased Pasteur effect), due to impaired respiratory chain function.[9] Warburg discovered in 1924 that tumor cells use anaerobic metabolism to generate lactate even in the presence of adequate oxygen.[10] Several transcription factors are involved in lactate administration to fibroblasts, including activating protein-1 and NF-κB. NF-κB is a protein complex consisting of a 65-kDa DNA binding subunit and an associated 50-kDa protein that is derived from the amino terminus of a precursor designated p105. NF-κB activation also blocks apoptosis in several cell types, and thus may promote cell survival after stress insults.

Hyaluronic acid metabolism and CD44 expression are regulated by lactate concentration in fibroblasts.[10] CD44 is the predominant receptor of hyaluronic acid[11,12] and mediates signaling that influences fundamental cell behavior including migration and survival.[13] The cytoplasmic domain of CD44 interacts with the cytoskeleton, and the extracellular domain is shed (i.e., ectodomain shedding), in response to ligand binding and protein kinase C activation as well as treatments with metalloproteinase activator 4-aminophenylmercuric acetate and cholesterol-extracting agent methyl-β-cyclodextrin.[14] Ectodomain shedding occurs by proteolytic mechanisms (e.g., by membrane type 1 matrix metalloproteinase [MT1-MMP]),[15–17] which allows a rapid change in the cell surface phenotype and generates soluble mediators that can act on other cells. Shedding is observed with a variety of proteins such as tumor necrosis factor α, Fas ligand, interleukin-6, L-selectin, transforming growth factor α, amyloid precursor protein and CD44.[14] The shed ectodomain of CD44 is known as soluble (s)CD44 and is toxic to human TM cells in cell culture.[18] Notably, sCD44 is significantly increased in the aqueous humor in primary open-angle glaucoma and normal tension glaucoma but not in secondary glaucomas.[19,20]

The purpose of this study was to challenge human TM cells with lactate to mimic the cell stress that may be present in primary open-angle glaucoma and then to observe NF-κB protein expression and nuclear translocation, MT1-MMP expression and activity, and the effects of lactate on cell viability and the shedding of sCD44.

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Materials and Methods

Cell Culture and Lactate Treatment

Human TM[21] cells derived from four individual normal donors (20, 33, 34, and 49 years of age) were grown in culture as previously described.[18] In brief, human TM cells were plated at a density of 100,000 cells per 35-mm well and grown until confluent in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS). The medium was changed to DMEM with 0.1% FCS and 1, 10, and 40 mM lactate (Sigma-Aldrich, St. Louis, MO) for 5 and 30 minutes and 1, 3, and 6 hours. The medium was removed from the cell cultures and stored at −80°C. The cells were washed twice with 1 mL of phosphate-buffered saline (PBS) and scraped from the wells, and cytoplasmic and nuclear fractions were isolated by a cell lysis kit (Pierce Biotechnologies, Inc., Rockford, IL) according to the manufacturer's instructions and stored at −80°C. Protein concentration was determined by a protein assay (Bio-Rad, Hercules, CA) using bovine serum albumin as a standard. sCD44 concentration was determined by ELISA as previously described.[20] In brief, 1 μg protein equivalent was treated with 1% Triton X-100 for 1 hour at 4°C, the volume was adjusted to 100 μL with sample dilution buffer, and sCD44 concentration was measured by ELISA according to the manufacturer's instructions (Bender Med Systems, Vienna, Austria).

The cells were photographed, counted with a Coulter counter, and stained with trypan blue to assess viability, as previously described.[18] The cell viability was expressed as the absolute number of viable cells in the experimental group (experimental percentage of cell viability times the total number of experimental cells) versus the absolute number of viable cells in the control group (control percentage of cell viability times the total number of control cells). Data are expressed as the mean ± SD ($n = 6$) and were analyzed by Student's *t*-test. $P < 0.05$ was considered statistically significant.

Western Blot Analysis

Western blot analysis was performed using antibodies specific for NF-κB p50 and p65, MT1-MMP, and CD44. Media and cytoplasmic and nuclear fractions were subjected to SDS gel electrophoresis under reducing conditions using 4% to 15% gradient gels (Bio-Rad). The proteins were transferred to nitrocellulose membranes and incubated with anti-NF-κB p50 (1:1000 dilution; Santa Cruz Biotechnologies, Inc., Santa Cruz, CA), anti-NF-κB p65 (1:1000; Santa Cruz Biotechnologies, Inc.), anti-active MT1-MMP (RP3MMP14, 1:5000 dilution; Triple Point Biologics, Inc., Forest Grove, OR), anti-pro form MT1-MMP (RP4MMP14, 1:5000, Triple Point Biologics, Inc.), or anti-CD44 antibody (BU52, 1:1000 dilution; Ancell, Bayport, MN) for 16 hours at 4°C. The membranes were rinsed, incubated with a goat anti-mouse-horseradish peroxidase (HRP) conjugate (1:3000 dilution; Bio-Rad), and visualized on x-ray film using enhanced chemiluminescence according to the manufacturer's instructions (GE Healthcare, Arlington Heights, IL).

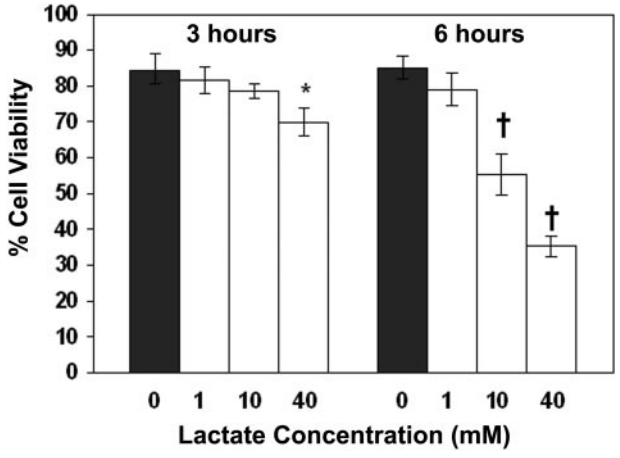

Figure 1. Dose and time dependence of lactate administration on human TM cell viability. The results are expressed as the mean ($n = 6$); error bars, SD; *$P < 0.05$, †$P < 0.01$ comparing treated with the control cells.

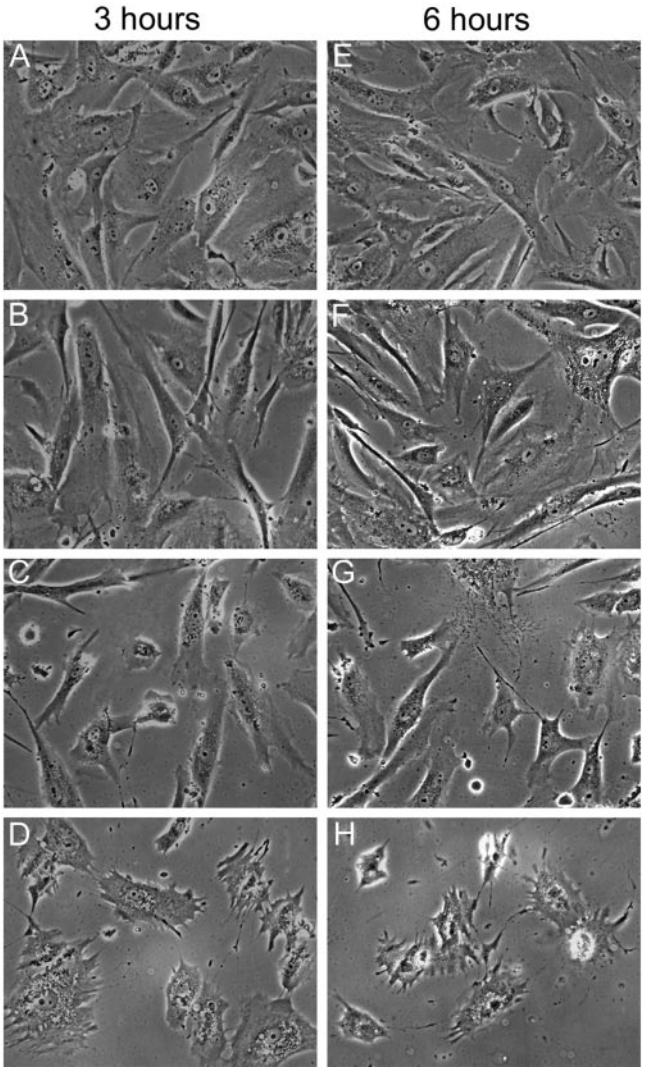

Figure 2. Phase-contrast microscopy of the effects of lactate on human TM cell morphology after 3 and 6 hours. (**A**) Control TM after 3 hours; TM treated with (**B**) 1 mM, (**C**) 10 mM, or (**D**) 40 mM lactate, after 3 hours; (**E**) control TM after 6 hours; and TM treated with (**F**) 1 mM, (**G**) 10 mM, or (**H**) 40 mM lactate, after 6 hours. Dead cells appear round and shiny. Magnification, ×400.

Results

Short-Term Effects of Lactate on Human TM Cell Viability

A dose- and time-dependent response to lactate was observed in human TM cell viability (Fig. 1). After 3 hours, cell viability was significantly decreased by incubation in 40 mM lactate ($P < 0.05$) compared with control cell viability. After 6 hours, cell viability was significantly decreased by incubation in 10 mM lactate and 40 mM lactate ($P < 0.01$). The cell viability of human TM cells treated with a low concentration (i.e., 1 mM lactate) was similar to that of control cells after 3 or 6 hours.

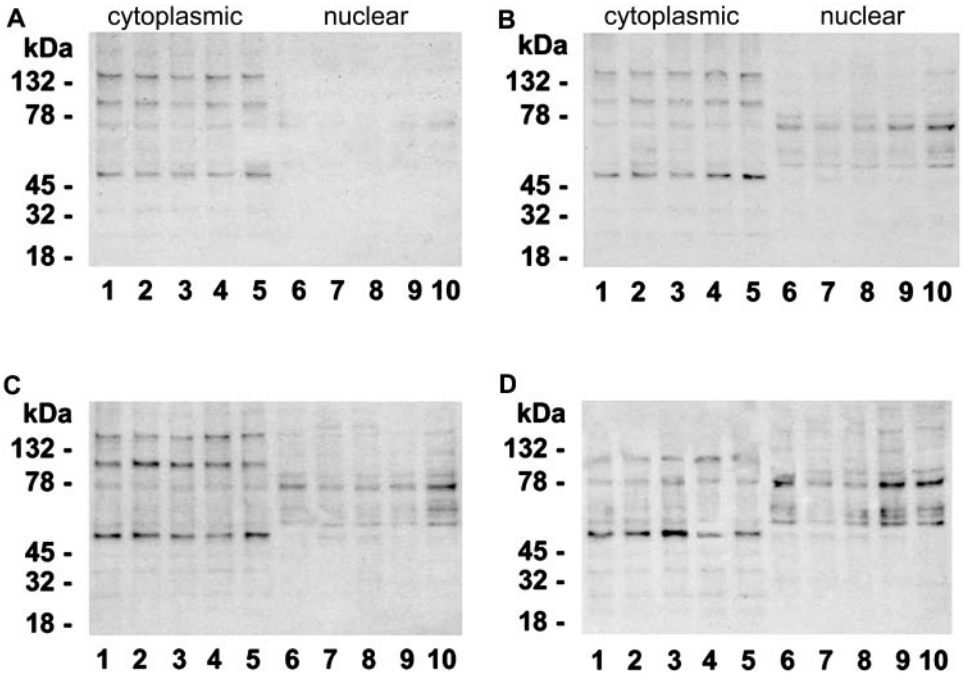

FIGURE 3. Western blot analysis of NF-κB p50 and p105 expression in human TM cells. (**A**) Control TM cells and (**B**) 1 mM, (**C**) 10 mM, or (**D**) 40 mM lactate-treated TM cells. Cytoplasmic fractions (7.5 μg protein equivalent), *Lane 1*: 5 minutes; *lane 2*: 30 minutes; *lane 3*: 1 hour; *lane 4*: 3 hours; *lane 5*: 6 hours; nuclear fractions (2.5 μg protein equivalent), *lane 6*: 5 minutes; *lane 7*: 30 minutes; *lane 8*: 1 hour; *lane 9*: 3 hours; *lane 10*: 6 hours. *Left*: molecular weight markers. Results of one of four representative experiments are shown.

Morphologic Changes in Lactate-Treated Human TM Cells

The morphology of human TM cells exhibited a time- and dose-dependent response to lactate treatment. Control human TM cells were uniform and squamous in appearance with stellate features (Figs. 2A, 2E). The morphology of human TM cells treated with 1 mM lactate was similar to that of control cells at 3 or 6 hours (Figs. 2B, 2F). As the concentration of lactate was increased to 10 and 40 mM, cell vacuolization, rounding, and death were observed (Figs. 2C, 2D, 2G, 2H).

Lactate Treatment and NF-κB Activation

A dose- and time-dependent expression and translocation of NF-κB was observed in lactate-treated cells. Western blot analysis of NF-κB using p50 antibody indicated immunopositive bands corresponding to 50 and 105 kDa in the cytoplasmic fraction but not in the nuclear fraction (Fig. 3A). Within 5 minutes of lactate treatment, p50 NF-κB immunopositive bands at approximately 55 and 78 kDa were observed in both cytoplasmic and nuclear fractions of all lactate-treated cells (Figs. 3B–D). Western blot analysis of the cytoplasmic fraction using NF-κB p65 antibody indicated a major immunopositive band corresponding to 65 kDa (Fig. 4). After 6 hours of lactate treatment, p65 NF-κB immunopositive bands corresponding to approximately 65 and 100 kDa were detected in nuclear fractions of all lactate-treated cells (Fig. 4).

MT1-MMP Expression

To determine MT1-MMP expression, the media and the cell lysate preparations of control and lactate-treated cultures were collected and analyzed. Western blot analysis using MT1-MMP antibody, which recognizes the catalytic domain of MT1-MMP, revealed an increase in the level of 54 kDa MT1-MMP in the cell lysates after 5 minutes of lactate treatment (Fig. 5A, lanes 2–4). A similar change in MT1-MMP was also observed in the cell lysates after 6 hours (Fig. 5B). Western blot analysis of the media of control cultures revealed a minimal immunopositive band in the media (Fig. 5A, lane 5) at the 5-minute time point. The immunopositive band present in the media was slightly increased in the lactate-treated cultures. After 6 hours, a low level of MT1-MMP immunopositive band was observed in the cell lysate preparations.

Western blot analysis using the pro form of MT-MMP-1 antibody, which recognizes the ectodomain of MT1-MMP, revealed minimal immunopositive material in the cell lysate and media of control cultures (Fig. 5C, lanes 1 and 5). An increase in the 57-kDa immunopositive band in the 40 mM lactate-treated cells was noted after 5 minutes of treatment (Fig. 5C, lane 4). After 6 hours, the cell lysates (Fig. 5D, lanes 2–4) and media (Fig. 5D, lanes 6–8) of lactate-treated cultures showed enhanced immunoreactivity. In the cell lysates, immunopositive bands of 34, 44, and 57 kDa were observed, whereas in the media, the 57-kDa protein was the major pro form of MT1-MMP.

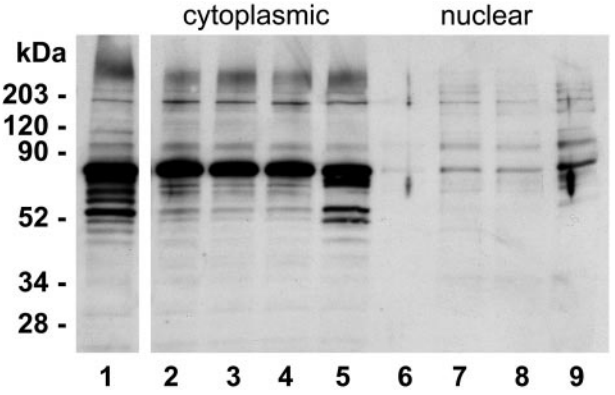

FIGURE 4. Western blot of NF-κB p65 in human TM cells after 6 hours of lactate treatment. Cytoplasmic fractions (7.5 μg protein equivalent), *lane 1*: A431 cell lysate, positive control; *lane 2*: control and *lane 3*: 1 mM, *lane 4*: 10 mM, and *lane 5*: 40 mM lactate; and nuclear fractions (2.5 μg protein equivalent), *lane 6*: control and *lane 7*: 1 mM, *lane 8*: 10 mM, and *lane 9*: 40 mM lactate. *Left*: molecular weight markers. Results of one of four representative experiments are shown.

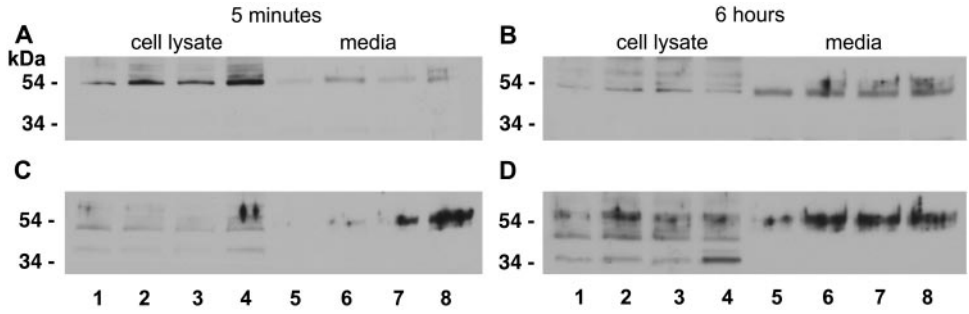

FIGURE 5. Western blot of MT1-MMP activation and lactate treatment of human TM cells. Active form of MT1-MMP after (**A**) 5 minutes and (**B**) 6 hours; pro-peptide form of MT1-MMP after (**C**) 5 minutes and (**D**) 6 hours. Cell lysates (7.5 μg protein equivalent), *lane 1*: control and *lane 2*: 1 mM, *lane 3*: 10 mM, *lane 4*: 40 mM lactate; and media (2.5 μg protein equivalent), *lane 5*, control and *lane 6*: 1 mM; *lane*7: 10 mM, and *lane 8*: 40 mM lactate. *Left*: molecular weight markers. Results of one of six representative experiments are shown.

sCD44 Release and Lactate Treatment

To determine whether sCD44 is released from the human TM cells in response to lactate treatment, the media from control and 1, 10, and 40 mM lactate-treated human TM cultures was analyzed by ELISA. In control media at 3 hours, the sCD44 concentration was 7.0 ± 1.0 ng/mL, whereas in the 1 mM lactate-treated media, it was significantly increased to 9.0 ± 1.0 ng/mL ($P < 0.05$). In contrast, the sCD44 concentration of the 10 and 40 mM lactate-treated media was statistically decreased ($P < 0.01$). In control media at 6 hours, the sCD44 concentration was 9.0 ± 1.0 ng/mL. Treatment with 1 mM lactate elevated the sCD44 level to 14.0 ± 3.8 ng/mL ($P < 0.05$). In contrast, the sCD44 concentration in 10 and 40 mM lactate-treated media was decreased ($P < 0.01$) after 6 hours. Western blot analysis of sCD44 after 6 hours showed an increase in the 32- and 55-kDa immunopositive bands in the 1 mM lactate-treated media, confirming the ELISA data and the release of sCD44 into the media (Fig. 6B).

The concentration of sCD44 in the media per viable cell for control and lactate-treated cells was determined to evaluate the release of sCD44 (Table 1). In control cells, the sCD44 concentration per viable cell increased significantly from 3 to 6 hours. In the 1 mM lactate-treated cells, the sCD44 concentration was greater than the control at both 3 ($P < 0.02$) and 6 ($P < 0.05$) hours. In contrast, the sCD44 concentration per cell in the 10 and 40 mM at 3 and 6 hours was considerably lower than that in control or 1 mM lactate-treated cultures.

The decrease in the media sCD44 concentration at the higher lactate treatment was examined by comparing the protein concentration of control and lactate-treated cells (Fig. 6C). In the higher doses of lactate treatment, the protein concentration in the cell medium was decreased, which was statistically significant ($P < 0.05$ for 10 mM and $P < 0.01$ for 40 mM lactate) at the 3-hour time point. The decrease in the protein concentration suggests that metabolic stress induced by lactate treatment results in decreased protein synthesis and/or release into the cell media.

DISCUSSION

Lactate treatment resulted in dose- and time-dependent effects on human TM cell viability, translocation of NF-κB, activation of MT1-MMP, and release of sCD44. Lactate concentration of 1 mM was tolerated by human TM cells, whereas higher concentrations of lactate were toxic to cells, resulting in decreased cell viability after 3 hours. The lactate concentrations used in this study thus represented a range below and above that in the normal aqueous. Extreme exercise has been shown to induce

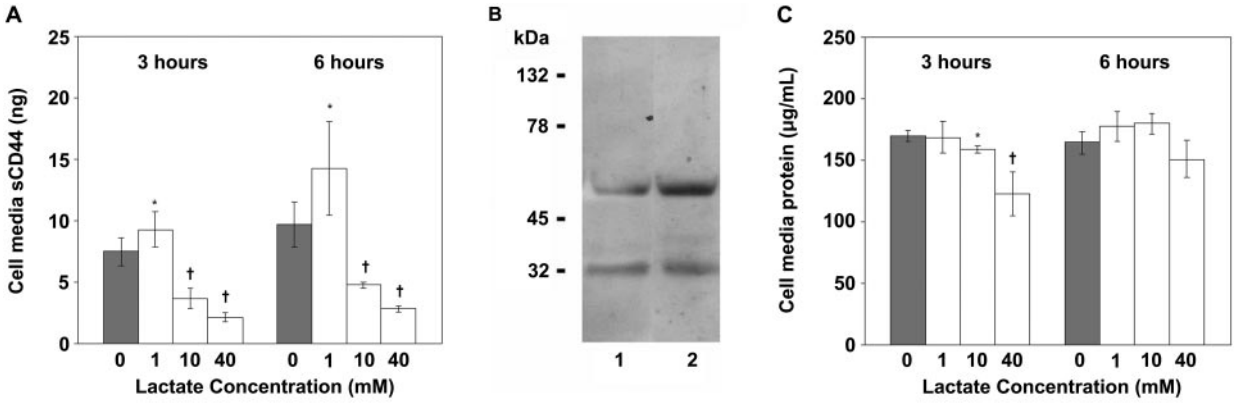

FIGURE 6. Protein and sCD44 concentration in the cell media of lactate-treated TM cells. (**A**) sCD44 concentration in the cell media. The *x*-axis is the dose of lactate and the *y*-axis is the concentration of sCD44 in the culture media as determined by ELISA. The results are expressed as the mean ($n = 6$); error bars, SD; $^*P < 0.05$, †$P < 0.01$ comparing lactate-treated media with control media. (**B**) Western blot analysis of media sCD44 after 6 hours with 20 μg protein equivalent and CD44 monoclonal antibody with chemiluminescent detection. *Left*: molecular mass. *Lane 1*: control media and *lane 2*: 1 mM lactate-treated media. Results of one of five representative experiments are shown. (**C**) Protein concentration in the cell media. The *x*-axis is the dose of lactate and the *y*-axis is the concentration of protein in the culture media as determined by protein assay. The results are expressed as the mean ($n = 6$); bars, SD; $^*P < 0.05$, †$P < 0.01$ comparing lactate-treated media with control media.

TABLE 1. Media sCD44*

		Lactate Dose					
Time	**Control**	**1 mM**	***P***	**10 mM**	***P***	**40 mM**	***P***
3 h	0.304 ± 0.036	0.382 ± 0.013	(0.02)†	0.182 ± 0.038	(0.02)†	0.119 ± 0.019	(0.001)†
6 h	0.376 ± 0.021	0.546 ± 0.102	(0.05)†	0.336 ± 0.020	(0.08)†	0.315 ± 0.020	(0.02)†
P	(0.04)‡	(0.05)‡		(0.003)‡		(0.0003)‡	

* sCD44 concentration in the media was determined by ELISA and is expressed as picograms per viable cell.

† The significance in the change in sCD44 concentration, as indicated in parentheses, was determined by Student's *t*-test comparing control to lactate-treated cells.

‡ The significance in the change in sCD44 concentration was also determined by Student's *t*-test comparing each treatment at 3 and 6 hours.

an increase in plasma levels of lactate to 6 to 10 mM.[22,23] The 1-mM lactate concentration used in this study is within physiologic limits, whereas the 10-mM lactate concentration is at the border of physiologic limits and the 40-mM lactate concentration is outside physiologic levels. Of note is that TM cells were grown in DMEM with no lactate and tested in 0.1% FCS. They were challenged with exogenous lactate, which has been shown to increase the NAD(P)H/NAD(P) ratio in a manner similar to hypoxia.[24,25] Whereas cells in situ are unaffected by 4.3 mM lactate in the aqueous,[26] the 1 mM lactate caused stress of TM cells in our culture system.

Within 5 minutes of lactate treatment, NF-κB was observed in the nuclear extract of human TM cells, indicating that NF-κB was activated and translocated to the nucleus. Nuclear translocation is essential for transcriptional activation of NF-κB target genes in response to a wide variety of stimuli such as cytokines, oxidant-free radicals, inhaled particles, ultraviolet irradiation, and bacterial or viral products.[27] The increased level of NF-κB in nuclear extracts indicated a time-dependent NF-κB activation in the lactate-treated cells. Notably, an increase in NF-κB nuclear translocation in TM occurs in all types of glaucoma.[3,28]

Lactate treatment also resulted in a dose-dependent increase in MT1-MMP expression in the media and cell lysates after 5 minutes and a marked increase of MT1-MMP in the media after 6 hours. The active form of MT1-MMP was detected by anti-MT1-MMP antibody which recognizes the catalytic domain of MT1-MMP. The pro form of MT1-MMP was detected by the pro anti-MT1-MMP antibody, which recognizes the ectodomain of MT1-MMP. The increased pro form of MT1-MMP shows that this protease is shed from the cells as a consequence of lactate treatment. MT1-MMP shedding generates an active enzyme, a phenomenon that is well known in cultures of a variety of cells, human sputum, and bronchoalveolar lavage fluid.[29,30]

MT1-MMP binds to CD44 through the hemopexin-like domain[31,32] and cleaves several cell surface proteins such as CD44 and syndecan-1,[33] both of which are present in TM cells.[19,34,35] MT1-MMP is upregulated by mechanical stretch in TM cells,[36] ischemia,[37,38] reactive oxidative species,[39] and cell injury.[40] MT1-MMP participates in the pericellular proteolysis and remodeling of the extracellular matrix[41] and also activates other members of the MMP family (e.g., proMMP-2 and proMMP-13) on the cell surface.[42] The regulation of the MT1-MMP protease activity involves a variety of inhibitors, formation of homo-oligomer complexes, and internalization.[29]

In this study, lactate treatment of human TM cells resulted in the ectodomain shedding of 32- and 55- kDa sCD44 into the media, presumably induced by MT1-MMP; these fragments are of the same molecular weight as those as in human aqueous. The 1-mM dose of lactate was the optimum dose to induce shedding of sCD44 into the media. Higher dosages of lactate resulted in decreased sCD44 release into the media and the cell death. sCD44 is released from the cell surface in response to ligand binding[13,29] and the activation of MT1-MMP.[11,43] Shedding of the extracellular portion of CD44 is the last step in the regulation of the molecule-releasing interaction between the ligand and cell.[13] Lactate treatment of fibroblasts for 20 hours has been shown to increase CD44 expression and sCD44 release.[10] Analysis of RNA from lactate-treated fibroblasts by reverse transcriptase–polymerase chain reaction further revealed that lactate activated genes in metabolic stress include increased transcripts of c-fos, c-jun, c-ets, hyaluronidase -1 and -2, CD44, and caveolin-1 mRNAs.[23] The release of sCD44 from the cell surface into the media is also followed by release of the transmembrane portion of CD44 by gamma secretase activity.[44] The released portion of the transmembrane CD44 (i.e., the intracytoplasmic domain) translocates to the nucleus and promotes the activation of NF-κB.[45] Our data suggest that the effects of lactate occur within minutes, with the initial activation of NF-κB and MT1-MMP, followed by the subsequent shedding of sCD44, and then the intracytoplasmic domain of CD44 could upregulates NF-κB expression.

The multifunctional CD44H transmembrane protein is widely distributed and once was considered to function primarily as a cell-adhesion molecule. CD44H is now recognized, however, as having broader functions involved in inflammatory and immune response,[46] as well as participating in phagocytosis,[47] cell signaling, and cell survival pathways.[48–50] Normally, CD44H is required for activation of certain high-affinity receptors (e.g., erbB2 phosphorylation and erbB2-erbB3 heterodimerization) for cell survival.[50] Proteolytic cleavage of the extracellular domain of CD44H releases sCD44 which has different biological functions than the intact CD44H[11,51,52] and the released sCD44 acts on other cells to adapt to their environment. If sCD44 interferes with CD44H activity, erbB2 becomes less active, which may result in the extrinsic pathway of apoptotic cell death.

Our results show that lactate treatment of human TM cells resulted in NF-κB activation, MT1-MMP activation, and the shedding of sCD44. Shedding of sCD44 may be a mechanism that human TM cells use to cope with cellular stresses present in primary open-angle glaucoma (POAG). Increased concentrations of sCD44, as seen in POAG aqueous, however, may have a detrimental cytotoxic effect on susceptible cells. In normal eyes, aqueous sCD44 is inactivated by hyaluronic acid,[18] but in conditions of impaired aqueous outflow and decreased hyaluronic acid such as in POAG, increasing sCD44 concentrations may lead to a series of detrimental cell responses that ultimately result in cell death.[18,53]

In the present study, low concentration of lactate treatment triggered NF-κB translocation, MT1-MMP activation, and shedding of sCD44 without loss of cell viability. Higher concentrations of lactate treatment also led to NF-κB translocation and MT1-MMP activation, but with a decreased level of media

sCD44, presumably as a consequence of severe metabolic stress and decreased protein synthesis of CD44. In keeping with this concept, the concentration of sCD44 was increased in the aqueous of patients with POAG with early stages of visual field loss, whereas sCD44 concentration was decreased in the aqueous of patients with POAG with advanced visual field loss, particularly in that of African-American patients. (Nolan MJ et al. *IOVS* 2005;46:ARVO E-Abstract 3777).

In summary, lactate treatment of human TM cells results in dose-dependent changes in sCD44 shedding that parallel the pattern of sCD44 concentration in patients with POAG. Lactate treatment of human TM cells in culture offers a useful model of metabolic stress for examining cell responses that occur in POAG. The cell model of metabolic stress using lactate is also applicable in evaluating potential control points to mitigate stress responses for the treatment of POAG.

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