Acid increases NHE8 surface expression and activity in NRK cells

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Joseph C, Twombley K, Gattineni J, Zhang Q, Dwarakanath V, Baum M. Acid increases NHE8 surface expression and activity in NRK cells. Am J Physiol Renal Physiol 302: F495–F503, 2012. First published November 16, 2011; doi:10.1152/ajprenal.00331.2011.—We previously demonstrated that there is a paucity of brush-border membrane NHE3 in neonates, the predominant Na+/H+ exchanger in the adult proximal tubule, while NHE8 is relatively highly expressed in neonates compared with adults. We recently showed that metabolic acidosis in neonatal rodents can increase brush-border membrane NHE8 protein expression and Na+/H+ exchange activity. To further examine the regulation of NHE8 by acid, we incubated NRK cells, which express NHE8 but not NHE3, with either acid or control media (6.6 vs. 7.4). There was an increase in Na+/H+ exchanger activity within 6 h of incubation with acid media assessed as the rate of sodium-dependent recovery of pH from an acid load (dph/dt). The acid stimulation persisted for at least 24 h. The increase in Na+/H+ exchange activity was paralleled by an increase in surface expression of NHE8, assessed by surface biotinylation and streptavidin precipitation. The increase in both apical membrane NHE8 protein expression and Na+/H+ exchange activity with pH 6.6 media compared with 7.4 media was not affected by actinomycin D or cycloheximide consistent with an increase in surface expression independent of mRNA or protein synthesis. Furthermore, there was no increase in total cellular NHE8 protein abundance or mRNA abundance with acid media. Finally, we demonstrate that the increase in surface expression of NHE8 with acid media was blocked by colchicine and cytochalasin D and mediated by acid increasing the rate of exocytosis. In conclusion, NHE8 surface expression and activity are regulated by acid media by increasing the rate of trafficking to the apical membrane.

METHODS

Cell culture. Normal rat kidney cells (NRK cells) were purchased from American Type Culture Collection (Manassas, VA). Cells were cultured in high-glucose DMEM (GIBCO, Grand Island, NY) supplemented with 5% fetal calf serum and 1 mM sodium pyruvate at 37°C in a 95% O2-5% CO2 environment and supplemented with penicillin (100 U/ml) and streptomycin (100 U/ml) as previously described by our laboratory (28). The cells were rendered quiescent when confluent by incubation in serum-free DMEM/Ham’s F12 (Sigma, St. Louis, MO) adjusted to a pH of 7.4 for 24 h. They were then washed extensively to remove the serum and incubated in the serum-free DMEM/Ham’s F12 that was adjusted to pH 6.6 or maintained at a pH of 7.4. The media was adjusted to an osmolality of 295 mosmol/kgH2O. The bicarbonate concentration of the pH 6.6 media was 5 mM and the 7.4 had a bicarbonate concentration of 19 mM. Unless otherwise specified, all chemicals were obtained from Sigma.

cDNA synthesis and real-time PCR. Total cellular RNA was isolated from NRK cells using GenElute Mammalian Total RNA Miniprep Kit per the manufacturer’s instructions (Sigma). RNA (2 μg) was treated with DNase I and the product was used to synthesize cDNA using random hexamer primers and reverse transcriptase (Stratagene, La Jolla, CA) at an annealing temperature of 25°C for 10 min, extension at 42°C for 50 min, and termination at 70°C for 15 min.

Real-time PCR was performed using an iCycler PCR Thermal Cycler (Bio-Rad, Hercules, CA) to quantify relative mRNA abundance. Primers for NHE8 were mixed with cDNA and SYBR green
master mix (Bio-Rad) using the manufacturer’s instructions. The PCR conditions were denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s for 40 cycles. 28s was used as housekeeping gene used to normalize the expression of NHE8 using the method described by Vandesompele et al. (24). NHE8 primers were (forward) 5'- AAGGCTATTTCGCGTTGAGCACA-3', (reverse) 5'- AGAGAAAACAGGCACGCTCCTCA-3', and 28S (forward) 5'-TTG AAA ATC CGG GGG AGA G-3', (reverse) 5'-ACA TTG TTC CAA CAT GCC AG-3'.

**SDS-PAGE and immunoblotting.** NRK cells were rinsed with PBS three times followed by lysis with RIPA buffer [150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 1% Triton-X-100, 0.5% deoxycholate, 1% SDS, and 0.5% deoxycholate containing protease inhibitors]. The protein lysates were centrifuged at 14,000 g for 30 min at 4°C and the protein content was determined using the Bradford reaction. Proteins were heated to 37°C for 2 min, separated on an 8% polyacrylamide gel using SDS-PAGE as previously described (14), and transferred to a polyvinylidene difluoride membrane (Immobilon, Millipore, Billerica, MA) at 400 mA at 4°C for 1 h (7, 10, 21). The blots were blocked with Blotto (1% nonfat milk and 0.05% Tween-20 in PBS, pH 7.4) for at least 1 h. The blots were then incubated with a monoclonal NHE8 antibody (a gift from Orson Moe) overnight at 4°C at a 1:5 dilution. The blots were washed in Blotto followed by the addition of the secondary antibody, a horseradish peroxidase-conjugated donkey anti-mouse antibody at 1:10,000 dilution (Santa Cruz Biotechnology). Enhanced chemiluminescence was used to detect bound antibody. In blots where total protein was assessed, equal loading of the samples was verified using an antibody to β-actin at a 1:15,000 dilution (Sigma). Relative protein abundance was assessed using densitometry.

**Measurement of NHE8 in total cell lysates.** Confluent NRK cells in 100-mm plates were washed twice with ice-cold PBS and lysed with RIPA lysis buffer [150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 1% Triton-X-100, 0.5% deoxycholate, and 0.1% SDS] containing a protease inhibitor protease cocktail (1:1,000 dilution) and 100 μg/ml phenyl-methane-sulfonyl fluoride. The samples were centrifuged at 13,000 g for 30 min at 4°C and the supernant was used for immunoblotting using 50 μg of protein per sample. The protein was heated to 60°C for 2 min before SDS-PAGE as described above.

**Cell surface NHE8 expression.** The protocol for cell surface biotinylation has previously been described from our laboratory (10). Confluent NRK cells were rinsed with ice-cold PBS. The cells were biotinylated by adding 1.5 mg/ml sulfo-NHS-SS-biotin (Pierce, Rockford, IL) in 10 mM triethanolamine (pH 7.4), 150 mM NaCl, and 2 mM CaCl2 and incubated on a horizontal rotator for 60 min at 4°C. Plates were washed twice with 6 ml of quenching buffer (PBS with 100 mM glycine, 1 mM MgCl2, and 0.1 mM CaCl2,) for 20 min at 4°C. Cells were then washed once more with ice-cold PBS. NRK cells were then lysed with RIPA buffer containing protease inhibitors, centrifuged at 13,000 g, and the supernant was diluted to 2.5 mg/ml protein with RIPA buffer containing protease inhibitors. The cell lysates containing equal amounts of protein were then incubated overnight with streptavidin-agarose beads (Pierce) at 4°C. Streptavidin-agarose beads were then washed as previously described (10, 28) and biotinylated proteins were heated to 85°C in loading buffer to release the proteins that were then assayed by immunoblot.

In experiments designed to determine whether RNA or protein synthesis was necessary for acid to increase surface NHE8, confluent NRK cells were preincubated with serum-free media containing actinomycin D (5 × 10^-6 M) or cycloheximide (10 μg/ml) for 1 h (10, 25). Similarly, in experiments designed to examine whether microtubules or the actin cytoskeleton were involved in trafficking of NHE8, cells were preincubated for 1 h with 10^-4 M colchicine or 10^-6 M cytochalasin D at pH 7.4 (18, 27). Cells were then exchanged into serum-free media at either pH 7.4 or pH 6.6 containing either actinomycin D, cycloheximide, colchicine, or cytochalasin D and incubated for 24 h. Surface NHE8 was then assayed as above.

**NHE8 exocytosis.** The assays used to assess exocytosis have been described previously by our laboratory (10). NRK cells were rinsed with PBS and incubated at 4°C with 1.5 mg/ml sulfo-NHS-SS-acetate.
using the pH-sensitive dye 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM; Molecular Probes, Eugene, OR), as described previously (15). NRK cells were incubated with 10 μM BCECF-AM (BCECF-acetoxyethyl ester) for 20 min at 37°C, and intracellular pH (pHi) was determined from the ratio of fluorescence (excitation: 500 and 450 nm, emission: 530 nm) in a computer-controlled spectrofluorometer (Photon Technology International, Birmingham, NJ). The 500/450 fluorescence ratio was calibrated to pHi using the K+/nigericin technique (6, 17). Na+/H+ exchange activity was determined as the initial rate of change in pHi after an acid load upon addition of sodium (15, 28). Briefly, NRK cells were initially incubated in sodium-free solution with nigericin (10 μM) upon addition of sodium (15, 28). Addition of NH4Cl led to an increase in intracellular pH. After steady state was achieved, this solution was replaced with a sodium-free solution and pH decreased rapidly due to dissociation of the NH4+ to NH3 and H+. This solution was then replaced with 25 mM sodium-containing solution and the rate of recovery of pH was determined as above.

Measurement of basal pHi. NRK cells on coverslips were rendered quiescent after they became confluent in serum-free media with a pH of 7.4 for 24 h after which they were incubated in media with pH of 7.4 or 6.6 for 24 h. The coverslips were incubated with BCECF-containing solution for 20 min after which they were transferred to a cuvette containing serum-free media containing (in mM) 130 NaCl, 7.4 or 6.6 for 24 h. The coverslips were incubated with BCECF-containing solution for 20 min after which they were transferred to a cuvette containing serum-free media containing (in mM) 130 NaCl, 30 HEPES, 5 KCl, 5 glucose, 5 alanine, 1.1 calcium chloride, 1.54 MgCl2, 1.5 Na2HPO4, and adjusted to pH 6.6 or 7.4 with hydrochloric acid or sodium hydroxide. The pHi of the cells was then measured using the spectrophotometer.

Determination of buffer capacity. The buffer capacity was calculated as described previously by Boyarsky et al. (11) in explicit detail. We determined the buffer capacity by measuring the change in pHi in response to a change in intracellular NH4+ using the following equation: $\beta = \Delta[pH]/\Delta[pHi].$

![Fig. 2. Effect of acid incubation on apical membrane NHE8 protein expression in vitro.](http://ajprenal.physiology.org/)
Specifically, we assessed the change in pH, after removing 10 mM NH$_4$Cl (NaCl replacement) from the bathing solution initially containing 20 mM NH$_4$Cl.

Statistical analysis. All data are expressed as means ± SE. Comparisons were made using an unpaired Student’s $t$-test. A $P$ value of $\leq 0.05$ was considered significant.

RESULTS

In the first series of experiments, we examined whether NHE8 mRNA in NRK cells was affected by the pH of the media after incubation for 24 h. The pH of the media did cause a change in pH$_i$. After incubation with 7.4 media for 24 h, the pH$_i$ was 7.63 ± 0.07 and 6.97 ± 0.10 in cells incubated at pH 6.6 ($P < 0.01$). As shown in Fig. 1A, there was no difference in NHE8 mRNA expression compared with 28s in NRK cells incubated for 24 h at pH 6.6 compared with 7.4. There was also no effect of media pH on total cellular NHE8 protein abundance (Fig. 1B) in NRK cells incubated for 24 h. This was also true for cells incubated in the presence of actinomycin D or cycloheximide (Fig. 1, C and D, respectively).

The total cellular NHE8 does not necessarily correlate with the amount of NHE8 on the apical membrane. We used surface biotinylation followed by streptavidin precipitation and immunoblotting to determine whether acid incubation increased surface expression of NHE8. As shown in Fig. 2, there was a time-dependent increase in NHE8 expression on the apical membrane of NRK cells incubated at pH 6.6 compared with media that had a pH of 7.4. The expression was comparable at 3 h, but NHE8 expression was higher at pH 6.6 than 7.4 at 6, 12, and 24 h of incubation.

In the next series of experiments, we assessed Na$^+$/H$^+$ exchanger activity by measuring the sodium-dependent recovery from an acid load. As shown in Fig. 3A, there was no difference in dpH/dt at 3 h in cells incubated at pH 6.6 and 7.4. There was a small but statistically significant increase in Na$^+$/H$^+$ exchanger activity in cells incubated with an acid pH compared with cells incubated at pH 7.4 at 6 h and a more pronounced increase at 12 h which was sustained in cells incubated in an acid medium for 24 h (Fig. 3, B-D). In cells incubated for 24 h, the dpH/dt was 0.06 ± 0.01 in the 7.4 group and 0.05 ± 0.01 in the presence of 10$^{-6}$ M EIPA ($P = N S$), which is comparable to the inhibition we previously found for NHE8 (28). Typical tracings of pH recovery from cell acidification using the nigericin technique are shown in Fig. 4. To validate our findings using another technique, we assessed the rate of recovery from an acid load induced by incubation and removal of 20 mM NH$_4$Cl. The dpH/dt was 0.04 ± 0.01 in cells incubated at pH 7.4 for 24 h and 0.14 ± 0.05 pH U/min for cells incubated at pH 6.6 ($P = 0.01$). The difference in rates of recovery from an acid load between the 7.4 and 6.6 group was not due to a difference in buffer capacity as this was comparable in the two groups (3.6 ± 0.3 and 3.3 ± 0.5 for the 7.4 and 6.6 groups, respectively).

In the next series of experiments, we examined the effect of actinomycin D on surface NHE8 expression and Na$^+$/H$^+$ exchanger activity. NRK cells were incubated at pH 7.4 and 6.6 for 24 h in the presence of actinomycin D to inhibit mRNA synthesis. The stimulation of Na$^+$/H$^+$ exchanger activity and increase in surface NHE8 expression in cells incubated at the lower pH media remained in the presence of actinomycin D. These results are shown in Fig. 5.

We next examined the effect of media pH on the surface expression of NHE8 as well as Na$^+$/H$^+$ exchanger activity.

Fig. 3. Effect of acid incubation on Na$^+$/H$^+$ exchange activity in NRK cells. Na$^+$/H$^+$ exchange activity was assessed in NRK cells using the K$^+$/nigericin technique. There was no pH recovery in the absence of sodium. The rate of cell pH was assessed after the addition of 25 mM sodium. The dpH/dt was comparable at pH 6.6 and 7.4 at 3 h as shown in A. In these experiments, there was no difference in the initial cell pH nor the intracellular pH (pH$_i$) nadir from which pH recovery was measured after addition of sodium. The initial cell pH in the 7.4 group was 7.64 ± 0.01 in the control group and 7.66 ± 0.01 in the 6.6 group. The pH$_i$ nadir was 6.64 ± 0.01 and 6.63 ± 0.01 in the 7.4 and 6.6 pH groups, respectively. The rate of recovery from an acid load was faster in cells incubated at pH 6.6 compared with 7.4 for 6, 12, and 24 h as shown in B, C, and D, respectively.

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when protein synthesis was inhibited with cycloheximide. These results are shown in Fig. 6. After incubation at pH 6.6 for 24 h in the presence of cycloheximide, the expression of NHE8 was greater than cells incubated at 7.4. Similarly, the Na\(^+/H^+\) exchanger activity was also faster in cells incubated at pH 6.6 in the presence of cycloheximide compared with that at 7.4. Thus, the effect of acid pH on surface expression of NHE8 and Na\(^+/H^+\) exchanger activity was not dependent on mRNA or protein synthesis.

The effect of colchicine, an inhibitor of cytoskeleton microtubule-mediated trafficking, on NHE8 by acid pH was next examined. As shown in Fig. 7, the surface expression of NHE8 and Na\(^+/H^+\) exchanger activity was comparable after 24 h of incubation at pH 6.6 and 7.4 in the presence of colchicine. This indicates that microtubules are likely involved in the acid-mediated increase in exocytosis by acid pH. The effect of acid on NHE8 surface expression and Na\(^+/H^+\) exchanger activity was also affected by cytochalasin D, which disrupts the actin cytoskeleton as shown in Fig. 8.

The increased surface expression of NHE8 after incubation with acidic media in the presence of actinomycin D and cycloheximide without an increase in total cellular NHE8 is...
consistent with an increase in trafficking of NHE8 to the apical membrane. We next examined whether acid incubation affected exocytosis by first labeling the NRK cells with sulfo-NHS-acetate to saturate all the NHS-reactive sites on the apical membrane. We then incubated the cells in either pH 6.6 or 7.4 media and performed surface biotinylation as in METHODS. The biotinylated surface NHE8 is that which was intracellular at the beginning of the experiment and not accessible to sulfo-NHS-acetate. The NHE8 retrieved by biotinylation is that which was inserted to the apical membrane during the 24-h incubation at pH 7.4 or 6.6. As shown in Fig. 9, A and B, there was greater surface NHE8 in the cells incubated at pH 6.6 than 7.4 consistent with acid media increasing exocytosis of NHE8 at 24 h. As shown in Fig. 9, C and D, there was also an increase in surface expression of NHE8 when cells were incubated in pH 6.6 compared with 7.4 when the exocytosis assay was performed in the presence of actinomycin D.

**DISCUSSION**

There are a number of protective mechanisms that occur in response to metabolic acidosis including an increase in proximal tubule bicarbonate reabsorption mediated in large part by an increase in Na\(^+/\)H\(^+\) exchange activity (1, 13, 19, 22, 26). In the adult proximal tubule, apical Na\(^+/\)H\(^+\) exchange is predominantly via NHE3 (9, 23, 26). Na\(^+/\)H\(^+\) exchange in the neonatal proximal tubule is likely predominantly mediated by NHE8, which is highly expressed on the neonatal proximal tubule brush-border membrane and which decreases in abundance at the time of weaning in the rat and mouse concordant with the increase in NHE3 expression (9, 23).

Neonates have a lower serum bicarbonate concentration than adults and are prone to metabolic acidosis due to diseases such as diarrhea. We recently examined whether neonatal mice and rats could respond to a metabolic acidosis with an increase in proximal tubule acidification (23). Both mice and rats were studied at a time when NHE8 was highly expressed on the apical membrane and there was a paucity of NHE3. Three days of acid loading resulted in a 10-meq/l decrease in the serum bicarbonate concentration. There was an increase in Na\(^+/\)H\(^+\) exchanger activity with acid loading in both mice and rats. In rats brush-border membrane vesicles were studied and the increase in Na\(^+/\)H\(^+\) exchange activity was highly EIPA sen-

**Fig. 6.** Effect of acid incubation on apical membrane NHE8 protein abundance and Na\(^+/\)H\(^+\) exchange activity with inhibition of protein synthesis. NRK cells were incubated with 10 μg/ml cycloheximide to inhibit protein synthesis for 1 h and then an additional 24 h at pH 6.6 or 7.4 also in the presence of cycloheximide. A: effect of incubation with the 2 pHs on surface NHE8 expression. A typical immunoblot is shown below. B: effect of the different pH media on Na\(^+/\)H\(^+\) exchange activity in the presence of cycloheximide. C: typical immunoblot where the protein expression was greater in cells incubated at pH 6.6 compared with cells incubated at 7.4 (both in the presence of cycloheximide).
sitive consistent with an upregulation of NHE8 and not NHE3 by acidosis. As in the adult rat, there was no change in NHE3 mRNA abundance in mice and actually a decrease in NHE3 mRNA abundance in the neonatal rats with acidosis (1, 23). Acid loading did not affect NHE8 mRNA abundance in either neonatal mice or rats. In both neonatal mice and rats, there was an increase in total protein and brush-border membrane NHE3 protein abundance. However, NHE8 expression was increased on the brush border without a change in the total cellular content suggesting that trafficking may be involved in the regulation of NHE8 by acid. In this study, we used NRK cells that express NHE8 and we induced intracellular acidification by incubation with a media of pH 6.6 compared with pH 7.4 to study the mechanism for the increase in NHE8 by acid.

Previous studies examined the mechanism for the stimulation in Na\(^+\)/H\(^+\) caused by metabolic acidosis using OKP cells, a cell line that like adult proximal tubules expresses NHE3 but does not express NHE8 (3, 27). Incubation of OKP cells in acid compared with control media for 24 h resulted in an increase in Na\(^+\)/H\(^+\) exchanger activity, NHE3 mRNA and NHE3 protein abundance (2, 3, 27). Incubation of OKP cells with an acid media resulted in an increase in exocytosis of NHE3 to the apical membrane (27). In OKP cells incubation in acid media resulted in an increase in NHE3 mRNA after 12 h of incubation that was comparable to the increase at 24 h (3). This is in contrast to the present study where there was no increase in NHE8 mRNA abundance in NRK cells even after 24 h of incubation of cells in acid media compared with control 7.4 pH media. Furthermore, in the present study the acid-induced effect on NHE8 was still found in the presence of actinomycin D.

There were other differences in the regulation of NHE3 and NHE8 by acid media. Incubation with acid media increased NHE3 total protein abundance at 24 h but not before that time (27). This is in contrast to the present findings examining the effect of NHE8 in NRK cells where NHE8 total protein abundance was not affected by the pH of the media even after 24 h. In OKP cells, there was an increase in surface NHE3 protein abundance as early as 6 h after incubation in acid media compared with control which was reflected by an increase in Na\(^+\)/H\(^+\) exchanger activity at this time point (3, 27). The increase in Na\(^+\)/H\(^+\) exchanger activity in OKP cells was not inhibited by cycloheximide, suggesting that mechanisms other than mRNA and protein synthesis were involved in the acute stimulation of Na\(^+\)/H\(^+\) exchanger activity.
exchange activity by acid (27). In the present study, there was an acid-induced increase in Na\(^+/\)H\(^+\) exchange activity and surface NHE8 expression in the presence of cycloheximide. Thus, the effect of acid on NHE8 surface protein expression does not require protein synthesis.

The effect of acid on trafficking of NHE3 to the apical membrane has been studied previously (27). Using the same exocytosis assay as in the present study, NHE3 trafficking to the apical membrane was stimulated by acid media at 6 h of incubation; well before the increase in mRNA and total protein abundance by acid. There was a concomitant increase in Na\(^+/\)H\(^+\) exchange activity in OKP cells at 6 h as well. The increase in Na\(^+/\)H\(^+\) exchange activity was blocked by both colchicine and latrunculin B consistent with microtubules and actin cytoskeleton being involved in trafficking. Thus, in OKP cells acid incubation increases NHE3 and Na\(^+/\)H\(^+\) exchange activity by both trafficking and mRNA and protein synthesis. The effect of acid on trafficking to the apical membrane occurs before the increase in NHE3 mRNA and protein abundance.

In the present study, we also demonstrate a concordant time-dependent increase in surface NHE8 protein expression and Na\(^+/\)H\(^+\) exchange activity. We demonstrate that this occurs in the presence of cycloheximide and actinomycin D. The acid-induced increase in surface NHE8 protein abundance and Na\(^+/\)H\(^+\) exchanger activity was blocked by both colchicine and latrunculin B consistent with microtubules and actin cytoskeleton being involved in trafficking. Thus, in OKP cells acid incubation increases NHE3 and Na\(^+/\)H\(^+\) exchange activity by both trafficking and mRNA and protein synthesis. The effect of acid on trafficking to the apical membrane occurs before the increase in NHE3 mRNA and protein abundance.

In summary, the present study examined the mechanism for the increase in NHE8 with acid media. In response to acid media, NRK cells had a time-dependent increase in Na\(^+/\)H\(^+\) exchange activity and surface NHE8 protein abundance with no change in NHE8 total cellular protein or mRNA. Our results are consistent with our previous in vivo studies showing that brush-border membrane NHE8 and Na\(^+/\)H\(^+\) exchange activity increase with metabolic acidosis, but there was no change in NHE8 mRNA and total cellular NHE8 protein abundance. Furthermore, the increase in apical membrane NHE8 protein abundance and Na\(^+/\)H\(^+\) exchange activity occurred in the presence of actinomycin D and cycloheximide demonstrating that the acid-induced increase in NHE8 surface expression occurred without mRNA or protein synthesis. The increase in NHE8 surface activity by acid was mediated by an increase in exocytosis. These data demonstrate that trafficking is the predominant and likely sole mechanism for mediating the acid-induced regulation of NHE8.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: C.J., K.T., J.G., Q.Z., and V.D. performed experiments; C.J., K.T., J.G., Q.Z., and M.B. analyzed data; C.J., J.G., and M.B. interpreted results of experiments; C.J. and M.B. prepared figures; C.J., K.T., J.G., Q.Z., and V.D. approved final version of manuscript; M.B. conception and design of research; M.B. drafted manuscript; M.B. edited and revised manuscript.

REFERENCES


