Conditionally immortalized human proximal tubular epithelial cells isolated from the urine of a healthy subject express functional calcium-sensing receptor

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Di Mise A, Tamma G, Ranieri M, Svelto M, van den Heuvel B, Levchenko EN, Valenti G. Conditionally immortalized human proximal tubular epithelial cells isolated from the urine of a healthy subject express functional calcium-sensing receptor. Am J Physiol Renal Physiol 308: F1200–F1206, 2015. First published February 5, 2015; doi:10.1152/ajprenal.00352.2014.—The calcium-sensing receptor (CaSR) is a G protein-coupled receptor, which plays an essential role in regulating Ca2+ homeostasis. Here we show that conditionally immortalized proximal tubular epithelial cell line (ciPTEC) obtained by immortalizing and subcloning cells exfoliated in the urine of a healthy subject expresses functional endogenous CaSR. Immunolocalization studies of polarized ciPTEC revealed the apical localization of the receptor. By Western blotting of ciPTEC lysates, both monomeric and dimeric forms of CaSR at 130 and 250 kDa, respectively, were detected. Functional studies indicated that both external calcium and the positive CaSR alloseric modulator, NPS-R568, induced a significant increase in cytosolic calcium, providing a high sensitivity of the endogenous receptor to its agonists. Calcium depletion from the endoplasmic reticulum using cyclopiazonic acid abolished the increase in cytosolic calcium elicited by NPS-R568, confirming calcium exit from intracellular stores. Activation of CaSR by NPS-R significantly reduced the increase in cAMP elicited by forskolin (FK), a direct activator of adenylate cyclase, further confirming the functional expression of the receptor in this cell line. CaSR expressed in ciPTEC was found to interact with Gαq as a downstream effector, which in turn can cause release of calcium from intracellular stores via phospholipase C activation. We conclude that human proximal tubular ciPTEC express functional CaSR and respond to its activation with a release of calcium from intracellular stores. These cell lines represent a valuable tool for research into the disorder associated with gain or loss of function of the CaSR by producing cell lines from patients.

the extracellular calcium-sensing receptor (CaSR) is a G protein-coupled receptor, originally cloned from the bovine parathyroid gland and successively identified in various organs (6, 7, 45). Besides the parathyroid gland, the key CaSR-expressing organs are intestine, bone, and kidney (43, 45). The CaSR senses changes in extracellular calcium concentrations and regulates parathyroid hormone (PTH) secretion and renal tubular calcium reabsorption to maintain serum calcium levels within the normal range. Ligand binding to the CaSR results in conformational changes of the intracellular loops, G protein-dependent stimulation of phospholipase C (PLC), causing an accumulation of inositol 1,4,5-trisphosphate (IP3) and rapid release of calcium ions from intracellular stores. The increase in intracellular calcium results in activation of protein kinase C, and CaSR also activates the mitogen-activated protein kinase (MAPK) pathway (4, 34, 36).

A significant limitation in studies of CaSR function in the kidney has arisen from the difficulty of clearly defining the patterns of CaSR expression along the nephron. Although all studies agree that the CaSR is expressed along the basolateral membrane of the thick ascending limb of Henle (TAL), a key site of regulated calcium reabsorption (24, 35, 37), some groups have reached different conclusions regarding the nature and level of CaSR expression in other sites. Along the nephron, Riccardi et al. (37) showed a specific receptor localization on the apical surface of the proximal tubule (PT). In contrast, Loupý et al. (24) reported that CaSR expression within the kidney is restricted to the TAL. A possible explanation for this discrepancy may be due to the use of commercial antibodies against specific fragments of the receptor. Riccardi and colleagues (8, 37, 38), using polyclonal antibodies against both full-length CaSR and specific regions of the exofacial domains coupled with antigen retrieval, were successful in obtaining a clear apical staining of the CaSR at the base of the brush border of PT.

In the kidney, the CaSR performs different tasks depending on the various tubular segments in which it is located (3, 20, 34, 44). In the PT, CaSR located on the apical membrane senses the increase in calcium luminal concentrations and inhibits cAMP production induced by PTH. In PTs, PTH causes phosphate excretion by internalization and degradation of phosphate reabsorption carriers in subapical vesicles derived from brush border (1, 48). In the TAL, activation of CaSR expressed on the basolateral membrane (35) inhibits calcium paracellular reabsorption by blocking claudin-16 by activation of claudin-14 transcription (15). In the cortical distal convoluted tubule, CaSR is expressed on the basolateral membrane, where it reduces calcium-active reabsorption, inhibiting calcium pump activity (plasma membrane Ca2+-ATPase, PMCA) (2, 13). In the collecting duct, CaSR is expressed on the apical membrane of the principal and intercalated cells (35, 45), where, during acute vasopressin action, its activation reduces...
aquaporin 2 (AQP2)-mediated water reabsorption and urinary concentration (31). CaSR has been shown also to reduce the vasopressin-induced AQP2 expression, via a calmodulin-dependent mechanism (9).

Regarding the physiological role of CaSR expressed in the PT, recent interesting studies performed in rats and in vitro perfused mouse PTs demonstrated a role of the CaSR in enhancing proximal tubular fluid absorption and urinary acidification by stimulation of luminal Na+/H+ exchanger (NHE) activity (10, 49). Activation of NHE will favor the ionization of calcium so that the ionized calcium is delivered to the distal portions of the nephron, where it would be more easily absorbed, thus avoiding calcium precipitation along more distant segments of the nephron. Therefore, the new concept is that CaSR expressed in the PT would play an active role in modulating PT fluid absorption as well as acid secretion, and its possible involvement in prevention of renal stone disease has been postulated.

On the basis of this recent evidence, it would be interesting to clarify at the cellular level the molecular mechanism occurring in PT epithelial cells activated by increased luminal calcium concentration leading to enhanced fluid reabsorption in the PT, a process related to activation of CaSR. To this end, a cell line that would be eligible is represented by the conditionally immortalized human proximal tubular epithelial cells (ciPTEC), whose isolation from urine and immortalization has been developed by Wilmer and coworkers (53). In recent studies, ciPTEC derived from patients were used to demonstrate the defect in Na-dependent phosphate reabsorption (52) in cystinosis and altered receptor-mediated endocytosis and the mechanisms of endosomal acidification occurring in them (16). These cell lines therefore may represent a valuable tool for research into the disorder associated with CaSR mutations by producing cell lines from patients. ciPTEC can be maintained for at least 45 passages and present PT characteristics when cultured at the nonpermissive temperature of 37°C for 10 days (53).

We provide here the first evidence that ciPTEC endogenously express CaSR at protein level. Moreover, exposure of ciPTEC to clinically relevant concentrations of calcium or to the positive allosteric CaSR modulator NPS-R568 resulted in an increase in intracellular calcium and a decrease of FK-induced cytosolic cAMP levels, demonstrating the expression of a functional receptor. ciPTEC could aid in understanding the role of CaSR in proton secretion and fluid reabsorption in PTs.

**MATERIALS AND METHODS**

Materials. All chemicals were purchased from Sigma (Sigma-Aldrich, Milan, Italy). Fura-2 AM was obtained from Molecular Probes (Life Technologies, Monza, Italy). NPS-R568 was kindly gifted by Amgen (Amgen Dompe, Milan, Italy). Media for cell culture were from Lonza (Lonza, Milan, Italy). Antibodies. Monoclonal CaSR antibody recognizing amino acid 15–29 at the extracellular NH2 terminus (17, 47) was from Sigma-Aldrich. To detect AQP2, we used antibodies against the 20-amino-acid residue segment just NH2 terminal from the polyphosphorylated region of rat AQP2 (CLKGLEPDTDWEERVRQRQ) (19, 41). Mouse monoclonal NHE3 and rabbit anti-Na+/K+/2Cl− cotransporter (NKCC2) antibodies were from Chemicon (Millipore, Merck, Milan, Italy). Rabbit polyclonal anti-AQP1, mouse anti-zonula occludens 1 (ZO-1) and mouse anti-Gαi2 antibodies were obtained from Santa Cruz Biotechnologies (TebuBio, Milan, Italy). Secondary goat anti-mouse-488-conjugated antibody and streptavidin-488 and streptavidin-488-conjugated antibody and streptavidin-488 conjugate antibodies conjugated with Alexa Fluor (Molecular Probes, Eugene, OR). Secondary rabbit anti-mouse-biotin, goat anti-rabbit, and goat anti-mouse antibodies were purchased from Sigma-Aldrich.

Proximal tubular epithelial cell line generation. Primary cells were cultured as described by Wilmer and coworkers (51) by collecting midstream urine. The cell line used in this study was derived from a female healthy donor, born in 1989, who was 12 yr old when her urine was taken. Parental consent was obtained.

Urine was centrifuged (223 g, 5 min, room temperature) within 5 h after collection. After being washed in PBS and a second centrifugation step, urine sediment was resuspended in 3 ml proximal tubular epithelial cells culture medium (DMEM Ham’s F12) supplemented with 10% FBS, 100 IU/ml penicillin, 100 mg/ml streptomycin, 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, 36 ng/ml hydrocortisone, 10 ng/ml EGF, and 40 μg/ml triodothyronine (11). The suspension was transferred to a 25-cm2 tissue culture flask and placed at 37°C in a 5% CO2 incubator. The medium was refreshed every 2–3 days (53).

Immortalization, subcloning, and generation of ciPTEC. Primary cells were immortalized as described (53). Briefly, cells were infected with SV40T and human telomerase reverse transcriptase vectors using the amphotropic packaging cell line PA 317 (39), containing geneticin (G418) and hygromycin resistance (30, 40). Subconfluent cell layers were transferred to 33°C and selected by using G418 (400 μg/ml) and hygromycin B (25 μg/ml) for 10 days. After being cultured for 2 wk at 33°C, single-cell clones were visible and picked by using cloning discs drained in trypsin/EDTA. For the following experiments, cells were cultured at 33°C to 70% confluency, followed by maturation for 10 days at 37°C during which the cells formed a confluent monolayer. Experimental procedures were performed on the cloned cells between passages 15 and 40. Morphology of ciPTEC was evaluated by using phase-contrast microscopy.

Immunofluorescence microscopy. Immunofluorescence localization of CaSR in polarized ciPTEC was performed as previously described (22). ciPTEC were cultured on polyester Transwell inserts and, after 10 days of maturation at 37°C, were fixed using 2% (wt/vol) paraformaldehyde in HBSS supplemented with 2% (wt/vol) sucrose for 5 min and permeabilized in 0.3% (vol/vol) Triton X-100 in HBSS for 10 min.

Cells were incubated with antibodies against the tight junction protein ZO-1 (1:200 dilution) and the CaSR (1:800 dilution) diluted in block solution containing 2% (wt/vol) BSA and 0.1% (vol/vol) Tween-20 in HBSS at 4°C overnight. Samples were treated with secondary goat-anti-mouse-Alexa 488 conjugate for ZO-1 and rabbit-anti-mouse-biotin antibody followed by streptavidin–488 for CaSR and mounted on glass slides with Mowiol. Images were obtained with a confocal microscope Leica TCS SP2 (Leica Microsystems, Heerbrugg, Switzerland).

Cell preparations. ciPTEC were seeded onto 60-mm dishes and grown at 37°C for 10 days and then were lysed in cell fractionation buffer (20 mM NaCl, 130 mM KCl, 1 mM MgCl2, 10 mM Hepes, pH 7.5) in the presence of proteases (1 mM PMSF, 2 mg/ml leupeptin, and 2 mg/ml pepstatin A) and phosphatases (10 mM NaF and 1 mM sodium orthovanadate) inhibitors. Cellular debris was removed by centrifugation at 12,000 g for 20 min at 4°C. The supernatants were collected and used for immunoblotting studies.

Immunoprecipitation. For immunoprecipitation experiments, ciPTEC were seeded onto 100-mm dishes and grown at 37°C for 10 days. Immunoprecipitation experiments were performed as described (23, 42). Briefly, ciPTEC were lysed with 1% Triton X-100, 150 mM NaCl, and 25 mM Hepes (pH 7.4) in the presence of protease inhibitors (1 mM PMSF, 2 mg/ml leupeptin, and 2 mg/ml pepstatin A). The supernatants were preclarified with 50 μl of immobilized protein-A and incubated overnight with anti-Gαi2 antibodies coupled to protein A-Sepharose. As negative control, lysates were incubated with nonspecific rabbit IgG. The immunocomplexes were washed three times,
Gel electrophoresis and immunoblotting. ciPTEC lysates were separated on 13% Bis-Tris acrylamide gels under reducing conditions. Protein bands were electrophoretically transferred onto Immobilon-P membranes (Millipore) for Western blot analysis, blocked in TBS-Tween-20 containing 3% BSA and incubated with primary antibodies overnight. Immunoreactive bands were detected with secondary antibody conjugated to horseradish peroxidase obtained from Santa Cruz Biotechnologies (Tebu-Bio, Milan, Italy). Membranes were developed using Super Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) with Chemidoc System (Bio-Rad Laboratories, Milan, Italy).

Video-imaging experiments. ciPTEC were grown on 40-mm glass coverslips at 37°C for 10 days and then were loaded with 4 μM fura-2 AM for 15 min at 37°C in DMEM. Ringer’s solution was used to perfuse cells during the experiment containing 120 mM NaCl, 4 mM KCl, 15 mM NaHCO3, 1 mM MgCl2, 15 mM Hepes, 0.5 mM Na2HPO4, 10 mM glucose, 1 mM CaCl2, 0.5 mM Na2HPO4, and 0.4 mM MgSO4, pH 7.4 (modified from Refs. 25 and 26). In fluorescence measurements, the coverslips with dye-loaded cells were mounted in a perfusion chamber (FCS2 Closed Chamber System; BIOPTECHS, Butler, PA), and measurements were performed using an inverted microscope (Nikon Eclipse TE2000-S microscope) equipped for single-cell fluorescence measurements and imaging analysis. The sample was illuminated through a ×40 oil immersion objective (NA = 1.30). The fura-2 AM-loaded sample was excited at 340 and 380 nm. Emitted fluorescence was passed through a dichroic mirror, filtered at 510 nm (Omega Optical, Brattleboro, VT), and captured by a cooled CCD camera (Cool SNAP HQ; Photometrics, Tucson, AZ). Fluorescence measurements were carried out using Metafluor software (Molecular Devices, MDS Analytical Technologies, Toronto, Ontario, Canada). The ratio of fluorescence intensities at 340 and 380 nm was plotted.

Fluorescence resonance energy transfer measurements. For evaluation of cAMP levels in ciPTEC, fluorescence resonance energy transfer (FRET) experiments were performed as described (41). Briefly, ciPTEC were seeded onto 20-mm glass coverslips at 37°C for 10 days and transiently transfected with a plasmid (0.4 μg of DNA/cm²) encoding the H96 probe containing cAMP-binding sequence of Epac1 between cyan fluorescent protein (CFP) and cp173Venus-Venus (gift from Dr. K. Jalink) (46), using Lipofectamine (1 μg/μl) according to the protocol provided by the manufacturer (Life Technologies). Experiments were performed 48 h posttransfection.

After overnight treatment with indomethacin (5 × 10⁻⁵ M), a prostaglandin synthesis inhibitor that reduced basal cAMP, cells were left under basal condition or stimulated with FK (10⁻⁵ M for 5 min) or alternatively with NPS-R568 (10 μM for 30 min or 20 μM for 15 min) and left unstimulated or stimulated with FK (10⁻⁵ M for 5 min). All treatments were performed in Ringer’s solution described above, containing 1.5 mM CaCl₂.

FRET measurements were carried out using MetaMorph software (Molecular Devices, MDS Analytical Technologies). Specifically, binding of cAMP to the Epac1 results in an intramolecular steric conformational modification causing an increase in the distance between the donor (CFP) and the acceptor (Venus) and a decrease of FRET process. CFP and Venus were excited at 430 and 480 nm, respectively; fluorescence emitted from CFP and Venus was measured at 480/30 and 545/35 nm, respectively. FRET from CFP to Venus was determined by excitation of CFP and measurement of fluorescence emitted from Venus. Corrected normalized FRET values were determined accordingly to Tamma (41).

RESULTS

Characterization of ciPTEC. Because a mixture of cell types can be exfoliated in urine, a characterization study was performed after subcloning the cell line to confirm its PT origin (12). At 37°C, cell monolayers of the subclones expressed ZO-1 protein, indicating the epithelial origin of cells with development of tight junctions (Fig. 1). The presence of PT-specific proteins AQP1 and NHE3 was demonstrated by immunoblotting of cells cultured for 10 days at 37°C (Fig. 2, A and B) (29). Cells originating from other segments known to express CaSR that were contaminated were excluded by immunoblotting experiments performed using NKCC2 and AQP2 as markers of the TAL and the collecting duct, respectively. Whereas AQP2 (Fig. 2C) and both forms of NKCC2 at 250 and 290 kDa are shown as positive controls. AQP2 (Fig. 2C) and both forms of NKCC2 at 250 and 290 kDa are shown as positive controls.
CaSR stimulation caused an increase in \( \text{Ca}^{2+} \) already at 2 mM \([\text{Ca}^{2+}]_o\), consistent with its functional expression, proving a high sensitivity of the endogenous receptor to low concentrations of its agonists. For a more specific functional analysis, cells were treated with 10 \( \mu \text{M} \) NPS-R568, the positive allosteric CaSR modulator that increases the sensitivity of the receptor for calcium (27). This compound is known to act selectively on the CaSR (28). Under these conditions, a bell-shaped calcium response was observed (Fig. 4B). Statistical analysis of the fluorescence responses revealed that cytosolic calcium levels were 46.32 ± 2.28% (vs. ATP 100%, \( n = 23 \)) after stimulation with 2.5 \( \mu \text{M} \) \( \text{Ca}^{2+} \) and 37.48 ± 1.71% (vs. ATP 100%, \( n = 25 \)) after stimulation with 10 \( \mu \text{M} \) NPS-R568. The NPS-R568 effect was stereo selective. In fact, experiments using the \( S \) enantiomer, which is 10- to 100-fold less potent than the \( R \) enantiomers (28), showed that in cells treated with NPS-S568 (10 \( \mu \text{M} \)) the increase in cytosolic calcium was negligible (4.21 ± 0.54% vs. ATP 100%, \( n = 7 \), Fig. 4C).

To investigate the origin of the calcium release upon specific CaSR activation with NPS-R568, calcium was depleted from the endoplasmic reticulum (ER) using 40 \( \mu \text{M} \) cyclopiazonic acid, an inhibitor of the sarco-endoplasmatic reticulum calcium ATPase (SERCA). Under this experimental condition, the increase in cytosolic calcium elicited by NPS-R568 was abolished, confirming that activation of CaSR results in calcium release from intracellular stores, likely the ER (Fig. 5).

As discussed, in PT the CaSR expressed on the luminal membrane senses the increase in luminal calcium concentrations and inhibits cAMP production induced by PTH (35). To further evaluate CaSR signaling in ciPTEC, cAMP levels were measured by FRET experiments using the H96 probe containing cAMP-binding sequence of Epac1 between CFP and Venus. Cells were treated with 10 \( \mu \text{M} \) FK, a direct activator of adenylyl cyclase, for 5 min or with the positive allosteric CaSR modulator NPS-R568, 10 \( \mu \text{M} \) for 30 min or 20 \( \mu \text{M} \) for 15 min, or with both NPS-R568 10 \( \mu \text{M} \) or 20 \( \mu \text{M} \) and FK 10 \( \mu \text{M} \) in the last 5 min. CaSR activation with NPS-R signifi-

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**Fig. 3.** Expression and localization of calcium-sensing receptor (CaSR). A: Immunodetection of CaSR in homogenates of ciPTEC clones, after 10 days of maturation at 37°C. Specific anti-CaSR antibodies revealed the monomeric form at 130 kDa, the glycosylated monomeric one at 160 kDa, and the dimeric receptor at 250 kDa compared with CaSR expression in HEK293 lysates, transiently transfected with human CaSR wild-type used as positive control. B: Immunofluorescence localization of CaSR in polarized ciPTEC showing its predominant apical plasma membrane localization.

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**Fig. 4.** Effects of increasing concentration of CaSR agonists on \([\text{Ca}^{2+}]_o\) levels. Cells were grown for 10 days at 37°C and stimulated with increasing levels of \([\text{Ca}^{2+}]_o\). (A) and NPS-R568 10 \( \mu \text{M} \) (B). Cells were also treated with the much less potent stereoisomer NPS-S568 10 \( \mu \text{M} \) (C), confirming the specific activation of CaSR elicited by NPS-R568 (28). Fluorescence ratio 340/380 nm was recorded. Each trace is representative of 3–4 different experiments with similar results.
cantly reduced FK-induced cAMP increase in a dose-dependent manner (Fig. 6). No changes in cAMP levels were detected in NPS-R568 10 μM- or 20 μM-treated cells with respect to cells left under basal condition.

*CaSR expressed in ciPTEC interacts with Gq.* The extracellular calcium-sensing receptor belongs to the C family of the G protein-coupled receptors and has been shown to couple to Gq in several tissues (5, 6, 18, 50). To evaluate whether CaSR expressed in ciPTEC is functionally coupled with Gq, coimmunoprecipitation studies were performed. ciPTEC lysates were immunoprecipitated with specific anti-Gq antibodies, and immunoprecipitates were next probed with anti-CaSR antibodies, revealing positive CaSR bands in all conditions corresponding to the glycosylated monomeric form at 160 kDa and the dimeric receptor at ~250 kDa. These studies indicate that CaSR couples with Gq as a downstream effector in ciPTEC (Fig. 7).

**DISCUSSION**

In this study, we provide the first evidence that ciPTEC isolated from urine of a healthy volunteer endogenously express a functional CaSR.

The immortalization of noninvasively collected cells developed by Wilmer and coworkers (53) has enabled the production of human cells maintaining PT characteristics and proliferating for at least 45 passages. For the culture of renal cells from urine, Wilmer and co-workers have used the immortalization methodology by using SV40T, originally described by Racusen and colleagues (32). The authors have cultured ciPTEC clones from the urine of two patients with nephropathic cystinosis, an inherited disorder of PT transport attributable to lysosomal cysteine accumulation. The detailed characterization of the ciPTEC, including their viability, proliferation capacity, formation of a tight monolayer, and expression of multiple PT-specific endogenous organic ion transporters, indicates the feasibility of using urinary cells as a source for obtaining human renal material for in vitro research by collecting cells from the urine of patients (53). Moreover, the expression pattern and the function of the majority of PT transporters were similar between cells isolated from urine compared with those isolated from kidney tissue and immortalized using the same methodology (22).

In a very recent work, ciPTEC established from three patients affected by human Dent disease have been crucial for understanding the molecular defect in renal reabsorption, demonstrating that CLC-5 mutations have multiple
effects on endosomal acidification and receptor-mediated endocytosis (14).

The major goal of the present contribution was to evaluate and characterize ciPTEC for the functional expression of the CaSR. The availability of a human source of cells from patients affected by mutations of this receptor might be of great relevance for confirming, using a noninvasive ex vivo approach, our recent in vitro data explaining the molecular basis of gain-of-function variants of CaSR (33). Specifically, we have shown that renal cells expressing gain-of-function CaSR variants showed a significant increase in SERCA activity and expression and a reduced PMCA expression. This combined parallel regulation increases the ER to cytosol calcium gradient, explaining the higher sensitivity of CaSR gain-of-function variants to external calcium, leading to an exacerbated signaling (33).

In the aim of using ciPTEC obtained by immortalizing and subcloning cells exfoliated in the urine of a healthy subject as possible model systems for the study of human renal disease associated with CaSR mutations, in the present contribution, we characterized ciPTEC cells for functional CaSR expression. As a first step, cells obtained after subcloning were screened for the expression of ZO-1 of the AQPI and NHE3 proteins, proving their epithelial and PT origin, respectively. The specific derivation of this clonal cell line from PT was demonstrated by the absence of other tubular segment protein expression such as NKCC2 and AQPI, markers of the TAL and the collecting duct, respectively.

Isolated ciPTEC expressed both the monomeric and the mature glycosylated form CaSR receptor that localized at the apical plasma membrane. Of note, functional experiments showed that activation of CaSR expressed in ciPTEC, obtained by either raising the luminal calcium ion concentration or by the specific CaSR-positive allosteric activator NPS-R568 (28), caused a release of calcium from an intracellular store likely corresponding to the ER. Functional expression of CaSR in ciPTEC was further confirmed by its ability to reduce FK and glycerol and IP3, causing releases of calcium from intracellular stores; 2) Gi, resulting in adenylate cyclase inhibition; and 3) G12/13, causing activation of Rho kinase (21, 34). In addition to inhibiting adenylate cyclase via Gi, the CaSR can also reduce cAMP indirectly by reducing the activity of calcium-sensitive adenylate cyclase or activating phosphodiesterase (14). Our data obtained in ciPTEC indicate that the activation of CaSR expressed in PT causes an increase in intracellular calcium attributable to CaSR coupling to Gq, resulting in PLC activation and IP3-dependent release in intracellular calcium. By demonstrating that human renal proximal tubular cells, ciPTEC, harbor endogenous and functional CaSR, our study provides a human cell model relevant for research into the disorder associated with gain- or loss-of-function mutations of the CaSR by producing cell lines from patients.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: A.D.M. and M.R. performed experiments; A.D.M., G.T., B.v.d.H., E.N.L., and G.V. conception and design of research; M.R. analyzed data; E.N.L. and G.V. edited and revised manuscript; E.N.L. and G.V. approved final version of manuscript; G.V. drafted manuscript.

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