Expression of canonical transient receptor potential (TRPC) proteins in human glomerular mesangial cells

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MESANGIAL CELLS (MCs) reside in the glomerular tuft, strategically positioned for the regulation of glomerular hemodynamics (26, 35). Like vascular smooth muscle cells, MCs contract in response to ANG II and relax in response to nitric oxide (25, 35). Altered responsiveness of MCs to the vasoactive hormones is one of the major causes leading to certain severe renal diseases, such as diabetic nephropathy. Modulation of mesangial cell transport plays an integral role in mediating many physiological and pathological responses, including mesangial cell contraction, mesangial cell growth, and mesangial matrix accumulation. It has been documented that binding of both vasoactive hormones and growth factors to mesangial cell receptors promotes an increase in mesangial cell cytosolic Ca\(^{2+}\) (26, 27, 30, 35). This process involves both the release of intracellular Ca\(^{2+}\) stores and extracellular Ca\(^{2+}\) entry. The latter occurs via classic, voltage-gated Ca\(^{2+}\) channels, receptor-operated Ca\(^{2+}\) channels, and recently identified store-operated Ca\(^{2+}\) channels in the mesangial cell plasma membrane (22).

Although a variety of channels have been described in various mesangial cultures using patch-clamp techniques (22), the majority of channels involved in Ca\(^{2+}\) influx via the plasma membrane have not been defined at the molecular level. These channels include nonselective cation channels, receptor-operated Ca\(^{2+}\) channels, and store-operated Ca\(^{2+}\) channels. Recently, mammalian canonical TRP (transient receptor potential) channels (TRPCs) have been proposed as Ca\(^{2+}\)-permeable cation channels that are activated in response to stimulation of G protein-coupled receptors or receptor tyrosine kinases (1, 12, 13). TRPCs have been discovered in a variety of cell types, including vascular smooth muscle (8, 9, 34, 43). In addition, the seven TRPC proteins can assemble to form functional complexes.

store-operated Ca\(^{2+}\) entry; Ca\(^{2+}\) channel; Ca\(^{2+}\) signaling; glomeruli

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channels, or nonselective cation channels. Because this information is extremely devoid in human glomerular mesangial cells (HMCs), important cells relevant to physiological function and the pathophysiological process in kidneys, we have, in the present study, employed Western blotting. RT-PCR, confocal microscopy, immunohistochemistry, and coimmunoprecipitation to systematically assess subtypes of TRPC proteins present in HMCs and the physical interaction among them. Our data show that TRPC1, 3, 4, and 6 are expressed in HMCs at the protein level, whereas TRPC5 and 7 are only detectable at the messenger level. TRPC1 might have physical interaction with TRPC4 and 6 in this type of cell.

MATERIALS AND METHODS

Preparation of cultures of HMCs. HMCs used in this study were purchased from Cambrex (East Rutherford, NJ). The procedures and methods for culturing HMC were described previously (16). Briefly, HMCs were cultured in DMEM (GIBCO, Carlsbad, CA) supplemented with 25 mM HEPES, 4 mM glutamine, 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20% fetal bovine serum. Only subpassages less than 11 generations of cells were used in the present study.

Transient transfection. Lipofectamine and Plus reagents (Invitrogen, Carlsbad, CA) were used to transiently transfect trpc1–7 cDNA plasmids or trpc1 or 4 or 6 RNAi constructs into H293T cells (in 10-cm plates) following instruction provided by the manufacturer. The cells were lysed 24–48 h after transfection.

Immunoprecipitation and immunoblots. When cell monolayers were 80% confluent, the cells were washed twice with PBS and then lysed in 1 ml of 1% Triton X-100 buffer (per 10-ml plate) containing (in mM) 150 NaCl, 10 Tris-HCl (pH 7.5), 1 EGTA, 0.2 sodium orthovanadate, 0.2 phenylmethylsulfonyl fluoride, 0.5% NP-40, aprotinin (1 μg/ml), pepstatin (1 μg/ml), and proteinase inhibitor cocktail (Roche Applied Science, Indianapolis, IN). The cell lysates were centrifuged at 6,000 g for 15 min at 4°C. For coimmunoprecipitation experiments, the cell lysates were incubated for 2 h with specific TRPC antibody and complexes were captured for 1 h with 30-μl slurry of protein G or A (Amersham Biosciences, Piscataway, NJ) in 50 mM Tris-HCl. Immunocomplexes were then washed five times in lysis buffer. The cell lysates (without precipitation, for regular Western blotting) or immunoprecipitates (for coimmunoprecipitation) were

![Image](http://ajprenal.physiology.org/)

**Fig. 1.** Western blotting. Transient receptor potential channel (TRPC)1, 3, 4, and 6 were detected in native human mesangial cells (HMCs). However, TRPC5 and 7 were not detectable. A: H293T trpc1(+) indicates lysates from H293T cells transfected with human trpc1 expression plasmids, served as a positive control. H293T trpc1(−) represents lysates from H293T cells cotransfected with human trpc1 expression plasmids and human trpc1-RNAi constructs, serving as a negative control. B: left: H293T trpc3(+) indicates lysates from H293T cells transfected with human trpc3 expression plasmids, serving as a positive control. B: right: anti-TRPC3 antibody was preadsorbed by incubating the antibody with its blocking peptide for 1 h at room temperature, serving as a negative control. C: left: TRPC4 immunoblots in HMCs treated with human trpc4-RNAi constructs (trpc4(−)) or empty vectors (Con). C: right: TRPC4 immunoblots in H293T cells transfected with bovine trpc4 expression plasmids [H293T-trpc4(+) and in HMCs], HMC-trpc4(−) and H293T-trpc4(+) served as negative and positive controls for TRPC4, respectively. D: H293T trpc5(+) indicates lysates from H293T cells transfected with mouse trpc5 expression plasmids, serving as a positive control. H293T con represents lysates from H293T cells transfected with empty vectors, serving as a negative control. E: HMC trpc6(−) indicates lysates from HMC cells transfected with human trpc6-RNAi constructs, serving as a negative control. H293T trpc6(+) indicates lysates from H293T cells transfected with mouse trpc6 expression plasmids, serving as positive control for TRPC6. F: left: H293T trpc7(+) represent lysates from H293T cells transfected with mouse trpc7 expression plasmids, serving as a positive control. F: right: anti-TRPC7 antibody was preadsorbed by preincubating the antibody with its blocking peptide for 1 h at room temperature, serving as negative control. Antibodies used: anti-TRPC1, monoclonal mouse IgG, 1:1,000 dilution; anti-TRPC3–7, polyclonal goat IgG, 1:200 dilution. Immunoblots were visualized with ECL reagents at Femto level. Actin was used as loading control.
fractionated by 10% SDS-PAGE, transferred to nitrocellulose membranes, and probed with the indicated primary TRPC antibodies. Bound antibodies will be visualized with Super Signal West Femto Luminol/Enhancer Solution (Pierce Biotechnology, Rockford, IL.).

**TRPC antibodies and plasmids.** TRPC1 mouse monoclonal and rabbit polyclonal antibodies were obtained from Dr. L. Tsiokas’s laboratory (University of Oklahoma Health Sciences Center, Oklahoma City, OK). The specificity and efficiency of the antibodies have been described previously (23, 31). TRPC3–7 goat polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). TRPC3 and 6 rabbit polyclonal antibodies were from Alomone Labs.

**trpc Plasmids were obtained from following sources.** Human TRPC1-pGEX-3X and TRPC3-pcDNA3 were obtained from C. Montell (Johns Hopkins Medical School, Baltimore, MD). Bovine TRPC4-pcDNA3, mTRPC5-pcDNA3, and mTRPC6-pcDNA3 were obtained from L. Birnbaumer (National Institutes of Health). mTRPC7-pCneo was obtained from Y. Mori (Okazaki National Research Institute). Trpc1-RNAi construct (trpc1-pSHAG1–2219) was provided by Dr. L. Tsiokas (University of Oklahoma Health Sciences Center), and trpc4 and 6-RNAi construct was obtained from Dr. M. Villereal (University of Chicago, Chicago, IL).

**Fluorescent immunocytochemistry.** HMC cells were plated on 22 × 22.1-mm coverslips, fixed with iced acetone/methanol, and incubated with a TRPC antibody or two TRPC antibodies (for double staining) in PBS plus 2% heat-inactivated goat or fetal bovine serum and 0.2% Triton X-100 for 1 h. Three washes with PBS containing 2% goat or fetal bovine serum and 0.2% Triton X-100 were followed by blocking at 4°C overnight in blocking buffer containing mouse monoclonal anti-TRPC1, rabbit polyclonal anti-TRPC3 or 6, or goat polyclonal anti-TRPC4 antibody. To label glomerular mesangial cells, we also incubated the sections with rabbit polyclonal or mouse monoclonal anti-desmin antibody (depending on the host of TRPC antibodies) overnight at 4°C. The concentrations for all primary antibodies were 2–5 μg/ml. The sections were rinsed and incubated for 30 min at room temperature with Alexa Fluor 488 goat anti-rabbit IgG or donkey anti-goat IgG (Molecular Probes), depending on the primary antibodies. The concentrations of the secondary antibodies were 2 μg/ml. In control slides, equal amounts of rabbit IgG, mouse IgG, or goat IgG were used instead of the primary antibodies. All stainings were visualized under confocal laser-scanning microscope (Zeiss LSM410).

Normal human kidney tissues were obtained from archival nephrectomy specimens. Paraffin sections of each specimen were stained with hematoxylin and eosin and were examined to confirm the preservation of histological structures and to validate the normality. For immunostaining, the sections were cut at 5 μm and a standard protocol of xylene and graded ethanol was employed to deparaffinize and rehydrate. TRPC protein stainings were detected using the same protocol as described above for rat kidney sections.

**Ratiometric Ca2+ measurements.** Cells were harvested in PBS containing 0.5 mM EDTA, washed with PSS, and loaded with 2 μM
Fig. 3. Immunohistochemistry showing expression of TRPC proteins in rat (A and B) and human (C) kidney sections. A and B: glomerular mesangial cells were labeled with desmin. A: IgG were stained with red fluorescence while desmin (probed with anti-desmin rabbit IgG) was stained with green fluorescence. B: TRPC4 and 6 (probed with anti-TRPC4 and 6 goat IgG, respectively) were stained with green fluorescence and desmin (probed with anti-desmin mouse IgG) were stained with red fluorescence. C: TRPC1, 3, 4, and 6 (probed with corresponding anti-TRPC goat IgG) were stained with green fluorescence in human glomeruli. Rabbit IgG was used as a negative control for TRPC stainings. The brightfield image shows normal morphology of glomerulus treated with rabbit IgG (negative control). The bars inside imagings indicate 50 μm.
indo-1/AM in PSS containing 0.05% Pluronic F-127 (Molecular Probes) for 40 min at room temperature. After the 40-min incubation, cells were washed three times with a nominally Ca$_2^+$-free solution. About 2 x 10^6 cells were resuspended in 2 ml of Ca$_2^+$-free solution. Cells in Ca$_2^+$-free solution were first incubated with 1 μM TG for 30 min to deplete the internal Ca$_2^+$ stores and then Ca$_2^+$ entry was determined by Ca$_2^+$ readdition (10 mM CaCl$_2$). Ratiometric measurements representing free intracellular Ca$_2^+$ concentration ([Ca$_2^+$]$_i$) were obtained by a PTI QuantaMaster spectrofluorometer equipped with an excitation monochromator set at 350 nm and two emission monochromators set at 405 and 485 nm.

**RESULTS**

Expression of TRPC proteins. We employed regular Western blotting to detect subtypes of endogenous TRPC proteins expressed in HMCs. With specific TRPC antibodies, we were able to identify immunoblots probed with anti-TRPC1, 3, 4, and 6 antibodies in the lysates from HMCs (Fig. 1). The sizes of the bands (80–90 kDa) were a little lower than predicted. However, they were located at the same level as their corresponding positive controls (lysates from TRPC-transfected H293T cells). To further confirm the specificity of the immunoblots, we also Western-blotted lysates from H293T or HMCs cells transfected with trpc1- or 4-RNAi constructs for knocking down corresponding TRPC (Fig. 1, A and C) or lysates from HMCs with preadsorbing corresponding primary antibodies (Fig. 1, B and E). As shown in Fig. 1, the immunoblots were either completely blocked or significantly reduced by the specific gene silencing or antigen competition. As reported by many investigators (3, 8, 31), immunoblotting endogenous TRPC proteins in native tissues is very difficult, presumably because of immunogenicity of TRPC antibodies or inherent properties of TRPC proteins. In the present study, we had to use a Femto chemiluminescent substrate to obtain clear TRPC bands. In contrast to TRPC1, 3, 4, and 6, TRPC5 and 7 were not detectable in HMCs (Fig. 1) even though a ~90-kDa band was clearly shown in the lysates from trpc5- and trpc7-transfected H293T cells. The specific TRPC5 and 7 immunoblots were also unable to be detected in the lysates of HMCs using the antibodies from W. P. Schilling (Case Western Reserve University School of Medicine, Cleveland, OH; data not shown).

Expression of TRPC proteins was also detected with fluorescence from immunocomplexes of specific TRPC protein antibodies and their Fluor-conjugated secondary antibodies using confocal laser-scanning microscopy. In agreement with the results from Western blotting, immunofluorescence stainings specific for TRPC1, 3, 4, and 6, but not TRPC5 and 7, were observed (Fig. 2). All of the expressed TRPCs displayed ubiquitous subcellular distribution, showing predominant intracellular localization with a pattern consistent with the endoplasmic reticulum and/or vesicles. In addition, TRPC1 and TRPC4 stainings were also clearly seen in the region of the plasma membrane while TRPC6 staining appears mostly localized to the nucleus.

In vivo expression of TRPCs in rat and human glomeruli. Because of the inaccessibility of mesangial cells in vivo, their function and cell biology have been mostly studied after several generations of growth in a culture environment. However, culture condition could change the phenotypes of mesangial cells from those in in vivo environment (15, 22). Therefore, the results obtained from cultured HMCs might not necessarily represent physiological presence of TRPCs in this type of cells. In particular, a recent study reported that expression of TRPCs in arterial smooth muscle might be associated with organ culture per se (4). Thus immunohistochemistry was performed in rat kidney sections and isotypes of TRPCs iden-
identified in cultured HMCs (TRPC1, 3, 4, and 6) were detected with specific anti-TRPC antibodies. Glomeruli were easily distinguished by their characteristic circular morphological aspect bordered by peripheral lumen. Glomerular mesangial cells were identified with positive staining with antibody against desmin. Consistent with the data described in cultured cells, stainings specific for TRPC1, 3, 4, and 6 were detected in desmin-labeled cells (Fig. 3, A and B). The specificity of the stainings was confirmed by failure in detecting stainings in the samples treated with control immunoglobulins (Fig. 3A). Not surprisingly, TRPC stainings were also found in the regions inside glomeruli, which were not stained by desmin antibody, suggesting that TRPCs also reside in other types of glomerular cells.

Expression of TRPC proteins in glomeruli was also detected in human kidney tissue sections by immunohistochemistry. Although there is no specific marker for mesangial cells in in vivo human glomeruli, the diffuse pattern of TRPC stainings indicates that TRPC1, 3, 4, and 6 are strongly and widely expressed in human glomerular cells, most likely including mesangial cells (Fig. 3C). In addition, TRPC stainings were also found in renal tubular epithelia. These results are consistent with the findings from recent studies which demonstrated expression of TRPC proteins in glomerular podocytes and renal tubule epithelial cells (11, 33).

**Heteromeric interaction between endogenous TRPCs in HMCs.** It has been reported that both exogenously expressed and endogenous TRPC proteins form multimeric protein assembly giving rise to biophysically and functionally discernible channel entities (1, 10, 13, 20). Thus we examined possible heteromerization between the natively present TRPCs in HMCs. Using coimmunoprecipitation as shown in Fig. 4, when anti-TRPC1 antibody was used to pull down TRPC1, TRPC4 and 6, but not TRPC3, were coimmunoprecipitated (Fig. 4A). However, neither IP of TRPC4 pull down TRPC3 or TRPC6 (Fig. 4B) nor IP of TRPC6 pull down TRPC3 (Fig. 4C). These data suggest a selective interaction of TRPC1 with TRPC4 and TRPC1 with TRPC6 in HMCs.

The selective interaction between TRPC1 and TRPC4 and 6 was verified by immunofluorescent double staining. Similar to Fig. 2, stainings for TRPC1, 3, 4, and 6 were seen in multiple fixed HMCs (Fig. 5). Overlaying TRPC1 signal (red) with TRPC3 or 4 or 6 (green) shows clear colocalization (yellow) between TRPC1 and TRPC4 and 6, but not TRPC3 (Fig. 5).

**Mediation of store-operated Ca$^{2+}$ entry by TRPC1 in HMCs.** We selected TRPC1 as a representative of TRPC channel proteins to detect their physiological function in mesangial cells because TRPC1 is the founding member of TRPC family and is highly expressed in HMCs. Emerging evidence demonstrated that specific TRPC proteins might be candidates of store-operated Ca$^{2+}$ channel (21, 32). Therefore, we tested the role of TRPC1 in store-operated Ca$^{2+}$ entry using ratiometric measurement of [Ca$^{2+}$]$_i$ in response to Ca$^{2+}$ readmission, a typical protocol of testing store-operated Ca$^{2+}$ entry. Internal Ca$^{2+}$ stores were depleted by incubating HMCs with 1 μM thapsigargin for 30 min in the nominally Ca$^{2+}$-free solution. As shown in Fig. 6A, readdition of Ca$^{2+}$ into the bathing solution induced a remarkable rise of [Ca$^{2+}$]$_i$ in control HMCs. However, this response was significantly attenuated by knocking down TRPC1 and enhanced by overexpressing TRPC1 (Fig. 6, A and B), suggesting an essential role of TRPC1 in mediating store-operated Ca$^{2+}$ entry.

**DISCUSSION**

In the present study, we employed multiple approaches and found that in HMCs, 1) TRPC1, 3, 4, and 6, but not TRPC5 and 7, were expressed at protein level; 2) TRPC1 physically interacted with TRPC4 and 6; 3) the proteins of TRPC1, 3, 4, and 6 were also found in rat and human glomeruli; and 4) TRPC1 participated in store-operated Ca$^{2+}$ entry.

![Fig. 4. Coimmunoprecipitation, showing interaction between the isoforms of TRPCs. A: immunoprecipitating TRPC1 with mouse monoclonal TRPC1 antibody and immunoblotting TRPC3, 4, or 6 with goat polyclonal antibodies. Input indicates cell lysates (without TRPC1 pull down). B: immunoblotting TRPC3 or TRPC6 with rabbit polyclonal antibodies in cell lysates (Input) or TRPC4 immunoprecipitates. C: immunoblotting TRPC3 with rabbit polyclonal antibody in cell lysates (Input) or TRPC6 immunoprecipitates. IP: immunoprecipitation; IB, immunoblotting. All inputs shown here were 1/20 of the proteins used for IP.](http://ajprenal.physiology.org/10.2302/ajprenal.org)
It has been documented that TRPC proteins exist almost ubiquitously in mammalian tissues. However, the expression of TRPC isoforms is tissue and cell type specific and may also be species specific (8, 9, 45). For instance, high levels of TRPC1, 3, and 5 mRNA were found in human cerebellum (34). Human parotid gland ductal cells endogenously expressed TRPC1, 3, and 4, but not TRPC5 or 6 (20). However, TRPC1, 3, 4, 5, and 6 mRNA and protein were detected in rat renal resistance vessels, whereas TRPC2 and 7 mRNA were not expressed (8). The data in the present study revealed that human-originated mesangial cells expressed the proteins of TRPC1, 3, 4, and 6. These results were further supported by immunohistochemistry data from rat and human kidney sections. Incapability of identifying TRPC5 and 7 proteins indicates that either TRPC5 and 7 is deficient in HMCs because of deficiency in posttranscriptional mechanism or the amounts of TRPC5 and 7 proteins are below the detectable level. Our results are not completely in agreement with those from a recent study by Wang et al. (45) in which TRPC1 and TRPC4 mRNA were the only two TRPC messengers identified in mouse glomerular mesangial cells. This discrepancy might be due to species difference (human vs. mouse). Indeed, rat glomeruli, in which glomerular

![Fig. 5. Selective colocalization of TRPC1 with TRPC4 and 6 in HMCs. TRPC1 (red signals) was probed with anti-TRPC 1 mouse monoclonal antibody (1F1, 1:500) followed by Alexa Fluor-568-conjugated goat anti-mouse secondary antibody (1:1,000) while TRPC3, 4, and 6 (green signals) were stained with corresponding rabbit polyclonal antibodies (1:100) followed by Alexa Fluor-488-conjugated goat anti-rabbit secondary antibody (1:1,000). Colocalization of TRPCs was indicated by yellow signals from overlaying red and green signals (right and bottom). The bars inside imagings indicate 50 μm.](image)

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![Fig. 6. Suppression of store-operated Ca^{2+} entry by inactivating TRPC1 in HMCs. A: representative experiment, showing the effect of Ca^{2+} addition (10 mM CaCl\(_2\), added as indicated) on intracellular [Ca^{2+}] (shown as 405/485 fluorescence ratio of indo-1/AM) in trpc1-RNAi construct transfected [TRPC(-)] and untransfected (Control) HMCs incubated in a nominally Ca^{2+}-free solution with 1 μM thapsigargin for 30 min. B: summary bar graph from experiment shown in A. TRPC1(+) indicates HMCs transfected with trpc4 cDNA. *Significant difference from control; n in each group indicates the sample size.](image)

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mesangial cells are located, express TRPC1, 3, 5, and 6 protein, but not TRPC4 and 7 (8). The species-dependent TRPC expression is also seen in other tissues. For example, TRPC3 and 6 are not detectable in adult rat brain (37) but highly expressed in human central nervous system (34).

 Fluorescence resonance energy transfer measurements and immunoprecipitation experiments demonstrated that the seven TRPC proteins (TRPC1–7) can assemble to form heteromers (10, 13, 36, 37). The heteromeric interactions between the TRPC proteins are proposed to occur between members of two groups of TRPCs: TRPC1/4/5 and TRPC3/6/7. Consistent with those studies, we found that TRPC1 was associated with TRPC4 in HMCs. In addition to this known interaction between TRPCs, in the present study we also found that TRPC1 physically interacted with TRPC6. Exceptions to the TRPC protein interaction governed by the principals described by Hofmann et al. (13) have been reported by several groups. For instance, that TRPC1 and TRPC3 coassemble to form a heteromeric complex has been reported in exogenously expressed HEK293 cells and in native human parotid gland ductal cells (19, 20). The novel heteromeric associations between endogenous TRPCs were also described by Strübing et al. (37) in embryonic brain. The novel interaction between TRPC1 and TRPC6 found in the present study suggests that this heteromeric TRPC protein complex might underlie a channel mechanism specific for HMCs. It is true that diversity of TRPC function and regulation in different types of tissues or cells is derived from diverse assembly of TRPC subunits (1, 19, 20, 36, 37). The tissue- and cell type-dependent expression profile of TRPCs (9, 34) also fits the diverse properties of store-operated or receptor-operated channels found in a variety of type of cells given that TRPCs are potential molecular candidates of these channels. Different assemblies of TRPC isoforms found in the present study (TRPC1 with TRPC4, and TRPC1 with TRPC6) might underlie differential molecular entities of different types of ion channels (nonselective cation channel, receptor-operated Ca\(^{2+}\) channel, or store-operated Ca\(^{2+}\) channel) present in HMCs. Another interesting finding in this study, which contradicted the results from Hofmann et al. (13), is that no interaction between TRPC3 and TRPC6 was detected even though the two isoforms of TRPCs are expressed in HMCs. We have used different anti-TRPC3 and TRPC6 antibodies (goat IgG and rabbit IgG) from different sources (Santa Cruz Biotechnology and Alomone Lab) and the interaction between TRPC3 and TRPC6 was never observed in immunoprecipitation or reverse immunoprecipitation. We speculate that the TRPC3-TRPC6 interaction might not exist in HMCs and each of the TRPCs might have another partner for its specific function.

 As founding members of the TRP superfamily, TRPCs take part in a wide range of physiological functions, including nerve growth (5, 17, 44), vascular tone (7, 14, 46), permeability of vascular endothelium (39), cell proliferation (38, 49), and mechanosensation (2). TRPC proteins might also be associated with development of certain diseases, such as pulmonary hypertension (18, 48). Glomerular mesangial cells have important physiological and pathophysiological relevance, and the function of mesangial cells is controlled by a variety of ion channels, including nonselective cation channel, receptor-operated and store-operated Ca\(^{2+}\) channels. However, the molecular entities of these channels are still unknown. Because expressed or endogenous individual TRPCs or TRPC complexes with differential assembly behave as nonselective cation channels or receptor-operated Ca\(^{2+}\) channels or store-operated Ca\(^{2+}\) channels depending on tissue and cell type, we speculate that the existing TRPCs found in the present study might constitute a functional entity of the three types of important channels in HMCs. This speculation was supported by the findings that TRPC1 and TRPC4 stainings were clearly seen in the region of the plasma membrane (Figs. 2 and 5), implying that the two subtypes of TRPCs might be important components of cation channels in the cell membrane of HMCs. Our functional data provide further support for the notion by showing mediation of store-operated Ca\(^{2+}\) entry via TRPC1 protein in HMCs (Fig. 6). Furthermore, immunocytochemistry revealed that TRPC6 is mostly localized to the nucleus of HMCs (Figs. 2 and 5). This unique localization of TRPC6 might imply an important role of this TRPC isoform in regulating mesangial proliferation and contractile function by modulating particular processes of gene transfer and transcription. Apparently, more functional and mechanistic studies are required to explore the physiological function of the TRPC proteins and their regulation under normal and disease states.

 In conclusion, our results suggest that HMCs specifically express isoforms of TRPC1, 3, 4, and 6 proteins. These isoforms of TRPCs might selectively assemble together to form functional complexes, which might underlie diverse channel mechanisms found in mesangial cells.

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GRANTS

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TRPC PROTEINS IN MESANGIAL CELLS


