Expression of immunoglobulin G in human proximal tubular epithelial cells

ZHENLING DENG[1*], ZIYANG JING[1,2*], YANHONG GUO[1], JUNFAN MA[3], HUIGE YAN[3], ZHAN SHI[3], HUI DENG[1], YAOXIAN LIANG[1], SONG WANG[1], ZHUAN CUI[1], YUEJUAN PAN[1], XIAOYAN QIU[3] and YUE WANG[1]

[1]Department of Nephrology, Peking University Third Hospital, Beijing 100191; [2]Department of Nephrology, Hainan General Hospital, Haikou, Hainan 570311; [3]Department of Immunology, School of Basic Medical Sciences, Peking University, Beijing 100191, P.R. China

Received July 7, 2020; Accepted January 20, 2021

DOI: 10.3892/mmr.2021.11966

Abstract. Proximal tubular epithelial cells (PTECs) have innate immune characteristics, and produce proinflammatory factors, chemokines and complement components that drive epithelial-mesenchymal transition (EMT). Our previous studies revealed that human mesangial cells and podocytes were able to synthesize and secrete immunoglobulin (Ig)A and IgG, respectively. The aim of the present study was to evaluate the expression of Igs in PTECs. Firstly, IgG was detected in the cytoplasm, the cell membrane and the lumen of PTECs in the normal renal cortex by immunohistochemistry. Secondly, Igγ gene transcription and V(D)J recombination were detected in single PTECs by nested PCR and Sanger sequencing. Thirdly, Igγ, Igκ and Igλ were clearly detected in an immortalized PTEC line (HK-2) by immunostaining and western blotting, in which RP215 (an antibody that predominantly binds to non-B cell-derived IgG) was used. In addition, Igγ, Igκ and Igλ gene transcripts, conservative V(D)J recombination in the Igγ variable region, recombination activating gene 1/2 and activation-induced cytidine deaminase were all detected in HK-2 cells. These data suggested that PTECs may express IgG in a similar manner to B cells. Furthermore, IgG expression was upregulated by TGF-β1 and may be involved in EMT.

Correspondence to: Professor Yue Wang, Department of Nephrology, Peking University Third Hospital, 49 Huayuanbei Road, Beijing 100191, P.R. China
E-mail: bjwangyue@sina.com

Professor Xiaoyan Qiu, Department of Immunology, School of Basic Medical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, P.R. China
E-mail: qiuxy@bjmu.edu.cn

*Contributed equally

Key words: proximal tubular epithelial cells, single cell, HK-2, IgG, epithelial-mesenchymal transition

Introduction

Proximal tubular epithelial cells (PTECs) are the most abundant cell type in the kidney, and have an important role in renal repair and/or the progression of chronic kidney diseases. PTECs exert immunological functions by expressing multiple Toll-like receptors (TLRs), such as TLR 1, 2, 3, 4 and 9 (1,2), and molecules associated with antigen-presenting cell function, including MHCII, CD74, CD80 and CD86 (3). These innate immune characteristics of PTECs enable them to act as immune responders to a wide range of stimuli, with the consequent production and release of bioactive mediators, including proinflammatory cytokines, chemokines and complement components, which drive interstitial inflammation and fibrosis (4). PTECs also express neonatal Fc receptor, and preserve the capacity of specific pH-dependent binding and transcytosis of immunoglobulin (Ig)G (5). However, to the best of our knowledge, it remains unknown as to whether PTECs express Igs.

It was previously hypothesized that Igs are produced solely by mature B cells and plasma cells, and that Igs act as antibodies to recognize and neutralize various pathogens. However, this theory has been challenged in recent decades, as increasing evidence has reported that Igs, including IgA, IgG and IgM, can be produced and secreted by non-B cells, such as human epithelial cancer cells (6,7) and normal non-B cells (8,9), as well as in immune-privileged sites, such as the eyes (10), central neurons (11,12), placenta (13), and the testis and epididymis (14).

Similar to B cell-derived Igs (B-Igs), non-B-Igs are also the products of Ig gene transcription and rearrangement, and display classic V(D)J recombination patterns with nucleotide additions at the junctions and somatic hypermutations (7,11,14). In contrast to B-Igs, non-B-Igs displays limited V(D)J recombination patterns and less diversity (7). Functionally, the non-B-Igs not only exert natural antibody activity in the skin and mucosa (8) but can also act as growth factors to promote cell proliferation and adhesion, and may enhance the initiation and metastasis of cancer by binding to integrins (15-17). For example, RP215 recognized cancer IgG executes its oncogenic function by interacting with the integrin α6β4 complex and activating the FAK and Src pathways (15).

Our previous study demonstrated that mesangial cells (18) and podocytes (19) can synthesize and secrete IgA and IgG, and participate in cell growth and cell adhesion *in vitro*. The present study aimed to evaluate the expression levels of Igs in PTECs and investigate its potential role in epithelial-mesenchymal transition (EMT).

Materials and methods

Cell culture and treatment. An immortalized PTEC line HK-2 was purchased from American Type Culture Collection. HK-2 cells were cultured in DMEM/F12 supplemented with 100 U/ml penicillin, 0.1 mg/ml streptomycin (all Gibco; Thermo Fisher Scientific, Inc.) and 10% fetal bovine serum (FBS; Australian origin; Biological Industries USA, Inc.) at 37˚C in an atmosphere containing 95% air and 5% CO_2. In order to avoid the interference of Ig in FBS, the medium was replaced with serum-free medium 24-48 h prior to cell harvest. HK-2 cells were treated with different concentrations (2, 5 and 10 ng/ml) of TGF-β1 (Sigma-Aldrich; Merck KGaA).

Single PTEC isolation and cDNA synthesis. A human kidney sample of macroscopically normal cortical tissue was obtained from a patient (male, 31 years old) undergoing nephrectomy as a result of renal carcinoma without obvious renal dysfunction. A single-cell suspension was prepared by digesting renal cortex with 1 mg/ml collagenase I (Sigma-Aldrich; Merck KGaA) at 37˚C for 20 min. PTECs were sorted using phycoerythrin (PE)-conjugated anti-CD10 (cat. no. 312203) and allophycocyanin (APC)-conjugated anti-CD13 (cat. no. 301705; both BioLegend, Inc.) by fluorescence-activated cell sorting (BD FACSAria II Special Order System) as previously described (20). The corresponding isotype control antibodies (cat. nos. 400111 and 400119; both BioLegend, Inc.) were used to exclude non-specific staining. Double positively labeled living cells were isolated as PTECs. A single PTEC was manually selected under an inverted light microscope using a capillary pipette and was then transferred to a 0.2-ml thin-wall PCR tube containing lysis buffer (21). Single PTEC RNA extraction and cDNA synthesis were carried out according to previously described methods (21). A total of five single PTECs were used to detect Ig gene transcription and rearrangement.

PCR amplification. Total RNA was extracted from HK-2 cells, peripheral blood mononuclear cells [PBMCs, isolated from a 31-year-old female healthy donor using Ficoll (cat. no. 7111011; Dakewe Biotech., Ltd.)] and kidney cortex (from the same patient used in single PTEC isolation) using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), and the RNA concentration was assessed using a NanoDrop spectrophotometer (NanoDrop; Thermo Fisher Scientific, Inc.). Subsequently, 2 μg total RNA was reverse-transcribed to cDNA using the RevertAid First Strand cDNA Synthesis kit (cat. no. K1622; Thermo Fisher Scientific, Inc.). PCR was performed using primers targeting the constant regions of Igγ, Igκ, Igλ and activation-induced cytidine deaminase (AID). Nested PCR was performed to amplify the variable region of Igγ, low-density lipoprotein receptor-related protein 2 (LRP2) and recombination activating gene (RAG)1 and RAG2. The PCR products were separated by electrophoresis on a 1.0% agarose gel and was visualized using GelRed (cat. no. 41003; Biotium, Inc.). The primers of AID, RAG1/2 and the constant regions of Igγ, Igκ, Igλ used in this study refer to primers used by Jing *et al* (19). The primers of Igγ variable region refer to primers used by van Dongen *et al* (22). The other primers used for PCR are listed in Table SI. The thermocycling conditions are listed in Table SII.

Sanger sequencing and analyses of sequencing data. PCR products of the Igγ variable region obtained from single PTECs, HK-2 cells and PBMCs were respectively cloned into a pGEM-T Easy Vector system I (cat. no. A1360; Promega Corporation), which was transformed into TOP10 Competent cells (CB104; Tiangen Biotech Co., Ltd.). Briefly, 5 μl ligation products were added to 30 μl TOP10 competent cells, incubated on ice for 30 min, heat shocked at 42˚C for 90 sec and incubated on ice for 5 min. Then, 500 μl LB was added and left to stand at 37˚C for 40 min before inoculating part of the bacterial liquid on Petri dishes coated with 0.1 mmol/l IPTG and 20 μg/ml X-Gal. Dishes were inverted at 37˚C overnight. In all, 5-16 white colonies per sample were chosen randomly, and sequenced using an ABI 3730XL Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The rearranged V(D)J sequences were compared with those in the basic local alignment search tool (https://www.ncbi.nlm.nih.gov/igblast/) to identify the best matching germline gene segments and junctions following primer trimming.

Western blot analysis. HK-2 cells were lysed in TSD lysis buffer [1% SDS, 50 mM Tris-HCl (pH 7.5), 50 mM DTT] containing a protease inhibitor cocktail (Applygen Technologies Inc.), sonicated at ice-water for 1 min (working 5 sec and resting 15 sec; 3 times) and lysed for 30 min at room temperature. Following centrifugation at 12,000 x g for 10 min at 4˚C, the protein concentration of the cell lysate was determined using a BCA kit (Applygen Technologies Inc.). Subsequently, 5X reducing loading buffer was added to the lysate, boiled at 100˚C for 10 min, and the samples were immediately used for western blot analysis. Serum, used as a positive control for Ig, was isolated from a healthy donor (the same donor as used in PBMCs) by centrifugation at 2,103 x g for 10 min at room temperature.

Western blotting was carried out according to standard procedures. Briefly, 30 μg proteins were separated by SDS-PAGE on 10% gels and were transferred onto a nitrocellulose membrane. Subsequently, the membrane was blocked in 5% skimmed milk at room temperature for 1 h and was incubated with primary antibodies at 4˚C overnight, including rabbit anti-human Igγ (cat. no. ab109489; 1:1,000), rabbit anti-human Igγ4 (cat. no. ab109493; 1:1,000), anti-Igκ (cat. no. ab124727; 1:10,000), anti-Igλ (cat. no. ab124719; 1:20,000), rabbit anti-human β-actin (cat. no. ab8227; 1:2,000) (all from Abcam), and RP215 monoclonal antibody (mAb) (donated by Professor Xiaoyan Qiu, Peking University, Beijing, China; 1:1,000), which specifically identified a carbohydrate-associated epitope on non-B-Igγ. The membrane was then incubated with goat anti-rabbit (cat. no. 926-32211) or anti-mouse (cat. no. 926-32210) IgG-IRDyeTM680CW secondary antibodies (both 1:10,000; both LI-COR Biosciences) at room temperature for 1 h. The signal was

detected using the Odyssey Imaging system and Odyssey V3.0 software (both LI-COR Biosciences). ImageJ software (version 1.8.0; National Institutes of Health) was used for semi-quantification.

IgG purification and mass spectrometry. After HK-2 cells had been cultured in DMEM/F12 without FBS for 48 h, the culture supernatant was collected after centrifugation at 2,103 x g for 10 min at 4˚C. The cell supernatant was purified by affinity chromatography using protein G Sepharose, according to the manufacturer's instructions (cat. no. 17-0618-02; Thermo Fisher Scientific, Inc.). The eluent was ultra-filtered to replace the elution buffer (0.1 M Glycine; pH 2.4) with PBS. The purified proteins were separated by SDS-PAGE on 10% gels, detected by western blotting, and further analyzed by mass spectrometry, performed by Beijing Protein Innovation Co., Ltd.

Immunofluorescence. HK-2 cells were cultured on cover-slips, which were fixed in cold undiluted acetone for 5 min at room temperature. Subsequently, the slides were washed twice in PBS and blocked with 5% FBS/PBS at room temperature for 20 min, after which they were incubated with primary antibodies at 4˚C overnight. The antibodies were the same as those used in western blotting: Rabbit anti-human Igγ (1:150), anti-human Igκ (1:250), anti-human Igλ (1:250) and RP215 mAb (1:200); PBS was used as a negative control. After washing in PBS, the slides were incubated with fluorescein isothiocyanate-labeled goat anti-rabbit (cat. no. A11008) or goat anti-mouse (cat. no. A11001) IgG antibodies (1:1,000; Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 1 h. Nuclei were stained with DAPI. Images were captured under a Leica DFC300 FX fluorescence microscope (Leica Microsystems GmbH).

Immunohistochemical staining. Paracancerous renal cortices were collected from four male patients (age, 38-49 years) with renal carcinoma. Normal paracancerous renal cortices following nephrectomy were fixed with 10% formalin for 48 h at room temperature, and then embedded in paraffin. Paraffin-embedded human kidney samples were cut into 3-μm sections, and deparaffinized and rehydrated through a series of graded ethanol concentrations. Antigen retrieval was performed by boiling in 0.05 M Tris-EDTA (pH 9.0) in a pressure cooker for 3 min. The sections were then incubated with 3% H_2O_2 solution for 10 min at room temperature to eliminate endogenous peroxidase and incubated with normal goat serum (cat. no. ZLI-9022, ZSGB-BIO, China) for 30 min at room temperature to block nonspecific antibody binding sites at room temperature. Subsequently, indirect immunohistochemical staining was performed with primary antibodies at 4˚C overnight, including RP215 mAb (1:200; 5 μg/ml), rabbit anti-human Igγ (1:2,000), anti-human Igκ (1:1,000), anti-human Igλ (1:1,000) (the same antibodies as used in western blotting). Sections without primary antibodies were used as negative controls. The slides were then incubated with undiluted horseradish peroxidase-labelled secondary antibodies (cat. nos. PV-6001 and PV-6002; both OriGene Technologies, Inc.) at room temperature for 30 min. Bound antibodies were detected using diaminobenzidine. Finally, the slides were counterstained with hematoxylin. Images were captured using a light microscope (x200 magnification).

Statistical analysis. Data are presented as the means ± standard deviation and were analyzed using SPSS 20.0 for Windows (IBM Corp.). All experiments were repeated 3 times. The differences among multiple groups were analyzed using one-way ANOVA and Tukey's post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

IgG expression in renal tubular epithelial cells of the kidney cortex. Normal renal cortexes, which were collected from donors undergoing nephrectomy as a result of renal cell carcinoma, were used to determine the IgG expression in renal tubular epithelial cells by immunohistochemistry using antibodies against human Igγ, Igκ, Igλ and RP215 (an antibody that predominantly binds to non-B-IgG). Positive staining of IgG heavy and light chains was not only detected in the PTECs but also in distal convoluted tubule epithelial cells, either in the cytoplasm, cell membrane or tubular lumen (Fig. 1).

Transcription and V(D)J recombination of IgG in single PTECs. To avoid the interference of residual blood, binding and transcytosis of IgG by PTECs in the kidney cortex, and to obtain direct evidence of IgG expression in PTECs, single PTECs were sorted from human kidney cortex using CD10 and CD13 co-labeling by flow cytometry (20). As shown in Fig. 2A, CD10/CD13 double-positive PTECs accounted for 4.1% of viable cells in the sample. The isolated PTECs were further confirmed using the specific marker gene LRP2, and B-cell contamination was eliminated by CD19. Igγ variable region transcripts were amplified in five single PTECs by nested PCR (Fig. 2B and C). Sanger sequencing results revealed that PTECs exhibited functional and conservative VDJ recombination of IgG heavy chain (Table I), indicating that IgG expression may occur in PTECs.

IgG heavy and light chain expression in HK-2 cells. Since it was difficult to detect IgG protein expression in a single PTEC and to obtain enough PTECs for western blotting, the HK-2 cell line, which is comprised of immortalized PTECs, was selected to further confirm IgG protein expression. Immunofluorescence analysis demonstrated positive staining of Igγ, Igκ and Igλ in the cytoplasm, and stronger positive staining of RP215 predominantly in the cytoplasm and cell membrane (Fig. 3A).

Subsequently, the expression of IgG heavy and light chains in HK-2 cells were detected by western blotting under reducing conditions. To eliminate FBS interference in the culture medium, medium containing FBS was blotted with corresponding antibodies and stained negative. A commercial IgG antibody was able to detect serum-derived IgG but not HK-2-derived IgG. By contrast, RP215 could detect HK-2-derived IgG, but not serum IgG. Both commercial IgG antibody and RP215 were able to detect Igγ at 55 kDa. An Igγ4 (36 kDa) band was detected in HK-2 cells, consistent with the predicted molecular weight. Furthermore, Igκ (25 kDa) and Igλ (50 kDa, dimer) expression was observed in the cell

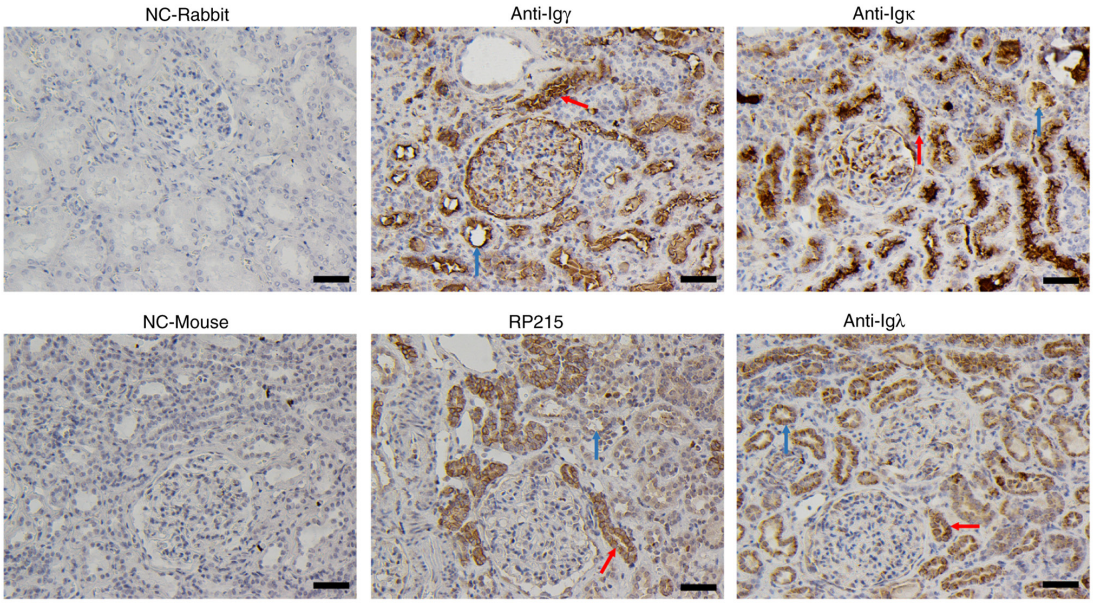


Figure 1. IgG expression in renal tubular epithelial cells of the kidney cortex. Representative images of Igγ, Igκ and Igλ expression in renal tubular epithelial cells from normal kidney cortex. IgG expression was detected by immunohistochemistry using antibodies against human IgG heavy and light chains. NC-Rabbit and NC-Mouse indicates PBS instead of primary antibody and goat anti-rabbit (NC-rabbit) or goat anti-mouse (NC-mouse) as the secondary antibody. The red arrows indicate proximal tubular epithelial cells and the blue arrows indicate distal convoluted tubular cells (scale bar, 100 μm). NC, negative control; Ig, immunoglobulin.

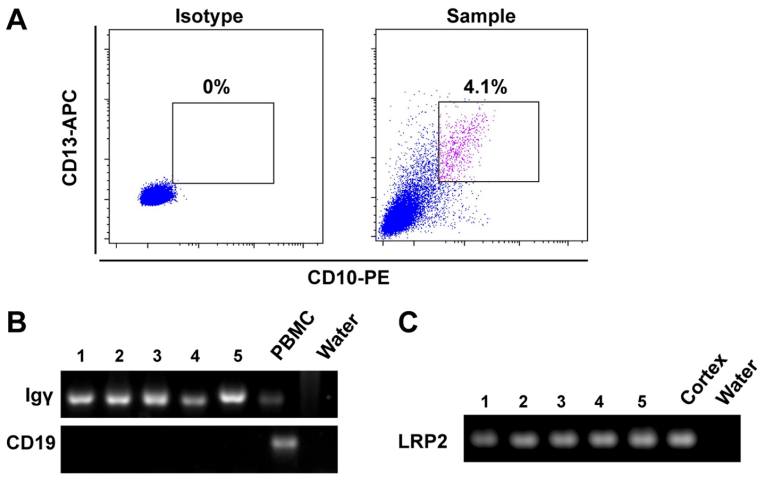


Figure 2. Rearranged IgG was detected in sorted single PTECs by nested PCR. (A) PTECs were sorted by FACS using antibodies against CD10-PE and CD13-APC. The corresponding isotype control antibody was used to exclude non-specific staining. FACS analysis revealed that 4.1% of cells were double-positive. PCR analysis of (B) Igγ and the B-cell marker gene CD19, and (C) PTEC marker gene LRP2. PBMCs were used as the positive control for IgG and CD19, and cDNA from kidney cortex was used as a positive control for LRP2. Water instead of cDNA was used as a negative control. Ig, immunoglobulin; PBMCs, peripheral blood mononuclear cells; PTECs, proximal tubular epithelial cells; LRP2, low-density lipoprotein receptor-related protein 2; FACS, fluorescence-activated cell sorting.

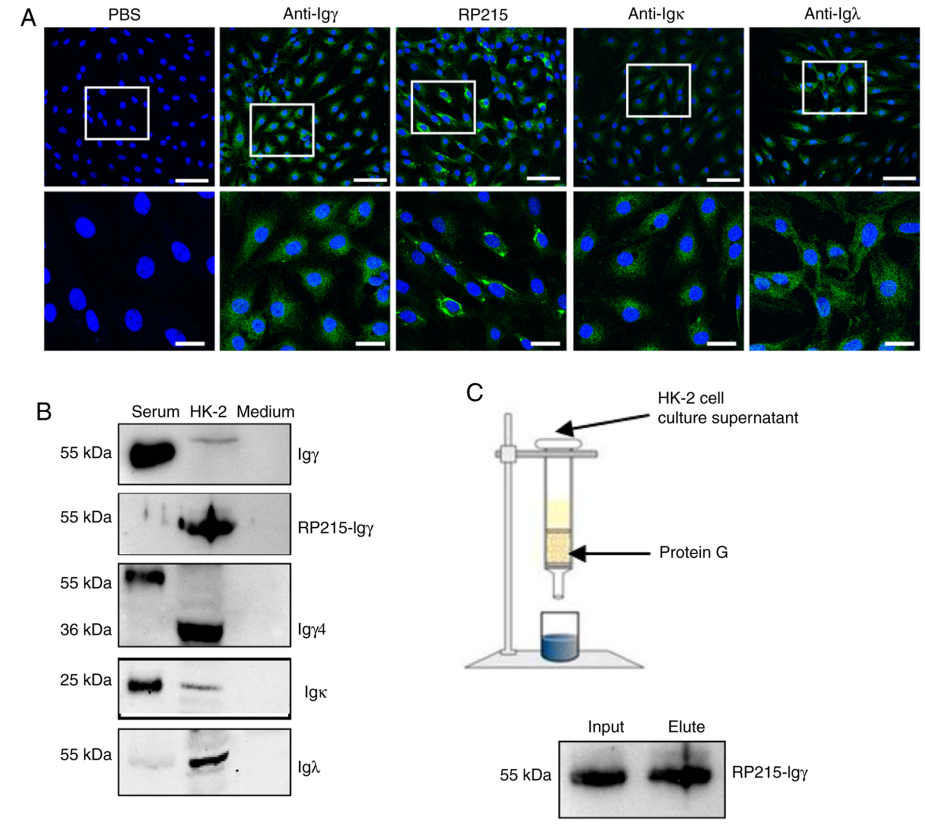
lysates (Fig. 3B). Subsequently, IgG in the cell supernatant was purified by protein G, confirmed by western blotting and sequenced by mass spectrometry, which demonstrated that the 55-kDa band contained fragments of the Igκ chain and Ig heavy chain variable region, according to the National Center for Biotechnology Information (NCBI) database (Fig. 3C-E). These data suggested that HK-2 cells produced and secreted IgG protein.

Transcription and V(D)J recombination of IgG heavy and light chains in HK-2 cells. Ig gene transcription and functional V(D)J recombination is a prerequisite for Ig expression. To confirm the expression of IgG in HK-2 cells, Igγ, Igκ and Igλ transcripts were assessed by amplifying the constant and variable regions in HK-2 cells (Fig. 4A and B). Sequencing of the constant PCR products exhibited high homology with the published sequence in the NCBI database. T-A cloning and Sanger sequencing demonstrated that the Igγ in HK-2 cells displayed conservative V(D)J recombination with VH4-4/D2-8/JH5. By contrast, the diversity of V(D)J recombination was observed in PBMCs, which eliminated primer bias (Table II). Similar to B cells, HK-2-derived IgG displayed typical productive V(D)J recombination with the V-D and D-J junctions (Fig. 4C). Moreover, the somatic hypermutations

Table I. V_HDJ_H recombination patterns of IgG in single PTECs.

Cell ID	Clones	V_HDJ_H usage	Productive	Identity with germlines (%)
1	6/6	IGHV1-46/IGHD5-12/IGHJ4	Yes	86.5-87.1
2	6/6	IGHV1-18/IGHD6-19/IGHJ4	Yes	91.5-92.7
3	6/6	IGHV1-2/IGHD1-1/IGHJ5	Yes	89.5-90.6
4	6/6	IGHV4-59/IGHD3-22/IGHJ3	Yes	91.1-91.7
5	6/6	IGHV1-46/IGHD5-12/IGHJ4	Yes	88.3-88.9

Igγ was amplified in five single PTECs, and V_HDJ_H patterns were sequenced by Sanger sequencing. Six clones in each single PTEC exhibited one functional V_HDJ_H recombination pattern, and cells 1 and 5 had identical V_HDJ_H recombination. PTECs, proximal tubular epithelial cells; Ig, immunoglobulin.



D Immunoglobulin kappa light chain (Homo sapiens)

1 DIQMTQSPST LSASVGDRVT ITCRASQSIN TWLAWYQQKP GKAPKLLMYK
51 ASSLESGVPS RFIGSGSGTE FTLTISSLQP DDFATYYCQQ YNSDSKMFGQ
101 GTKVEVKGTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
151 DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
201 LSSPVTKSFN RGEC

E Ig heavy chain variable region (Homo sapiens)

1 QVQLVESGGG LVKPGGSLRL SCAASGFTFS DYYMSWIRQA PGKGLEWVSY
51 ITSSSNYTNY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARHK
101 RPPRADYYYY GMDVWGQGTT VTVSS

Figure 3. Expression of IgG heavy and light chains in HK-2 cells. (A) Expression and cellular localization of Igγ, Igκ and Igλ in HK-2 cells was assessed by immunofluorescence staining. Green indicates positive staining of Igs and blue indicates nuclear staining by DAPI. Scale bar, 75 μm (upper panel), 25 μm (lower panel). (B) Expression of IgG heavy and light chains in HK-2 lysates was detected by western blotting under reducing conditions. Human serum was used as a positive control. The absence of a band in the medium eliminates the possibility of human Ig heavy chain and light chain expression in the culture medium containing 10% fetal bovine serum, which was considered as a negative control. (C) A 55-kDa band of Igγ was collected from the culture supernatant and purified by protein G. (D and E) Mass spectrometry results of the 55-kDa protein detected in the culture supernatant of HK-2 cells. The bold red sequences refer to the alignment of an amino acid sequence with Igκ chain protein and Ig heavy chain variable region protein in the National Center for Biotechnology Information database. Ig, immunoglobulin.

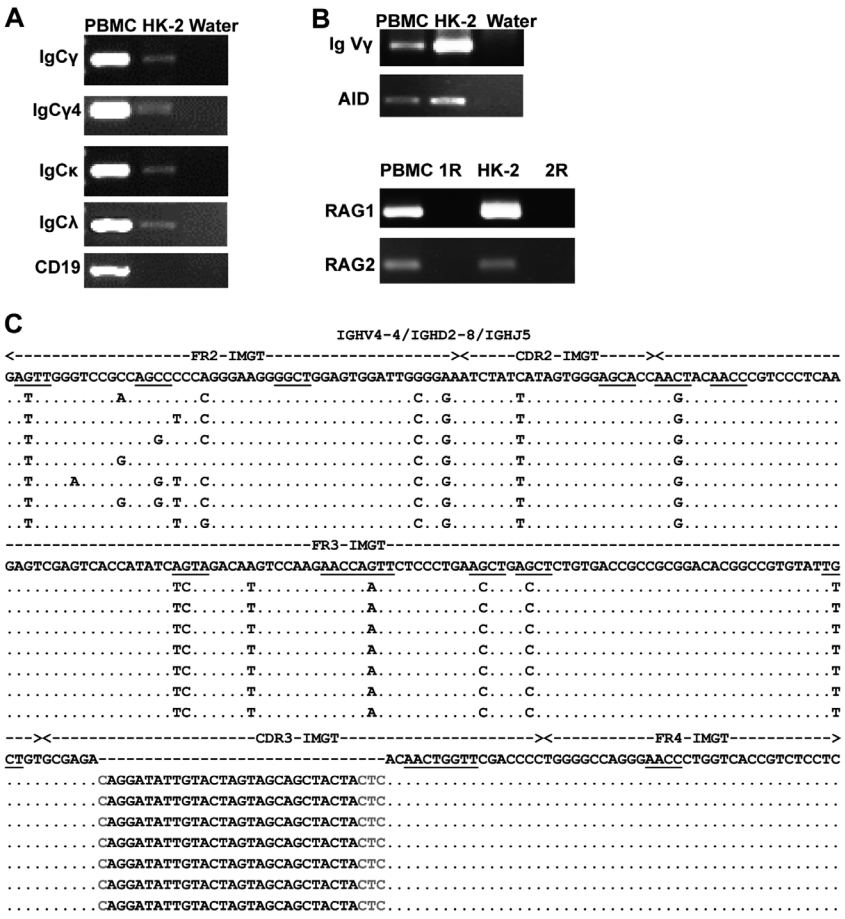


Figure 4. Transcription and V(D)J recombination of IgG heavy and light chains in HK-2 cells. (A) Transcription of IgCγ, IgCγ4, IgCκ and IgCλ were amplified by RT-PCR. CD19 was used to eliminate B-cell interference. (B) IgVγ and RAG1/RAG2 mRNA expression was amplified by nested RT-PCR, whereas the AID mRNA was amplified by RT-PCR. PBMCs acted as a positive control. Water acted as a negative control. R acted as a negative control. (C) Variable region sequences and mutations of IGHV4-4/IGHD2-8/IGHJ5. Identity with the homologous germline sequence is indicated by dots. Each nucleotide mutation is indicated. The mutation hotspots in germline genes are underlined. The red letters refer to the junctions. CDR, complementarity determining region; FR, framework region; R, cDNA template replaced by DNase-treated RNA; RT-PCR, reverse transcription-PCR; AID, activation-induced cytidine deaminase; RAG, recombination activating genes; IgC, immunoglobulin constant region; IgV, immunoglobulin variable region.

were detected in HK-2-derived Igγ (range, 6.8-8.4%) with 7 out of 15 hotspot motifs (RGYW/WRCY, W=A/T, R=A/G, Y=C/T) presenting with mutations.

In addition, AID transcripts (essential element for somatic hypermutation and class switch recombination in B cells), and RAG1 and RAG2 [necessary for V(D)J recombination] were detected in HK-2 cells (Fig. 4B), indicating that the mechanism underlying Ig synthesis in HK-2 cells may be similar to that in B cells.

TGF-β1 upregulates IgG expression in HK-2 cells. HK-2 cells were stimulated with various concentrations of TGF-β1 for 48 h. Immunofluorescence staining revealed that cytoplasmic IgG exhibited enhanced positive staining compared with the control group (Fig. 5A). Western blotting confirmed that IgG was significantly upregulated by TGF-β1 ($P<0.05$; Fig. 5B and C).

Discussion

The present study demonstrated that PTECs expressed IgG with gene transcription and functional conservative V(D)J recombination, which was similar to non-B-IgG. TGF-β1 upregulated IgG expression in HK2 cells.

To investigate whether PTECs expressed IgG, IgG was first detected in the cytoplasm, cell membrane and lumen of PTECs in the normal human renal cortex by immunohistochemistry; the results indicated that PTECs produced and secreted IgG. This is in contrast to our routine pathological examination, where no apparent IgG was detected in PTECs. This could be due to very weak PTEC staining, which was too low to be detected, particularly in immune-related glomerular diseases in which Igs are strongly positive in the glomeruli and PTEC staining may be unintentionally but artificially lost. IgG transcytosis from the circulation by PTECs via the Fc receptor was partially ruled out, as clear staining of IgG by RP215 was observed in the cell membrane and cytoplasm. These findings suggested that PTEC-derived IgG was similar to other non-B-IgG and may have unique glycosylated epitopes, which can be specifically recognized by RP215 instead of a commercial anti-IgG antibody (15). The finding that only part of tubular epithelial cells express IgG may be explained by dynamic expression at different cell cycles, which was similar to the expression pattern of mesangial cell-derived IgA (18). Co-staining of IgG with tubular markers (such as aquaporin 1 and aquaporin 3) would be useful for further studies to enhance the findings of the present study.

Table II. V_HDJ_H recombination patterns of IgG in HK-2 cells and PBMCs.

Name of cells	Clones	V_HDJ_H usage	Productive	Identity with Germlines (%)
HK-2	16/16	IGHV4-4/IGHD2-8/IGHJ5	Yes	91.6-93.2
PBMCs	2/10	IGHV3-7/IGHD4-17/IGHJ4	Yes	92.6
	1/10	IGHV3-7/IGHD3-10/IGHJ4	Yes	93.7
	1/10	IGHV3-23/IGHD3-10/IGHJ3	Yes	95.2
	2/10	IGHV3-30/IGHD3-10/IGHJ4	Yes	89.5-91.1
	1/10	IGHV3-33/IGHD3-22/IGHJ1	Yes	85.9
	1/10	IGHV4-4/IGHD6-19/IGHJ4	Yes	93.7
	1/10	IGHV4-59/IGHD6-19/IGHJ5	Yes	98.9
	1/10	IGHV5-51/IGHD3-22/IGHJ3	Yes	96.9

Sanger sequencing demonstrated that the Igγ in HK-2 cells displayed conservative V_HDJ_H patterns with IGHV4-4/IGHD2-8/IGHJ5 in all 16 clones. By contrast, V_HDJ_H patterns in PBMCs displayed more diversity, which eliminated primer bias. PBMCs, peripheral blood mononuclear cells; Ig, immunoglobulin.

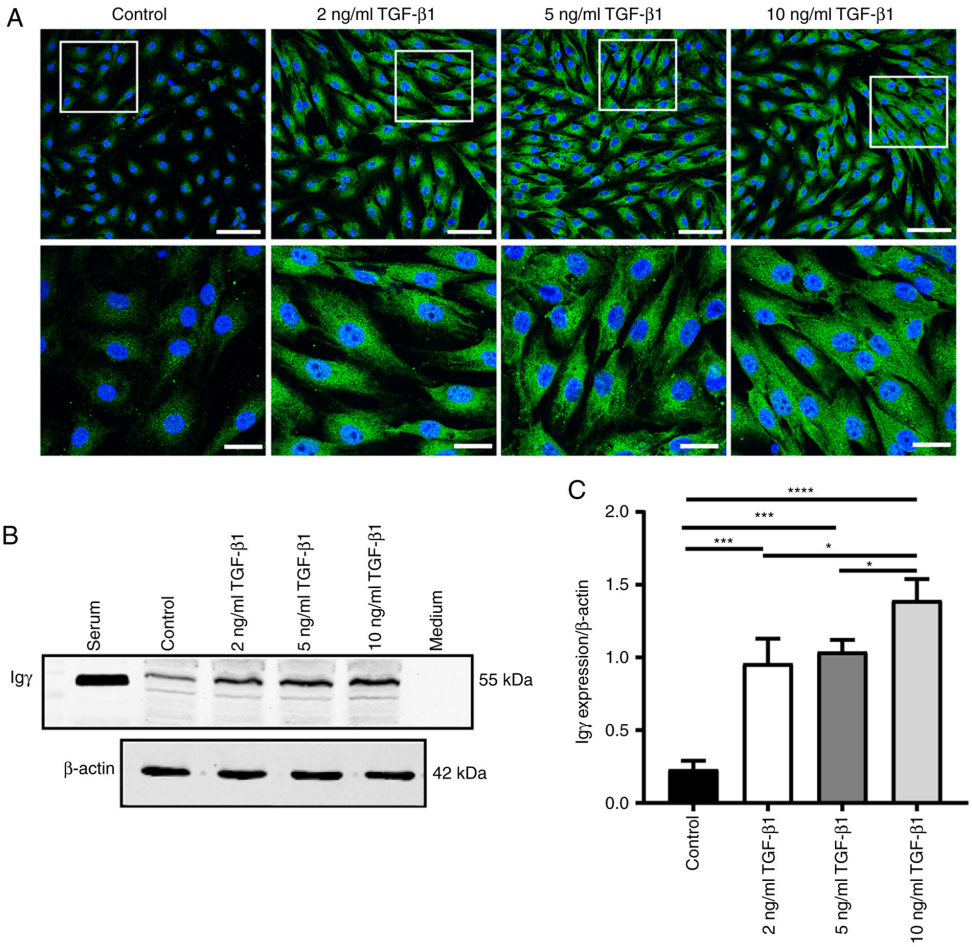


Figure 5. Expression of Igγ in HK-2 cells induced by TGF-β1. (A) HK-2 cells were stimulated by TGF-β1 and Igγ expression was assessed by immunofluorescence staining. Green indicates positive staining of Igγ, blue indicates nuclear staining by DAPI. Scale bar, 75 μm (upper panel), 25 μm (lower panel). (B) Representative western blotting images of Igγ in HK-2 cells treated with TGF-β1. Serum acts as the positive control. Cell culture medium containing 10% fetal bovine serum was used as a negative control. (C) Gray value of Igγ relative to β-actin upon treatment with different concentrations of TGF-β1. ****P<0.0001 and ***P<0.001 vs. untreated control group. *P<0.05 vs. 10 ng/ml TGF-β1. Ig, immunoglobulin.

Single cell RNA sequencing can clearly display the transcription of a specific gene in a given cell. Transcripts of the Igγ chain and V(D)J recombination were detected in single PTECs. Although only five single PTECs were used, the process was rigorous as the kidney cortex was collected far from the tumor, and each single cell was sorted by flow

cytometry with two PTEC-specific marker genes, reconfirmed using a third specific marker gene (LRP2) and B-cell contamination was eliminated. The results revealed that PTECs do not only present with IgG gene transcripts but also the classical V(D)J recombination in the variable region as B cells, such as the productive V(D)J recombination with the V-D and D-J junctions (Fig. 4), indicating that PTECs have the potential to produce IgG. In addition, the more conservative V(D)J recombination illustrated that PTECs present with IgG gene transcripts and V(D)J recombination that are similar to other non-B cells.

HK-2, an immortalized PTEC line, is easy to culture in large quantities. The present study confirmed IgG protein expression in cultured HK-2 cells; IgG was detected in HK-2 cells by both immunofluorescence and western blotting. Igγ4, a subclass of Igγ, was also detected at a band size consistent with the predicted molecular weight. Mass spectrometry revealed that the protein purified from cell supernatant using protein G contained fragments of the Ig heavy chain variable region and Igκ chain, providing evidence for IgG secretion by PTECs. IgG transcription in HK-2 cells further supported IgG expression in PTECs, and the conservative V(D)J recombination with IGHV4-4/IGHD2-8/IGHJ5 in HK-2 cells further supported the presence of IgG in HK-2 cells similar to other non-B cells.

The present study also investigated the underlying mechanism of IgG production in PTECs by examining the transcription of RAG1, RAG2 and AID in HK-2 cells. It was revealed that RAG1, RAG2 and AID were transcribed in HK-2 cells. Additionally, RAG1, RAG2 or AID transcripts have previously been detected in numerous other non-B cells, such as podocytes (19) and several cancer cell lines (23). These results suggested that non-B cells, including PTECs, may have similar mechanisms of Ig synthesis to B cells. However, whether AID and/or RAG1/2 are necessary genes for PTEC-derived IgG requires further investigation. In addition, follicular helper CD4 T cells are important to regulate germinal center B-cell differentiation into plasma cells and support the production of Igs (24). Our unpublished data revealed that non-B-Igs were still detected in T and B cell-deficient NOD-SCID mice, indicating that non-B-Igs were not entirely dependent on T helper cells. Whether T helper cells are required for PTECs to express IgG requires further research.

TGF-β1 has a key immunomodulatory role in Ig production. For example, TGF-β1 induced IgA class switching and secretion in stimulated B cells in mouse spleen (25) and human tonsil B cells (26). McIntyre *et al* (27) demonstrated that TGF-β1 selectively stimulated IgG2b secretion by lipopolysaccharide-activated B cells most likely by inducing an IgM to IgG2b class switch. Duan *et al* (28) demonstrated that TGF-β1 increased IgA expression by upregulating the transcription factor Ets-1 in epithelial cancer cells. The present study demonstrated that TGF-β1 upregulated IgG expression in HK-2 cells. Given that only IgG was detected in HK-2 cells, it was hypothesized that TGF-β1 induced IgG expression independent of Ig class switching. The regulatory mechanism of IgG production by TGF-β1 requires further investigation.

PTECs serve an important role in tubular interstitial fibrosis through EMT and TGF-β1 acts as a master profibrotic mediator. In the present study, the addition of TGF-β1 to cultured HK-2 cells increased IgG expression, indicating that HK-2-derived IgG may be positively associated with EMT. Previous studies have reported that cancer-IgG was associated with metastasis and promoted EMT by decreasing E-cadherin in salivary adenoid cystic carcinoma (29) and lung cancer (30). It is worth investigating whether PTEC-derived IgG may serve a role in renal tubular EMT and interstitial fibrosis under disease conditions, such as ischemia/reperfusion injury or chronic kidney disease.

In conclusion, to the best of our knowledge, the present study was the first to demonstrate that PTECs can express and secrete IgG, and TGF-β1 can upregulate IgG expression in HK-2 cells. However, the potential role of PTEC-derived IgG in tubulointerstitial fibrosis requires further investigation.

Acknowledgements

Not applicable.

Funding

This study was supported by grants from the National Natural Science Foundation of China (grant nos. 82070736, 91642109 and 81870488), the Hainan Natural Science Foundation (grant no. 819QN355), the Hainan Medical University Scientific Research Cultivation Foundation (grant no. HYPY201926) and the Key Support Projects of the National Natural Science Foundation's Major Research Program (grant no. 91642206).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

As the corresponding authors, YW and XQ conceived and designed the study. ZD participated in the research design, performed histological and single-cell experiments, prepared samples for mass spectrometry, analyzed data and wrote the manuscript. ZJ participated in the research design, most experiments regarding IgG expression in HK-2 cells and relevant data analysis. YG, JM, HD and YL performed partial cell line experiments. ZC and YP selected appropriate cases for single-cell experiments according to clinical characteristics. HY and ZS participated in single-cell experiments. SW participated in immunohistochemical staining, analyzed tissue staining data and drafted and revised the manuscript. YW, ZD and ZJ confirmed the authenticity of all the raw data. All authors reviewed the manuscript and revised data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study conformed to the principles of the Declaration of Helsinki, and was approved by the Medical Ethics Committee of Peking University Third Hospital (approval no. S2020121) and conducted in accordance with the protocol. All donors voluntarily donated kidney cortexes

and provided written informed consent prior to donating the kidney cortex to the study. All methods were carried out in accordance with relevant guidelines and regulations. These samples were strictly anonymized. Human serum and PBMCs, used as positive controls in western blotting or reverse transcription-PCR in the present study, were obtained from the blood of a healthy volunteer, who provided written informed consent for sampling.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Schlondorff DO: Overview of factors contributing to the pathophysiology of progressive renal disease. Kidney Int 74: 860-866, 2008.
2. Donadio ME, Loiacono E, Peruzzi L, Amore A, Camilla R, Chiale F, Vergano L, Boido A, Conrieri M, Bianciotto M, *et al*: Toll-like receptors, immunoproteasome and regulatory T cells in children with Henoch-Schonlein purpura and primary IgA nephropathy. Pediatr Nephrol 29: 1545-1551, 2014.
3. Breda PC, Wiech T, Meyer-Schwesinger C, Grahammer F, Huber T, Panzer U, Tiegs G and Neumann K: Renal proximal tubular epithelial cells exert immunomodulatory function by driving inflammatory $CD4^+$ T cell responses. Am J Physiol Renal Physiol 317: F77-F89, 2019.
4. Liu BC, Tang TT, Lv LL and Lan HY: Renal tubule injury: A driving force toward chronic kidney disease. Kidney Int 93: 568-579, 2018.
5. Kobayashi N, Suzuki Y, Tsuge T, Okumura K, Ra C and Tomino Y: FcRn-mediated transcytosis of immunoglobulin G in human renal proximal tubular epithelial cells. Am J Physiol Renal Physiol 282: F358-F365, 2002.
6. Qiu X, Zhu X, Zhang L, Mao Y, Zhang J, Hao P, Li G, Lv P, Li Z, Sun X, *et al*: Human epithelial cancers secrete immunoglobulin g with unidentified specificity to promote growth and survival of tumor cells. Cancer Res 63: 6488-6495, 2003.
7. Zheng J, Huang J, Mao Y, Liu S, Sun X, Zhu X, Ma T, Zhang L, Ji J, Zhang Y, *et al*: Immunoglobulin gene transcripts have distinct VHDJH recombination characteristics in human epithelial cancer cells. J Biol Chem 284: 13610-13619, 2009.
8. Jiang D, Ge J, Liao Q, Ma J, Liu Y, Huang J, Wang C, Xu W, Zheng J, Shao W, *et al*: IgG and IgA with potential microbial-binding activity are expressed by normal human skin epidermal cells. Int J Mol Sci 16: 2574-2590, 2015.
9. Zhu Z, Zhang M, Shao W, Wang P, Gong X, Ma J, Qiu X and Wang B: Immunoglobulin M, a novel molecule of myocardial cells of mice. Int J Biochem Cell Biol 88: 172-180, 2017.
10. Niu N, Zhang J, Sun Y, Wang S, Sun Y, Korteweg C, Gao W and Gu J: Expression and distribution of immunoglobulin G and its receptors in an immune privileged site: The eye. Cell Mol Life Sci 68: 2481-2492, 2011.
11. Huang J, Sun X, Mao Y, Zhu X, Zhang P, Zhang L, Du J and Qiu XY: Expression of immunoglobulin gene with classical V-(D)-J rearrangement in mouse brain neurons. Int J Biochem Cell Biol 40: 1604-1615, 2008.
12. Niu N, Zhang J, Guo Y, Zhao Y, Korteweg C and Gu J: Expression and distribution of immunoglobulin G and its receptors in the human nervous system. Int J Biochem Cell Biol 43: 556-563, 2011.
13. Li J, Korteweg C, Qiu Y, Luo J, Chen Z, Huang G, Li W and Gu J: Two ultrastructural distribution patterns of immunoglobulin G in human placenta and functional implications. Biol Reprod 91: 128, 2014.
14. Huang J, Zhang L, Ma T, Zhang P and Qiu X: Expression of immunoglobulin gene with classical V-(D)-J rearrangement in mouse testis and epididymis. J Histochem Cytochem 57: 339-349, 2009.
15. Tang J, Zhang J, Liu Y, Liao Q, Huang J, Geng Z, Xu W, Sheng Z, Lee G, Zhang Y, *et al*: Lung squamous cell carcinoma cells express non-canonically glycosylated IgG that activates integrin-FAK signaling. Cancer Lett 430: 148-159, 2018.
16. Cui M, You L, Zheng B, Huang X, Liu QF, Huang J, Pan B, Qiu X, Liao Q and Zhao Y: High expression of cancer-derived glycosylated immunoglobulin G predicts poor prognosis in pancreatic ductal adenocarcinoma. J Cancer 11: 2213-2221, 2020.
17. Cui M, Hu Y, Zheng B, Zhang S, Zhang X, Wang M, Qiu XY, Liao Q and Zhao YP: Cancer-derived immunoglobulin G: A novel marker for differential diagnosis and relapse prediction in parathyroid carcinoma. Clin Endocrinol (Oxf) 92: 461-467, 2020.
18. Deng H, Ma J, Jing Z, Deng Z, Liang Y, A L, Liu Y, Qiu X and Wang Y: Expression of immunoglobulin A in human mesangial cells and its effects on cell apoptosis and adhesion. Mol Med Rep 17: 5272-5282, 2018.
19. Jing Z, Deng H, Ma J, Guo Y, Liang Y, Wu R, A L, Geng Z, Qiu X and Wang Y: Expression of immunoglobulin G in human podocytes, and its role in cell viability and adhesion. Int J Mol Med 41: 3296-3306, 2018.
20. Van der Hauwaert C, Savary G, Gnemmi V, Glowacki F, Pottier N, Bouillez A, Maboudou P, Zini L, Leroy X, Cauffiez C, *et al*: Isolation and characterization of a primary proximal tubular epithelial cell model from human kidney by CD10/CD13 double labeling. PLoS One 8: e66750, 2013.
21. Tang F, Barbacioru C, Nordman E, Li B, Xu N, Bashkirov VI, Lao K and Surani MA: RNA-Seq analysis to capture the transcriptome landscape of a single cell. Nat Protoc 5: 516-535, 2010.
22. van Dongen JJ, Langerak AW, Bruggemann M, Evans PA, Hummel M, Lavender FL, Delabesse E, Davi F, Schuuring E, García-Sanz R, *et al*: Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 concerted action BMH4-CT98-3936. Leukemia 17: 2257-2317, 2003.
23. Chen Z and Gu J: Immunoglobulin G expression in carcinomas and cancer cell lines. FASEB J 21: 2931-2938, 2007.
24. Crotty S: Follicular helper CD4 T cells (TFH). Annu Rev Immunol 29: 621-663, 2011.
25. Coffman RL, Lebman DA and Shrader B: Transforming growth factor beta specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. J Exp Med 170: 1039, 1989. J Immunol 182: 8-13, 2009.
26. van Vlasselaer P, Punnonen J and de Vries JE: Transforming growth factor-beta directs IgA switching in human B cells. J Immunol 148: 2062-2067, 1992.
27. McIntyre TM, Klinman DR, Rothman P, Lugo M, Dasch JR, Mond JJ and Snapper CM: Transforming growth factor beta 1 selectively stimulates immunoglobulin G2b secretion by lipopolysaccharide-activated murine B cells. J Exp Med 177: 1031-1037, 1993.
28. Duan Z, Deng H, Xu S, Jiang Y, Liu H, Li M, Hu D, Li W, Bode AM, Dong Z and Cao Y: Activation of the Ig Iα1 promoter by the transcription factor Ets-1 triggers Ig Iα1-Cα1 germline transcription in epithelial cancer cells. Cell Mol Immunol 11: 197-205, 2014.
29. Peng J, Wang HC, Liu Y, Jiang JH, Lv WQ, Yang Y, Li CY and Qiu XY: Involvement of non-B cell-derived immunoglobulin G in the metastasis and prognosis of salivary adenoid cystic carcinoma. Oncol Lett 14: 4491-4498, 2017.
30. Jiang C, Huang T, Wang Y, Huang G, Wan X and Gu J: Immunoglobulin G expression in lung cancer and its effects on metastasis. PLoS One 9: e97359, 2014.