Expression of ATP sensitive K⁺ channel subunit Kir6.1 in rat kidney

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ATP-sensitive K⁺ (KATP) channels in kidney are considered to play roles in regulating membrane potential during the change in intracellular ATP concentration. They are composed of channel subunits (Kir6.1, Kir6.2), which are members of the inwardly rectifying K⁺ channel family, and sulphonylurea receptors (SUR1, SUR2A and SUR2B), which belong to the ATP-binding cassette superfamily. In the present study, we have investigated the expression and localization of Kir6.1 in rat kidney with Western blot analysis, immunohistochemistry, in situ hybridization histochemistry, and immunoelectron microscopy. Western blot analysis showed that Kir6.1 was expressed in the mitochondria and microsome fractions of rat kidney and very weakly in the membrane fractions. Immunohistochemistry revealed that Kir6.1 was widely distributed in renal tubular epithelial cells, glomerular mesangial cells, and smooth muscles of blood vessels. In immunoelectron microscopy, Kir6.1 is mainly localized in the mitochondria, endoplasmic reticulum (ER), and very weakly in cell membranes. Thus, Kir6.1 is contained in the kidney and may be a candidate of mitochondrial KATP channels.

Key words: ATP-sensitive K⁺ channel, Kir6.1, immunohistochemistry, in situ hybridization, immunoelectron microscopy, kidney, rat

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A TP-sensitive K⁺ (KATP) channels, originally discovered by a patch-clamp technique in the cardiac muscle (Noma 1983), belong to the inwardly rectifying K⁺ channel superfamily. These channels have ATP sensitivity and weak inward rectification capacity (Aguilar-Bryan et al. 1998). In 1995, a pore-forming subunit was cloned (Inagaki et al. 1995), and was ubiquitously localized in tissues and cells. Initially, the subunit was called uKATP, and later was named as Kir6.1. It was detected in liver and skeletal muscle (Suzuki et al. 1997), brain (Lacza et al. 2003a; Zhou et al. 1999), heart (Lacza et al. 2003b; Zhou et al. 2005), human embryo kidney (HEK) 293 cells (Ammala et al. 1996; Braun et al. 2002; Kondo et al. 1998; Satoh et al. 1998), and in kidney as well (Anzai et al. 1996; Braun et al. 2002; Brochiero et al. 2002). The functional KATP channels need four pore-forming subunits of Kir6.x (Kir6.1 or Kir6.2) as well as four regulatory subunits of SURs (SUR1, SUR2A, or SUR2B) that form a hetero-octameric compound (Clement et al. 1997; Inagaki et al. 1996). In most excitable tissues KATP channels are activated when cell metabolism is impaired; thereby the cell is clamped in the resting state which conserves ATP, with the benefit of keeping the structural integrity of the cell (Quast 1996).

Although several lines of evidence including patch clamp techniques (Wang et al. 1995), RT-PCR (Brochiero et al. 2002), Northern blot analysis (Inagaki et al. 1995), Western blot analysis (Braun et al. 2002) and immunohistochemistry (Anzai et al. 1996; Braun et al. 2002) clearly revealed that Kir6.1 localized in kidney, and Kir6.1 mRNA increased significantly after renal ischemia (Sgard et al. 2000), a conflicting report claimed thatKir6.1 protein was not expressed in rat kidney (Sun et al. 2004). Thereby, it is important to elucidate whether or not Kir6.1 is localized in the kidney. Confirmation of its localization will lead to improved understanding of its functions in renal
tubules. The cellular and subcellular localization of Kir6.1 in renal tubular epithelial cells has not been established, although it was found in the mitochondria as well as in the surface plasma membrane of rat liver, skeletal muscle, brain neurons and glial cells, and cardiomyocytes (Lacza et al. 2003b; Singh et al. 2003; Suzuki et al. 1997; Zhou et al. 2005; Zhou et al. 1999).

The aim of the present study is to determine detailed information of Kir6.1 in renal tubular epithelial cells. Its expression was assessed by Western blot analyses with cellular fractions, its localization was observed by immunohistochemistry and in situ hybridization, and its subcellular localization was revealed by immunoelectron microscopy.

Materials and Methods

Generation of anti-Kir6.1 antibody

Rabbit anti-Kir6.1 antibody was raised against a synthetic 14 amino acid peptide, NH₂-(C)QFMT-PEGNQCPSES-OH, which corresponds to amino acid residues 411 to 424 of rat Kir6.1 (Gene No. D42145). The polyclonal peptide antibody production was processed according to Van Bueren et al. (1993), with some modifications. In brief, the synthetic peptide representative of rat Kir6.1 was coupled to the carrier protein keyhole limpet hemocyanin (KLH) via the N-terminal cysteine residue added to the peptide. Two Japanese white rabbits, 2.5-3.0 kg (Japan SLC, Hamamatsu, Japan) were injected with approximately 200 µg of peptide-KLH conjugate emulsified with an equal volume of Freund’s complete adjuvant at multiple intradermal sites, followed by 3 boosters once 2 weeks interval later by injection with the same dosage of the peptide-KLH conjugate emulsified with an equal volume of Freund’s complete adjuvant (Rockland Immunochemicals) at multiple intradermal sites, followed by 3 boosters once 2 weeks interval later by injection with the same dosage of the peptide conjugate emulsified in Freund’s incomplete adjuvant (Rockland Immunochemicals, Gilbertsville, PA, USA) three time. The antiserum was harvested 1 week after the final injection. The antiserum was purified by immunoaffinity column chromatography before using for Western blot analysis and immunohistochemistry.

Transfection and preparation of cells extract

COS-7 cells were transfected with rat Kir6.1 in the mammalian expression vector pFLAG-CMV5a by lipofectamine method according to the manufacturer’s instructions. Briefly, The PCR product, a full-length rat Kir6.1 cDNA was subcloned into BamHI site of pFLAG-CMV-5a vector (Sigma-Aldrich, St. Louis, MO, USA), designated as pFLAG-CMV-5a-rKir6.1. Twenty-four hours prior to transfection, COS-7 cells were plated at a density of 2.0×10⁴ cells/100-mm cell-culture dish (TPP, Trasadingen, Switzerland) in Dulbecco’s modified Eagle’s medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Gemini Bioproducts Woodland, CA, USA). Cells were incubated at 37°C in 5% CO₂ with 95% air and transferred with 4 µg of pFLAG-CMV-5a-rKir6.1 plasmid vector using 15 µL of LipofectAMINE™ 2000 Reagent (Invitro-gen, Carlsbad, CA, USA).

The transfected cells were washed with PBS 48 or 72 h after transfection, suspended in 0.25 M sucrose/20 mM Tris-HCl buffer, pH 7.4, containing 4 mM EDTA, 1 mM EGTA and 1 mM DTT with complete protease inhibitor cocktail tablets (Roche, Basel, Switzerland) and sonicated for 5 min with 50% duty cycle on ice with an Ultrasonic Processor (Sonics & Materials inc., Newtown, Connecticut, USA). The cell lysate was clarified by centrifugation (1500 x g for 10 min at 4°C), stored at -80°C until use.

Preparation of cRNA probes

Single strand riboprobes were labeled with digoxigenin prepared using Dig RNA Labeling Kit (Boehringer Mannheim, GmbH, Germany) according to the manufacturer’s instructions. The template for synthesis of digoxigenin (DIG)-labeled RNA probes was generated by using PCR amplification from pGEM-rat-Kir6.1 plasmid. The primer sequences were 5’-TCCATCTTGATTCAGACCTCCAAAAGAGTGAACTGTCGCACCAG-3’ and 5’-GGCGACAGGTCCGATACTTCGATCACCAGAACTCAGCAAACTGTCGACAGGATTGCAGCTGGGCGAAGGTCCGACTGTCGCACCAG-3’ and 5’-GGCGACAGGTCCGACTGTCGCACCAG-3’ corresponding to 1266-1310 and 1514-1558 of the rat Kir6.1 (D42145). PCR reaction was run with Taq polymerase (Takara, Otsu, Japan) for 34 cycles of 94°C x 30 sec denaturating, 42°C x 30 sec annealing, and 72°C x 1 min extension each, with a final extension of 7 min at 72°C. A 293-bp PCR product was obtained and subcloned into a TA cloning pCRII vector (Invitrogen, San Diego, CA, USA). The identity and orientation of the constructs were verified by sequence analysis (Takara, Otsu, Japan) and the product was identical to the published sequence of rat Kir6.1. After linearization of the construct with
BamHI (Toyobo, Osaka, Japan), the antisense RNA probe was generated using T7 RNA polymerase (Takara, Otsu, Japan). To generate a sense RNA probe, the construct was linearized with EcoRI (Boehringer Mannheim, GmbH, Germany), and transcription was performed using SP6 RNA polymerase (Takara, Otsu, Japan).

Animals and tissue preparations
Male Wistar rats (4 to 6 weeks) were used (Japanese SLC; Hamamatsu, Japan). The protocols for animal experimentation described herein were previously approved by the Animal Research Committee, Akita University; all subsequent animal experiments adhered to the Guidelines for Animal Experimentation of the University.

The rats were anesthetized through peritoneal injection of pentobarbital sodium (Abbott Lab.; Chicago, Illinois, USA) as 50 mg per kilogram body weight, and 4% paraformaldehyde (PFA) buffered at pH 7.4 by 0.1 M phosphate buffered saline (PBS) was perfused through the left ventricle at room temperature. The excised kidneys were cut into thin slices and placed into the same fixative at 4°C over night and subsequently transferred into 30% sucrose in PBS at 4°C. For in situ hybridization histochemistry, kidneys were quickly removed and immediately frozen on powdered dry ice. Cryosections for both groups were cut at a thickness of 8-10 µm and thaw-mounted on MAS-coated glass slides (Matsunami Glass Ind. Ltd.; Kishiwada, Japan).

Subcellular fractionations
All procedures were carried out at 0-4°C. The kidneys were immediately excised from anesthetized rats and quickly washed with 0.9% NaCl solution. They were then cut into small pieces and homogenized with protein extraction buffer with 0.25 M sucrose in 50 mM Tris-HCl, pH7.4, containing protease inhibitor cocktail tablets (Roche Diagnostics GmbH, Mannheim, Germany). Subcellular fractions were extracted as described elsewhere (Itoh et al. 2002). Briefly, after centrifugation at 600 × g for 10 min, the precipitate was discarded, and the 600 g supernatant was centrifuged at 7000 × g for an additional 10 min. The 7000 g precipitate (P1) was re-dissolved in the buffer and centrifuged at 5000 × g for 10 min, and the 5000 g precipitate was used as the mitochondrial fraction. The 7000 g supernatant (S1) was centrifuged at 54,000 × g for 60 min, and the supernatant (S2) was centrifuged at 105,000 × g for an additional 60 min. The 54,000 g precipitate was used as the cell membrane fraction, the 105,000 g precipitate as the microsome fraction, and the supernatant as the cytoplasm fraction.

Immunoblotting
Proteins of harvest COS-7 cells transfected with pFLAG-CMV5a-rKir6.1 plasmid vector, COS-7 cells only, and whole rat kidney, heart, brain, liver, and testis, and subcellular fractions of kidney were denatured in a modified sample buffer (125 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% Bromophenol blue and 10% 2-mercaptoethanol). Electrophoresis was performed on 10 or 12% SDS-polyacrylamide gel. The proteins were subsequently transferred onto a polyvinylidine difluoride (PVDF) membrane (NENTM Life Science, Boston, CA, USA) by using a semi-dry transfer unit (Hoefer TE70 series, Amersham Pharmacia Biotech) according to the manufacturer’s instruction. The transferred PVDF membranes were then blocked with 5% Blot-QuickBlocker (Chemicon International, Inc.; Temecula, CA, USA) in PBS over night at room temperature. The PVDF membranes were incubated with rabbit anti-rat Kir6.1 antibody (Zhou et al. 2005) diluted to 1:1000, or mouse anti-FLAG M2 antibody (Sigma-Aldrich, St. Louis, MO, USA) diluted to 1:1000 for 1 h at 37°C. After rinsing with PBS-T (PBS containing 0.1% Tween-20), they were then reacted to HRP-conjugated donkey anti-rabbit IgG (Amersham Pharmacia Biotech) diluted to 1:3000, or HRP-conjugated sheep anti-mouse (Amersham Pharmacia Biotech) diluted to 1:6000 for 30 min at room temperature. Membrane was washed three times with PBS-T; bands were visualized with ECL Western blotting detection reagents (Amersham Pharmacia Biotech) according to the manufacturer’s instructions and exposed to X-OMAT™ film (Eastman Kodak, Rochester, NY, USA).

Immunohistochemistry
Cryosections of kidney were kept in PBS containing 0.3% Tween-20 for 45 min. Prior to incubation with the first antibody, sections were treated with 0.3% H2O2/methanol solution and ABC blocking kit (Vector Lab., Inc.) to reduce endogenous peroxidase reaction as well as non-specific binding with avidin-biotin complex. After incubation with 5%
normal goat serum for 1 h, the sections were reacted with rabbit anti-Kir6.1 antibody at a dilution of 1:500 for 12 h at room temperature. The sections were then treated with biotinylated goat anti-rabbit IgG (BA-1000, Vector Laboratories Inc., Burlingame, CA) at a dilution of 1:200 for 30 min, and then with ABC complex (Vectastain ABC kit, Vector Lab., Inc.) for 45 min according to the manufacturer’s instructions. Reactivity was visualized by incubating the sections in 0.001–0.005% DAB (3,3′-diaminobenzidine tetrahydrochloride) reaction with 0.003% H2O2 and counterstained with methyl green. Between the above steps, each section was carefully rinsed 3 times with PBS containing 0.05% Tween-20, except between the normal goat serum and the first antibody.

**Immunoelectron microscopy**

For immunoelectron microscopy, the sections that showed good immunoreaction to anti-Kir6.1 antibody mentioned above were post-fixed in 1% osmium tetroxide/PBS for 30 min, dehydrated in an ethanol series, and embedded (Vectastain ABC kit, Vector Lab., Inc.) for 45 min according to the manufacturer’s instructions. Reactivity was visualized by incubating the sections in 0.001–0.005% DAB (3,3′-diaminobenzidine tetrahydrochloride) reaction with 0.003% H2O2 and counterstained with methyl green. Between the above steps, each section was carefully rinsed 3 times with PBS containing 0.05% Tween-20, except between the normal goat serum and the first antibody.

**In situ hybridization**

Fresh frozen sections of rat kidneys were fixed in 4% paraformaldehyde for 15 min and then digested with 10 µg/mL proteinase K at room temperature for 5 min. They were refixed in 4% paraformaldehyde for 10 min, treated with 0.2N HCl for 10 min for inactivation of internal alkaline phosphatase, and acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine pH 8.0 for 10 min. After dehydration with a graded series of ethanol and air drying, hybridization was performed at 50°C for 16 h under a parafilm coverslip with the hybridization buffer, which contained 50% deionized formamide, 10% dextran sulfate, 10 mM Tris HCl pH 7.6, 200 µg/mL salmon sperm DNA, 1 × Denhardt’s solution, 600 mM NaCl, 0.25% SDS, 1 mM EDTA, and 0.1–0.5 µg/slide cRNA probe. After rinsing and treatment with 50 µg/mL RNase at 37°C for 30 min, hybridized digoxigenin-labeled probes were detected by Nucleic Acid Detection Kit (Boehringer Mannheim, GmbH, Germany) according to the manufacturer’s instructions. After color reaction, sections were rinsed with 10 mM Tris HCl pH 7.6 and 1.0 mM EDTA, post-fixed with 4% paraformaldehyde in PBS, rinsed with distilled water, and sealed without counter staining. For the control experiment, the same procedure was employed with a sense cRNA probe.

**Electron microscopy of the mitochondrial fractions**

In order to confirm the purity of the mitochondrial fractions used in the immunoblotting, pellets obtained from the 5000 g precipitate were fixed in 2% glutaraldehyde for 2 h, followed by 1% O.O- for 2 h. Between the above steps, the pellets were rinsed carefully, then dehydrated with a graded acetone series and embedded in Quetol 812. Thin sections were cut and directly examined under an electron microscope.
Results

The anti-Kir6.1 antibody recognized a prominent 50 kDa protein band and a weak small band below in the extracts of COS-7 cells transfected with rat Kir6.1 cDNA (Figure 1A, lane 2) but no remarkable signal was detected in extracts of COS-7 cells only (Figure 1A, lane 3). The extracts of COS-7 cells transfected with rat Kir6.1 cDNA was also detected by mouse anti-FLAG M2 antibody (Figure 1A, lane 1). This result indicated that the cellular transfection was successfully and the specificity of anti-Kir6.1 antibody is qualified. In rat kidney extract the anti-Kir6.1 antibody recognized a prominent 50 kDa band and a weak small band below (Figure 1B, right lane). The specificity of binding to Kir6.1 was further confirmed by the fact that the detection signal was completely removed by pre-absorption with the immunizing peptide antigen (Figure 1B, left lane). This antibody recognizes a 50 kDa band in all examined tissues such as kidney, brain liver, and testis except heart which showed a ~ 43 kDa band, and a weak 50 kDa band (Figure 1C).

In kidney cellular fractions, Kir6.1 was expressed prominently in the mitochondrial fraction (Figure 1D, mit), weakly in the microsomal fraction (Figure 1D, ms), and very weakly, if at all, in the cell membrane fraction (Figure 1D, cm).

The purity of mitochondrial fractions was confirmed by electron microscopy. In the electron micrograph, most of the visual field was covered with intact mitochondria (Figure 1E).

By immunohistochemistry, Kir6.1 protein was widely distributed in the renal cortex and medulla (Figure 2A). It was expressed in podocytes, mesangial cells of glomerulus, and epithelial cells of renal tubules (Figure 2B, C). Some of the moderate immunoreactivity with Kir6.1 was observed as fine granular or punctate reaction products in the cytoplasm of proximal tubules and distal tubules (Figure 2B). It was expressed only weakly in the basolateral membrane or apical membrane. The immunoreactivity was also expressed in collecting ducts in the medulla (Figure 2D). Immunoreactivity was also expressed in the smooth muscles and endothelium of blood vessels within the kidney (Figure 2E). No significant immunoreactivity could be observed in the rat kidney when anti-Kir6.1 antibody was pre-absorbed with the immunizing peptide antigen (Figure 2F).

By in situ hybridization histochemistry, the localization of Kir6.1 mRNA in the kidney was detected with antisense cRNA probes as purple deposition of Kir6.1 mRNA.
reaction products of alkaline phosphatase with nitroblue tetrazolium chloride (NBT). The Kir6.1 mRNA was widely expressed in the renal cortex (Figure 3A) and the medulla (Figure 3B) as well as blood vessels (Figure 3C), as observed as the distribution of Kir6.1 protein. In the renal cortex, the glomerulus (Glo) expresses Kir6.1 at a weak level in the cells located within the Bowman’s capsule (Figure 3A). The proximal convoluted tubule (PCT), distal convoluted tubule (DCT), and collecting ducts express Kir6.1 moderately (Figure 3A, B). Sections treated with sense probes expressed no significant reactions (Figure 3D).

Under electron microscope, the punctate immunoreaction products for Kir6.1 observed by light microscopy were localized in the mitochondria in the renal tubular epithelial cells (Figure 4A, B). Some endoplasmic reticulum and small vesicles, as well as microvilli, were also observed to be immunopositive in the apical portion of epithelial cells (Figure 4A, B).

**Discussion**

Among various K⁺ channels, Kir6.1 is distinct because of its ATP sensitivity and its ubiquitous distribution in various cells and organs including kidney as detected with Northern blot and PCR analysis (Inagaki et al. 1995; Brochiero et al. 2002). A new polyclonal anti-Kir6.1 antibody was applied to investigate the detailed distribution of Kir6.1 protein in rat kidney. The specificity of the antibody was assessed by analyzing cells transfected with the corresponding gene and Western blot analysis. This antibody recognized a prominent 50 kDa band and a faint small band in COS-7 cells transfected with Kir6.1 and kidney extraction. The immunopositive bands were eliminated after pre-absorption with immunizing peptide antigen, further proved the specificity of the new anti-Kir6.1 antibody. The Kir6.1 protein was widely expressed in renal tubules both in cortex and medulla, which was confirmed by in situ hybridization with Kir6.1 cRNA probe. In addition, Kir6.1 was also expressed
in all tissues examined herein, such as heart, brain, liver and testis. The size differences of detected signals between heart (about 43 kDa) and other tissues (about 50 kDa) may be due to the post translational modifications in different tissues.

The results of the present study demonstrated that Kir6.1 protein not only localizes in the cell membrane but also in the mitochondrial and microsomal fractions of the kidney. The Kir6.1 is widely distributed in various renal tubular segments including glomerulus, proximal and distal tubules and collecting ducts by immunohistochemistry and in situ hybridization histochemistry. The subcellular localization of Kir6.1 is revealed in the mitochondrial and ER, and in the cell membrane by immunoelectron microscopy.

Previously, K$_{ATP}$ channels were shown to be expressed in the mitoplasts of the inner membrane of liver mitochondria (Inoue et al. 1991). Since then, two conflicting views have been proposed. Kir6.1 was localized in the mitochondria in skeletal muscles (Suzuki et al. 1997) and in neurons and glial cells of rat brain (Lacza et al. 2003a; Zhou et al. 1999), in the isolated mitochondrial fraction of mouse cardiomyocytes (Lacza et al. 2003b), and in the isolated ventricular myocytes (Singh et al. 2003), but some reports have claimed that there was no Kir6.1 in mitochondria with gene transfer technique (Seharaseyon et al. 2000) and immunoblotting analysis for cellular fractions (Kuniyasu et al. 2003). Focusing on these conflicting results, we recently observed that Kir6.1 was expressed in mitochondria in rat cardiomyocytes by immunoblotting analysis in cellular fractions and immunoelectron microscopy (Zhou et al. 2005). With the same antibody, Kir6.1 was also shown to be expressed in kidney cell fractions including mitochondria. Thus, Kir6.1 not only localizes in the mitochondria of cardiomyocytes but also in the mitochondria of renal tubular epithelial cells, regardless of the lower expression level of the Kir6.1 mRNA in kidney (Inagaki et al. 1995).

Recently, a new report claimed that Kir6.1 was not expressed in rat kidney by Western blotting (Sun et al. 2004). These investigators explained that the translation of Kir6.1 was limited in kidney,
but it may be up-regulated by hypoxic or ischemic stresses (Sun et al. 2004). We think that the negative reaction of kidney to anti-Kir6.1 antibody in these investigator's report may be due to the quantity of material applied for immunoblot, since the actin band detected in spleen was very heavy while those in liver and kidney were very light. If the applied concentration of sample was increased, the signal would be detectable in kidney by their anti-Kir6.1 antibody.

The observation of Kir6.1 in the kidney mitochondria in the present study could not deny its expression in the cell membrane, even though the detectable immunoreactivity is low. Previous studies with patch-clamp technique had shown that ATP-sensitive K+ channel was localized in the basolateral membrane of rabbit proximal convoluted tubule and the cortical thick ascending limb (Beck et al. 1993; Hurst et al. 1993; Hurst et al. 1992; Tsuchiya et al. 1992), and in the apical membrane of rat cortical collecting tubule and rabbit thick ascending limb of Henle's loop (Wang et al. 1990a; Wang et al. 1990b). These channels play important roles in kidney as 1) maintenance of cell-negative potential, 2) K+ recycling at the cell membrane, 3) K+ secretion and 4) cell volume regulation (Kawahara and Anzai 1997). It is well established that the Kir6.1 may not co-localize with Na+/K+ ATPase but will be regulated by a common cellular mechanism, which coordinates the epithelial Na+ transport and the basolateral Na+/K+ ATPase activity.

There is now convincing evidence that the mitochondrial volume is regulated by the balance between mitochondrial K\text{ATP} channel (mitoK\text{ATP}) and the K+/H+ antiporter, because the K+ cycle across the inner membrane consists of K+ influx through the mitoK\text{ATP} and electroneutral K+ efflux by K+/H+ antiporter. The steady-state volume of the mitochondrial matrix is therefore maintained by the secondary active, energy consuming process of a K+/H+ antiporter (Garlid 1994), and mitochondrial K+ cycle, while in turn, volume appeared to play a key role in regulating cellular bioenergetics, such as activity of the electron transport chain (Garlid 1996). Thus, our finding of an intracellular localization of Kir6.1 protein in mitochondria may support the hypothesis that mitoK\text{ATP} may play a key signal role in providing an energy during epithelial ion transportation.

In conclusion, Kir6.1 was verified to exist in the kidney in various renal tubular epithelial cells, mesangial cells and podocytes of glomeruli, and endothelium and smooth muscles of renal blood vessels. The subcellular localization was mainly in the mitochondria. Although only weak immunoreaction was observed in the cell membrane of renal epithelial cells in this study, the membrane K\text{ATP} channel plays roles in the connection of membrane potential and cell metabolism no matter how low its level of expression. The Kir6.1 may be a molecular member of membrane K\text{ATP} channel in kidney, and it may also be a candidate for a mitochondrial K\text{ATP} channel as well.

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