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Protein expression and gene mutation status of *KIT* and *PDGFRA* in renal cell carcinoma

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Summary. The author investigated protein expression and gene mutations of *KIT* and *PDGFRA* in 61 consecutive surgical cases of renal cell carcinoma (RCC). The cases of RCC consisted of 43 clear cell RCC (CCRCC), 9 chromophobe RCC (ChrRCC), or 9 papillary RCC (PaRCC). Normal distribution of KIT and PDGFRA protein was also examined in non-tumorous normal parenchyma (n=10). In normal kidneys, KIT was expressed in distal convoluted tubules and collecting ducts, and PDGFRA in distal and proximal convoluted tubules and collecting ducts. KIT expression was recognized in 9 ChrRCC (100%, 9/9), but not in 43 CCRCC (0%, 0/43) and 9 PaRCC (0%, 0/9). PDGFRA expression was recognized in 7 CCRCC (16%, 7/43) and 2 PaRCC (28%, 2/9), but not in ChrRCC (0%, 0/9). A molecular genetic analysis using PCR-direct sequencing was performed in selected 30 cases (ChrRCC=9, CCRCC=12, PaRCC=9): it revealed no mutations in *KIT* (exons 9, 11, 13, and 17) or *PDGFRA* (exons 12 and 18) genes in any cases examined. These results suggest that in normal renal parenchyma KIT is expressed in distal convoluted tubules and collecting ducts, and PDGFRA in proximal and distal convoluted tubules and collecting ducts, that KIT is expressed exclusively in ChrRCC and its incidence is 100%, that KIT-positive ChrRCC was negative for PDGFRA, that PDGFRA is expressed in a small percentage in CCRCC and PaRCC, and that mutations of *KIT* (exons 9, 11, 13, and 17) and *PDGFRA* (exons 12 and 18) are absent in RCC.

Key words: Renal cell carcinoma, *KIT, PDGFRA*

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Introduction

KIT and *platelet-derived growth factor receptor-α (PDGFRA)* genes, both mapped to 4q12, encode receptor tyrosine kinase oncoproteins called KIT (CD117) and PDGFRA, respectively (Miettinen and Lasota, 2005; Hirota and Isozaki, 2006). Both molecules are transmembranous oncoproteins involved in tumorigenesis, particularly in gastrointestinal stromal tumor (GIST) (Miettinen and Lasota, 2005; Hirota and Isozaki, 2006). *KIT* and *PDGFRA* genes are frequently mutated in some neoplasms such as GIST and germ cell tumors of the testis and ovary (Miettinen and Lasota 2005; Hirota and Isozaki, 2006).

Chromophobe renal cell carcinoma (ChrRCC), renal oncocytoma, and renal angiomyolipoma are known to frequently express KIT protein (Makhlouf et al., 2002; Sulzbacher et al., 2003; Lin et al., 2004; Miliara et al., 2004; Pan et al., 2004; Huo et al., 2005; Kato et al., 2005; Kruger et al., 2005; Petit et al., 2005; Sihto et al., 2005; Wang and Mills 2005; Sengupta et al., 2006; Liu et al., 2007; Memeo et al., 2007). KIT mutations in renal cell carcinoma (RCC) are controversial: several reports reported no *KIT* mutations in RCC (Miliara et al., 2004; Pan et al., 2004; Kruger et al., 2005; Petit et al., 2005; Sihto et al., 2005; Sengupta et al., 2006). while one report showed KIT mutations in RCC (Lin et al., 2004). With regard to PDGFRA, little is known about PDGFRA expression and *PDGFRA* mutations in RCC. In addition, there are only a few studies of normal distribution of KIT and PDGFRA (Miliaras et al., 2004; Kato et al., 2005).

The author investigated normal distribution of KIT and PDGFRA in normal kidneys, and protein expression and gene mutational status of *KIT* (exons 9, 11, 13, and 17) and *PDGFRA* (exons 12 and 18) in 61 RCC.

Materials and methods

The author retrieved 61 consecutive nephrectomy kidneys of RCC from archival surgical files of the last 17 years in our hospital. The mean age of the patients with RCC was 67.4±7.6 years, and male to female ratio was 43:18. The 61 cases of RCC consisted of 43 clear cell RCC (CCRCC), 9 ChrRCC, and 9 papillary RCC (PaRCC). The criteria for differential diagnosis of RCC subtypes was adopted according to the WHO blue book (Eble et al., 2004), and the 61 nephrectomy kidneys of RCC were re-evaluated. Several sections were obtained from the tumor and non-tumorous parenchyma in each case.

The sections were fixed in 10% formalin and embedded in paraffin blocks. Several 3-μm sections were cut, and one of them was stained with hematoxylin and eosin. Colloidal iron stain was employed in the ChrRCC cases. The remaining sections were immunohistochemically examined, using the Dako's Envision method as previously reported (Terada et al., 2002; Terada and Kawaguchi, 2005), for KIT (Dako, Glostrup, Denmark) and PDGFRA (Santa Cruz, CA, USA). In the ChrRCC cases, immunostainings of cytoketatins (AE1/3, CAM5.2, and cytokeratin no.7) AE1/3, Dako Corp, Glustrup, Denmark; CAM5.2 Beckton-Dickinson, CA, USA; CK7, clone N1626, Dako Corp) were performed.

A molecular genetic analysis of *KIT* gene (exons 9, 11, 13, and 17) and PDGFRA gene (exons 12 and 18) was performed by the PCR direct sequencing method, as previously reported (Terada 2008, 2009a-c, 2010, 2011), in 30 selected cases (ChrRCC=9, CCRCC=12, PaRCC=9). The exons of both genes were selected because they are frequent mutation sites in GIST and other tumors (Miettinen and Lasota, 2005; Hirota and Isozaki, 2006). In brief, genomic DNA was extracted from paraffin blocks with proteinase K digestion and phenol/chloroform extraction, and subjected to PCR for 40 cycles (94°C for one minute, 52°C for one minute, 72°C for one minute), using a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, ABI, CA). The primers are shown in Table 1. The annealing temperature was 53°C. PCR products were extracted and subjected to a computed automatic DNA sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems, ABI, CA). Two cases of gastric GISTs were used as positive controls, and two uterine leiomyomas as negative controls.

Results

Normal kidney parenchyma

Normal non-tumorous parenchyma of ten cases was

Table 1. Primer sequence.

	Forward	Reverse
KIT exon 9	5'-TCC TAG AGT AAG CCA GGG CTT-3'	5'-TGG TAG ACA GAG CCT AAA CAT CC-3'
KIT exon11	5'-GAT CTA TTT TTC CCT TTC TC-3'	5'AGC CCC TGT TTC ATA CTG AC-3'
KIT exon 13	5'-GCT TGA CAT CAG TTT GCC AG -3'	5'-AAA GGC AGC TTG GAC ACG GCT TTA-3'
KIT exon 17	5'-CTC CTC CAA CCT AAT AGT GT-3'	5'-GTC AAG CAG AGA ATG GGT AC-3'
PDGFRA exon12	5'-TTG GAT ATT CAC CAG TTA CCT GTC-3'	5'-CAA GGG AAA AGC TCT TGG-3'
PDGFRA exon 18	5'-ACC ATG GAT CAG CCA GTC TT-3'	5'-TGA AGG AGG ATG AGC CTG ACC-3'

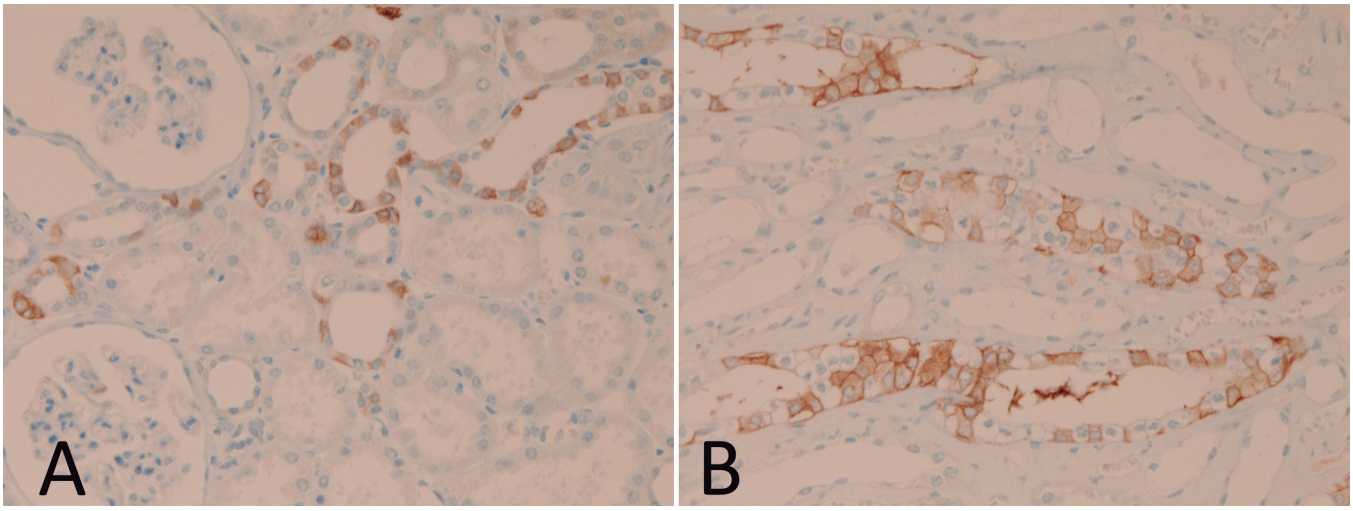

Fig. 1. A. Distal convoluted tubules are positive for KIT. KIT immunostaining. **B.** Collecting ducts are positive for KIT. KIT immunostaining. x 200

studied. KIT was expressed in distal convoluted tubules (Fig. 1A) and collecting ducts (Fig. 1B). PDGFRA was expressed mildly in the proximal and distal convoluted tubules and collecting ducts.

Renal cell carcinoma

In the 61 cases of RCC, KIT expression was recognized only in ChrRCC (9/9, 100%) (Fig. 2A,B). All cases of ChrRCC were positive for colloidal iron (Fig. 2C) and cytokeratins (AE1/3, CAM5.2, and CK7) (Fig. 2D). KIT expression was absent in CCRCC (0%, 0/43) and PaRCC (0%, 0/9). PDGFRA expression was not recognized in ChrRCC (0%, 0/9) (Fig. 3A), but it was noted, in small percentage, in CCRCC (16%, 7/43) (Fig. 3B) and PaRCC (22%, 2/9) (Fig. 3C).

Mutations of *KIT* gene (exons 9, 11, 13, and 17) and PDGFRA gene (exons 12 and 18) were absent in all the 30 cases. The positive control of gastric GISTs showed a point mutation of *KIT*, and negative control of uterine leiomyomas showed no mutations of *KIT* and *PDGFRA*.

Discussion

In normal adult kidneys, there are only two studies of the distribution of KIT and PDGFRA (Miliaras et al., 2004; Kato et al., 2005) reported KIT expression was present in renal tubules. Kato et al. (2005) reported that KIT was especially expressed in distal nephrons. There have been no reports on PDGFRA distribution in normal adult kidneys. In the present study, KIT was especially expressed in distal convoluted tubules and collecting ducts, while PDFGRA in proximal and distal convoluted tubules and collecting ducts. Much more studies on normal distribution of KIT and PDGFRA in the kidney are required.

KIT is expressed in various tumors, including GIST, mast cell neoplasm, melanoma, germ cell tumor,

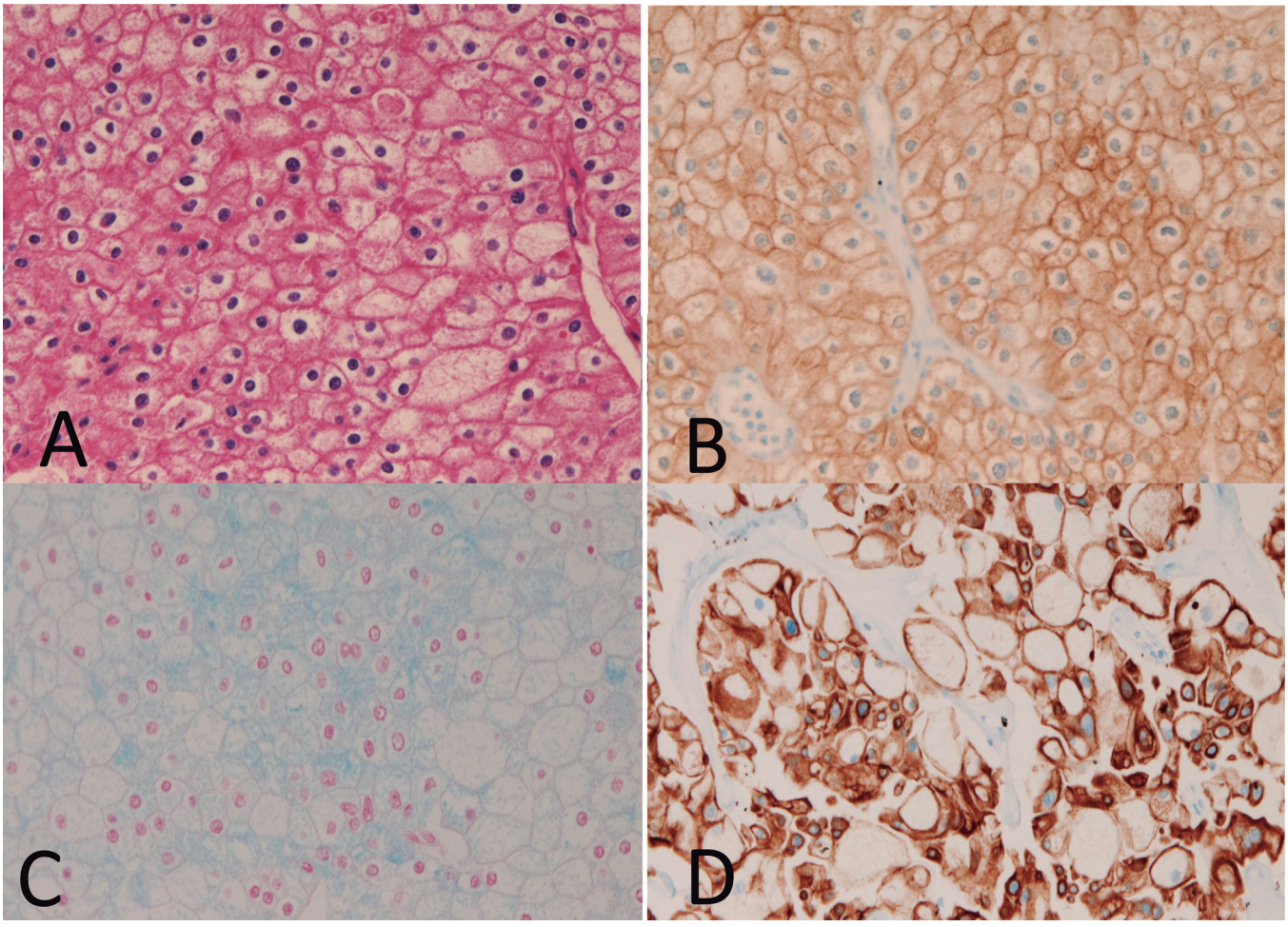

Fig. 2. A. HE morphology of chromophobe renal cell carcinoma. HE. **B.** Strong membranous expression of KIT in chromophobe renal cell carcinoma. KIT immunostaining. **C.** Colloidal iron is positive in chromophobe renal cell carcinoma. Hale's colloidal iron. **D.** Cytokeratin no.7 is positive in chromophobe renal cell carcinoma. Immunostaining. x 200

hematopoietic malignancies and small cell lung carcinoma (Miettinen and Lasota, 2005; Hirota and Isozaki, 2006). In RCC, it is well known that ChrRCC expresses KIT (Makhlouf et al., 2002; Sulzbacher et al., 2003; Miliara et al., 2004; Pan et al., 2004; Huo et al., 2005; Kruger et al., 2005; Petit et al., 2005; Wang and Mills, 2005; Sengupta et al., 2006; Liu et al., 2007; Memeo et al., 2007) Most studies reported that KIT is preferentially expressed in ChrRCC (Pan et al., 2004; Kruger et al., 2005; Wang and Mills, 2005; Liu et al., 2007). The incidence of KIT expression in ChrRCC ranges from 57% to 100%. However, there are a few reports in which CCRCC and PaRCC infrequently express KIT (Miliaras et al., 2004; Huo et al., 2005; Memeo et al., 2007), and one report showed 100% expression of KIT in PaRCC (Lin et al., 2004). In the present study, KIT was expressed preferentially in ChrRCC, and was negative in CCRCC and PaRCC. The incidence of KIT expression in ChrRCC was 100% in the present study, similar to the results of Kruger et al. (2005), Wang and Mills (2005), and Memeo et al. (2007). The present study suggests that KIT is expressed preferentially in ChrRCC with an incidence of 100%, and that ChrRCC arises from distal convoluted tubules which were positive for KIT.

Recently, imatinib, a targeting anti-KIT kinase inhibitor, is now used in GIST and certain other KIT-expressing tumors. Although the author does not know whether imanitib is now used in ChrRCC, imatinib is a potential molecular targeting agent (Stec et al., 2009). Since ChrRCC was positive for KIT in all cases in the current study, it appears that imatinib can be used for the therapy of ChrRCC, in particular in metastatic ChrRCC (Stec et al., 2009).

Expression of PDGFRA has rarely been reported in RCC. Sulzbacher et al. (2003) reported PDGFRA expression in CCRCC. In the present study, CCRCC and PaRCC expressed PDGFRA protein, although the incidence was low. In contrast, PDGFRA expression was absent in ChrRCC in the present study. The present

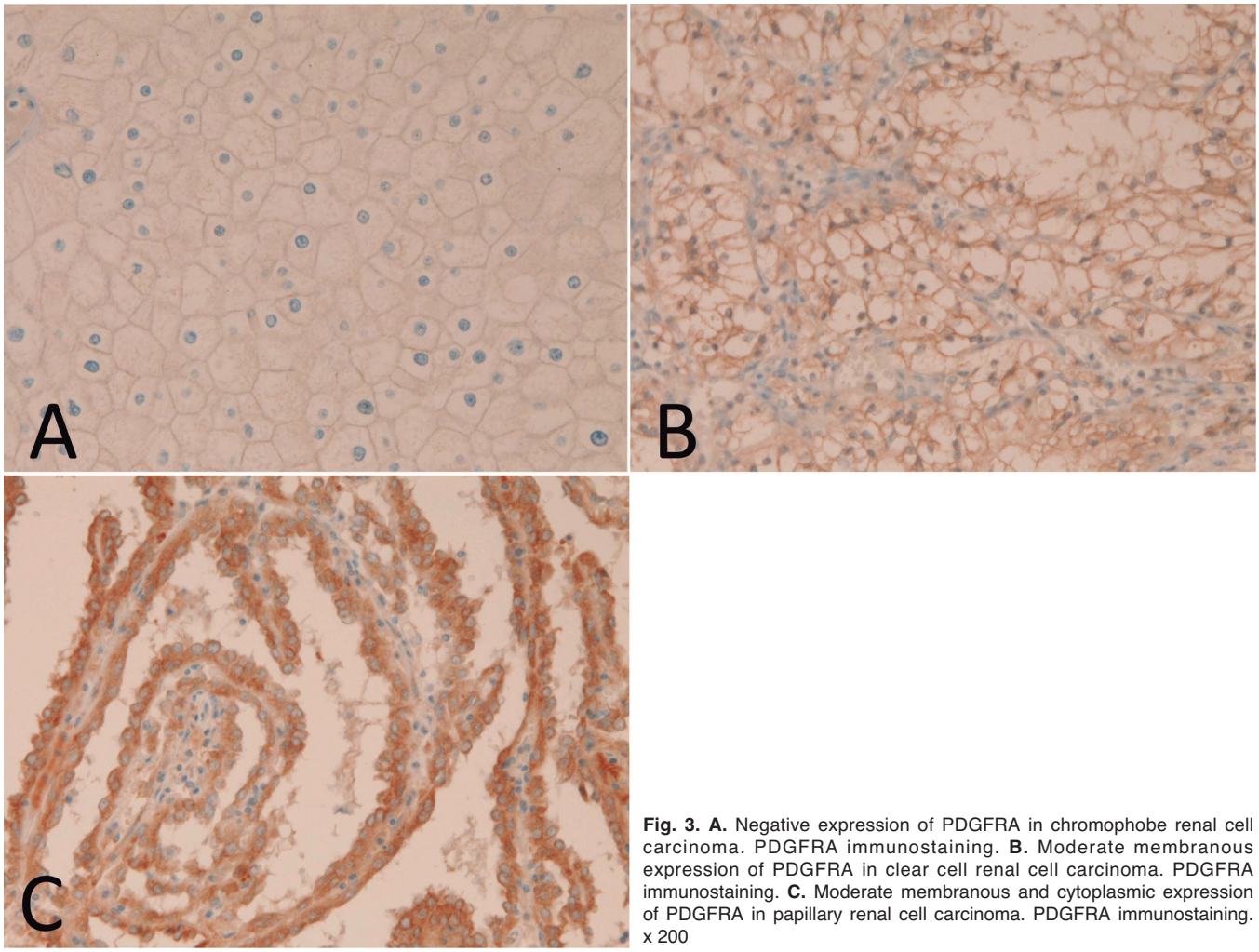

Fig. 3. A. Negative expression of PDGFRA in chromophobe renal cell carcinoma. PDGFRA immunostaining. **B.** Moderate membranous expression of PDGFRA in clear cell renal cell carcinoma. PDGFRA immunostaining. **C.** Moderate membranous and cytoplasmic expression of PDGFRA in papillary renal cell carcinoma. PDGFRA immunostaining. x 200

study suggests that CCRCC and PaRCC infrequently express PDGFRA protein and ChrRCC does not.

There have been several studies on *KIT* mutations in RCC. Lin et al. (2004) found point mutations in intron 17 of *KIT* gene in 94% of PaRCC. Sengupta et al. (2006), using PCR-direct sequencing, reported no *KIT* mutations in high grade RCC. Petit et al. (2005) reported no mutations of *KIT* (exons 9 and 11) in ChrRCC by the use of PCR-direct sequencing. Kato et al. (2005) also reported no *KIT* mutations, with the use of PCR-SSCP technique, in any types of RCC. Kruger et al. (2005) reported no mutations of exon 17 of *KIT* in RCC and oncocytoma. Pan et al. (2004) found no mutations of *KIT* (exons 9,11,13, and 17), by PCR-direct sequencing, in ChrRCC and oncocytoma. In the present study, no mutations of *KIT* (exons 9, 11, 13 and 17) were identified in the 30 RCC cases. Taken together, it can be concluded that RCC has no *KIT* mutations at least in exons 9, 11, 13, and 17. *KIT* expression without *KIT* mutations is thought to be due to *KIT* gene amplification (Sihto et al. 2005).

There are only two studies on *PDGFRA* mutations in RCC; Kato et al. (2005) showed no mutations of PDGFRA with the use of PCR-SSCP analysis. Sihto et al. (2005) reported, using PCR-direct sequencing, no mutations in exons 11 and 17 of PDGFRA gene in seven cases of RCC. In the present study, no *PDGFRA* mutations were recognized in any types of RCC, suggesting that *PDGFRA* mutations (exons 12 and 18) are absent in RCC.

Finally, it is very important to investigate the status of KIT phosphorylation and KIT downstream proteins in cases with KIT expression but without *KIT* mutations. However, this is beyond the scope of this paper. This important investigation is mandatory in order to understand KIT signaling and tumorigenesis.

In summary, the present study suggests that in normal renal parenchyma KIT is expressed in distal convoluted tubules and collecting ducts and PDGFRA in proximal and distal convoluted tubules and collecting ducts, that KIT is expressed exclusively in ChrRCC and its incidence is 100%, that KIT-positive ChrRCC was negative for PDGFRA, that PDGFRA is expressed in a small percentage in CCRCC and PaRCC, and that no mutations of *KIT* and *PDGFRA* are present in RCC.

The author declares no conflict of interest.

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