Evaluation of the KIT/Stem Cell Factor Axis in Renal Tumours

MARKUS HORSTMANN[1*], JOERG HENNENLOTTER[1], LISA M. GEIGER[1], ULRICH VOGEL[2], HEIDI SCHMID[3], URSULA KUEHS[1], ARNULF STENZL[1] and JENS BEDKE[1]

[1]Department of Urology, [2]Institute of Pathology and
[3]Interfaculty Institute for Biochemistry, Eberhard Karls University, Tuebingen, Germany

Abstract. *Aim: To investigate the expression of the KIT/stem cell factor (SCF) axis in different renal cell carcinoma subtypes with regard to targeted therapies. Materials and Methods: The expression of KIT and SCF were immunhistochemically assessed in 40 clear cell (ccRCC), 25 papillary (pRCC) and 19 chromophobe carcinomas (chRCC); 27 oncocytomas and 32 benign kidney parenchyma specimens differentiated into distal tubules (DT) and proximal tubules (PT). Results: The expression of KIT was significantly higher in chRCC and oncocytoma compared to ccRCC and pRCC. All tumours exhibited a significant increase of membranous to cytoplasmic KIT expression, with the highest in ccRCC and pRCCs. SCF was expressed in all tumour subgroups, with the highest in oncocytomas and pRCC. SCF correlated positively with the cytoplasmic expression of KIT. A higher tumour stage correlated to lower KIT expression in ccRCC. Conclusion: Simultaneous expression of SCF and KIT in renal tumours, which seems to undergo a shift from the cytoplasm to the cell membrane, suggests paracrine and autocrine mechanisms in KIT activation, with different, as yet unknown, regulatory mechanisms in the different tumour entities.*

Renal cell cancer (RCC) is the seventh most common malignancy in men and the ninth in women. In Europe an estimated 88,400 new patients were diagnosed with this disease in 2008 (1). In comparison to other urological malignancies, renal cell cancer represents the tumour entity, with the highest death rate. During the past decades, knowledge on molecular biology of renal tumours has increased tremendously. This has led to the development of several new targeted therapies that have opened-up a new era of treatment for patients with metastasized disease. One of the targets addressed but rarely described is KIT, also known as c-Kit or CD 117 (cluster of differentiation 117) which is a 145–150 kDa glycoprotein encoded by the *KIT* gene that is localized on chromosome 4 (4q11-q21). KIT is a transmembrane receptor tyrosine kinase that belongs to the family of class III receptors, including colony-stimulating factor 1 and platelet-derived growth factor (PDGF) (2).

The tyrosine kinase KIT is activated by its known ligand, stem cell factor (SCF), also known as KIT ligand, mast cell growth factor or steel-factor. SCF is a glycoprotein with a molecular weight ranging from 28 kDa to 40 kDa, encoded on chromosome 12q22-12q24 (3). Binding of SCF to KIT leads to the activation of the extracellular binding domain with subsequent receptor dimerization and phosphorylation of the intracellular kinase domains. Thereby the ligand pair SCF-KIT activates several signal transduction pathways including the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway, the phosphoinositide 3-kinase inhibitor (PI3K), protein kinase B (AKT) and mammalian target of rapamycin (mTOR) pathway and the rat sarcoma oncogene (RAS), rapidly accelerated fribrosarcoma (RAF), extracellular-signal-regulated kinases (ERK) and mitogen-activated protein kinases (MAPK) pathway. It furthermore interacts with the regulatory subunit p85 of phosphoinositide-3 kinase (PI3K) (4) and MAPK which consequently trigger downstream pathway activation (5). In cellular homeostasis in health, KIT activation is essential as complete loss of KIT function in mice results in death *in utero* or perinatally. Loss of function due to mutation or due to a heterozygous state results in anaemia and reduced fertility (6). In health, the receptor ligand pair SCF-KIT plays an essential role in the development, maintenance and proliferation of several tissues and cell systems. It is considered essential in gametogenesis (6), melanogenesis and early stages of haematopoiesis (8). *In vitro* and *in vivo*, SCF can stimulate the proliferation of mature and immature mast cells. In

**Present address:* Department of Urology, Kantonsspital Winterthur, Brauerstr. 15, 8401 Winterthur, Switzerland.

Correspondence to: Dr. med. Marcus Horstmann, Department of Urology, Kantonsspital Winterthur Brauerstr. 15, 8401 Winterthur, Switzerland. E-mail: marcushorstmann@gmx.ch and PD. Dr. med. Jens Bedke, Department of Urology, University of Tuebingen, Hoppe-Seyler-Str. 3, 72070 Tuebingen, Germany. E-mail: bedke@live.com

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Table I. *Patients' characteristics*.

	ccRCC	pRCC	chRCC	Oncocytoma
Patients (male/female), n	40 (22/18)	25 (18/7)	19 (10/9)	27 (15/12)
Mean age, years	61±2.0	68±2.3	59±3.0	64±2.9
Mean tumour size (range), cm	4.8 (1.7-12.5)	4.7 (1.8-13)	4.8 (1.3-12)	3.4 (1.0-7.8)
T 1 (a/b)	24 (15/9)	19 (13/6)	14 (8/6)	
T2	0	1	0	
T3 (a/b/c)	16 (4/10/2)	5 (3/2/0)	5 (2/3/0)	
N +	4	3	2	
M +	8	4	2	
G1	6	4	2	
G2	27	18	16	
G3	7	3	1	

T: n; N+: n; M+: n; G: n.

malignant tumours, KIT activation or mutations are involved in the progression of several types of cancer including germ cell tumours (9), bronchial cancer (10) or gastrointestinal stromal tumours (GIST) (11-14). For kidney cancer, several studies have described the expression pattern of KIT among different renal tumour entities. In chromophobe renal cell carcinoma (chRCC) and oncocytoma, KIT is overexpressed (15-18). This fact led to the postulation that KIT might serve as a marker to differentiate chRCC from papillary (pRCC) or clear cell renal cell carcinoma (ccRCC) as the latter two usually exhibit a reduced KIT expression.

In renal disease, the simultaneous protein expression of SCF and KIT has been described for mast cell-associated inflammation in glomerulonephritis (19) and for mRNA expression by polymerase chain reaction (PCR) for chRCC (20). A detailed analysis of SCF and KIT expression among the different renal tumour entities has, to our knowledge, not yet been performed. Several multi-tyrosine kinase inhibitors, including sorafenib, sunitinib and pazopanib, are used in the medical treatment of metastatic RCC inhibit KIT. A detailed analysis of the SCF-KIT axis would give insight into the biological role of this axis in renal cell tumours.

Therefore, the aim of this study was to analyse the SCF and KIT expression in specimens of clear cell, papillary and chromophobe RCC in addition to benign oncocytoma and benign renal parenchyma.

Materials and Methods

Expression of SCF and KIT was determined in 111 renal tumour specimens using an immunohistochemical approach and the tissue microarray technique (TMA). Tumour specimens were collected at the University of Tuebingen between 2002 and 2007. They included 40 ccRCCs, 25 pRCCs, 19 chRCCs and 27 oncocytomas. Reference data were obtained from 32 specimens of normal renal parenchyma adjacent to tumours. These specimens were evaluated separately for epithelial cells of the distal tubule (DT) and the proximal tubule (PT). The study was approved by the Ethics Committee of the University of Tuebingen (approval no. 292/2008B01).

Patients' characteristics. The study included 111 (65 male and 46 female) patients. Their mean age was 63±1 years (range=18–91 years) at the time of surgery. The mean age of patients with ccRCC, pRCC, chRCC and oncocytoma was 61±2.0, 68±2.3, 59±3.0 and 64±2.9 years, respectively. All tumours were classified histopathologically according to the WHO classification (21) and staged according to the TNM system of 2002 (22). Tumours had been surgically resected either by open or laparoscopic, radical or partial nephrectomy (Table I).

TMA. Specimens were cut into square sections of 6 mm in length, formalin fixed (pH 7; Roti-Histofix, Roth, Germany), dehydrated and paraffin embedded for TMA preparation. After a primary evaluation of HE-stained slides, the TMA was constructed as previously described (23-24). A total of 2-4 cores (diameter 1 mm) were taken from each tumour with the same number of cores being taken from benign tissue.

Immunohistochemistry. TMA sections were de-paraffinized, rehydrated and immersed in 3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was accomplished by microwave heating in Dako S1700 Buffer Solution for KIT and in a Dako Target Retrieval Solution S1700 for SCF (Dako, Carpinteria, CA, USA) at 750 watts for 15 min. KIT expression was detected by a commercially polyclonal rabbit antibody to human CD117 antibody (Dako) and SCF expression by a sc-13126 mouse monoclonal antibody IgG2b (Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA). After 12 h of incubation at 4˚C the sections were washed in a buffer solution and incubated with secondary biotinylated antibodies (KIT: rabbit IgG AK, BA-1400; Linaris, Wertheim, Germany. SCF: anti-mouse IgG; Dako). The ABC system with DAB (Vector Laboratories, Burlingame, CA, USA) was used for visualization according to the manufacturer's instructions. Sections were briefly rinsed in distilled water, counterstained with Mayer's haematoxylin solution and mounted. For negative control, the primary antibody was replaced by non-immune serum.

Immunoreactive staining score. All TMA staining was evaluated digitally. TMA slides were scanned using Mirax Scan program

Table II. *Results of immunohistochemical staining for KIT and Stem Cell Factor (SCF) in 32 normal renal tissue specimen and 111 epithelial renal tumours*

	Membranous expression		Cytoplasmic expression	
KIT	Positive staining (%)	Mean IRS±SEM (range)	Positive staining (%)	Mean IRS±SEM (range)
DT	0	0.00±0 (0)	98	11.27±0.26 (0-12)
PT	0	0.00±0 (0)	100	8.90±0.28 (6-12)
ccRCC	10	0.33±0.23 (0-9)	57	1.29±0.23 (0-4.5)
pRCC	36	0.88±0.33 (0-6.25)	96	4.56±0.79 (0-12)
chRCC	100	7.69±0.76 (2-12)	100	6.04±0.53 (3-10)
Oncocytoma	92	6.75±0.64 (0-12)	100	9.18±0.37 (5-12)
SCF				
DT	5	0.67±0.46 (0-8)	80	4.54±0.72 (0-12)
PT	5	0.19±0.19 (0-4)	5	0.38±0.38 (0-8)
	Membranous and Cytoplasmic			
ccRCC	100	2.65±0.22 (0.6-8.0)		
pRCC	100	4.42±0.19 (3.4-7)		
chRCC	100	3.02±0.27 (1.2-5)		
Oncocytoma	100	4.48±0.22 (4-8)		

(Zeiss, Jena, Germany) and cores were visualized using the ACD See program (ACD Systems International Inc., Victoria, BC, Canada). All samples were evaluated by three independent investigators for membranous (m) and cytoplasmic (c) staining according to the immunoreactive staining score (IRS), described by Remmele *et al*. (25). To establish the IRS, the percentage of positively stained cells was first evaluated by using a 0-4 scoring system: 0% of positive cells resulted in a score of 0, fewer than 10% in a score of 1, 10-50% in score of 2, 51-80% in score of 3 and >80% in score of 4. Staining intensity was evaluated on a gradual scale ranging from 0-3 (negative/weak/moderate/strong). For the final IRS, the scores of intensity and staining were multiplied and the mean value per patient was calculated. Additionally, the percentage of specimen with no staining *vs*. that with positive staining was reported.

Statistical analysis. IRS was compared between RCC subtypes, oncocytomas and benign kidney tissue (PT and DT) and further correlated to tumour characteristics (T, N, M stage; tumour grading; and size) using linear regression analysis and Wilcoxon or Kruskal–Wallis tests with *post hoc* Tukey–Kramer analysis. A *p*-value of <0.05 was considered to show a significant difference (JMP version 8.0; SAS Inc., Cary, NC, USA). All data are presented as the mean±standard error of the mean (SEM) and percentage of positively stained cells.

Results

KIT and SCF expression in normal kidney tissue. In normal renal tissue, DT and PT exhibited no membranous expression of KIT while cytoplasmic staining was positive in nearly all DT (cIRS=11.27±0.26, 98%) and PT (cIRS=8.9±0.28, 100%; $p<0.05$, Table II). For SCF, membranous staining in DT (mIRS=0.67±0.46, 5%) and PT (mIRS=0.19±0.19, 5%) was weak. Cytoplasmic SCF expression was significantly higher in DT (cIRS=4.54±0.72, 80%) than in PT (cIRS=0.38±0.38, 5%, $p<0.05$) and also when compared to membranous SCF expression (Table II, Figure 1).

KIT and SCF expression in RCC subtypes and oncocytoma. Membranous and cytoplasmic expression of KIT differed between the tumour entities. Membranous and cytoplasmic expression of KIT were significantly ($p<0.05$) higher in chRCC (mIRS=7.69±0.76; cIRS=6.04±0.53) and oncocytoma (mIRS=6.75±0.64; cIRS=9.18±0.37) than compared to ccRCC (mIRS=0.33±0.23; cIRS=1.29±0.23 and pRCC (mIRS=0.88±0.33; cIRS=4.56±0.79). Interestingly, compared to healthy tissues all tumors exhibited a significantly ($p<0.05$) higher membranous KIT expression, which was the highest in ccRCCs and pRCCs compared to the increase in chRCCs and oncocytomas.

SCF was expressed in all tumour subgroups and in every tumour analysed. A distinctive evaluation between the cell compartments of the cytoplasm and the cell membrane was not possible, as all tumours exhibited homogenous membranous and cytoplasmic staining. The highest SCF expression was detected in oncocytomas (IRS=4.48±0.22), followed by pRCCs (IRS=4.42±0.19). These expression levels were significantly ($p<0.05$) higher than those in ccRCCs (IRS=2.65±0.22) and chRCCs (IRS=3.02±0.27) (Table II, Figure 1)

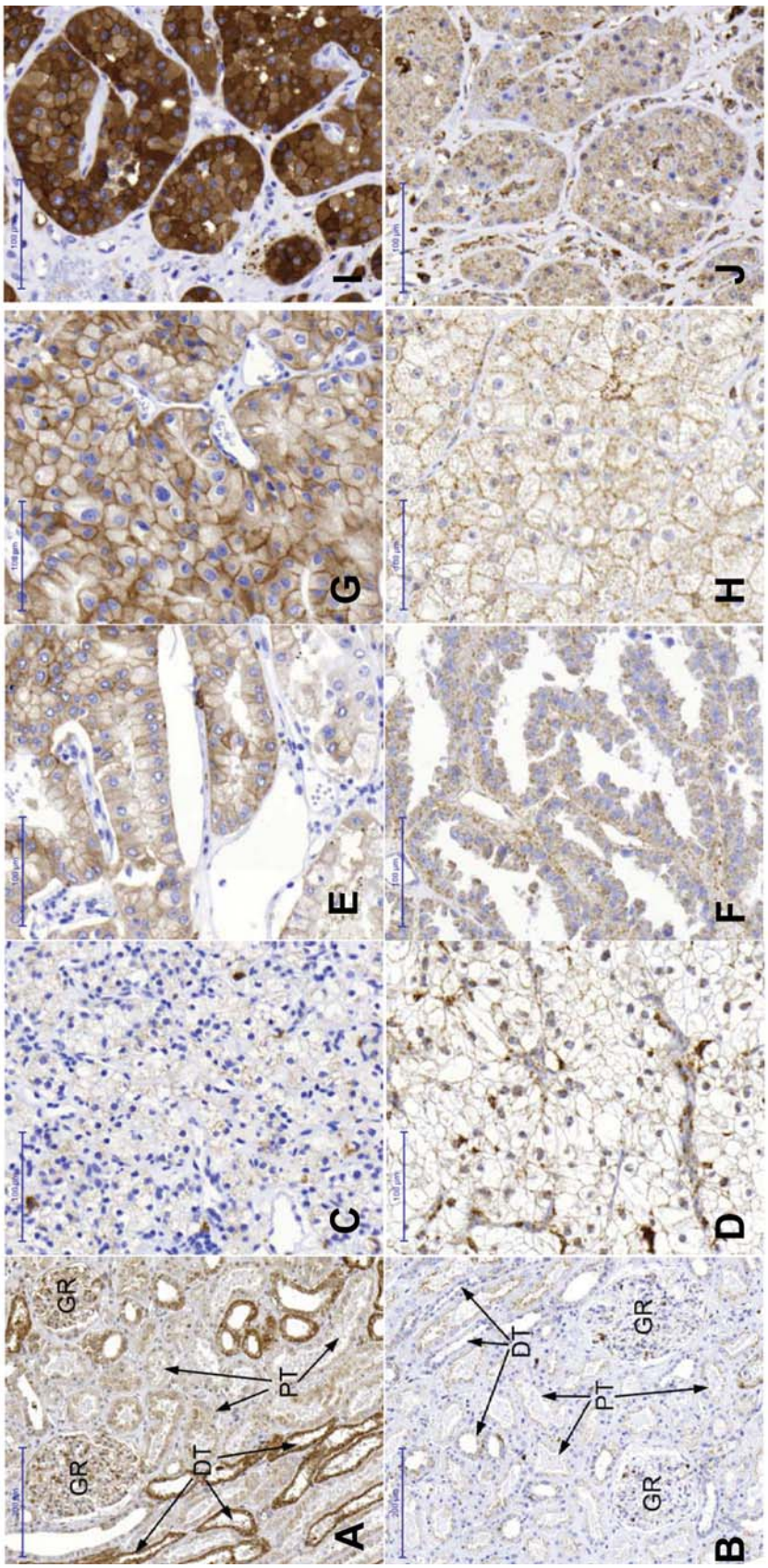

Figure 1. *KIT and stem cell factor (SCF) staining of renal tissues. A: benign renal tissue: proximal tubules (PT) with no membranous, but cytoplasmic staining; distal tubules (DT) with no membranous, but strong cytoplasmic staining for KIT; GR: glomerulus. B: benign renal tissue: PT with weak membranous and cytoplasmic staining for SCF. DT with weak membranous and moderate cytoplasmic staining for SCF. C: clear cell renal cell cancer (ccRCC) with focal weak membranous and weak cytoplasmic staining for KIT. D: ccRCC with minimal weak membranous and cytoplasmic staining for SCF. E: papillary RCC (pRCC) with weak membranous and moderated cytoplasmic staining for KIT. F: pRCC with moderate membranous and cytoplasmic staining for SCF. G: chromophobe (chRCC) with strong membranous and cytoplasmic staining for KIT. H: chRCC with weak to moderate membranous and cytoplasmic staining for SCF. I: Oncocytoma with strong membranous and cytoplasmic staining for KIT. J: Oncocytoma with moderate membranous and cytoplasmic staining for SCF.*

For a further analysis of any potential correlation between KIT and SCF, a regression analysis was performed. SCF expression correlated positively with the cytoplasmic expression of KIT in all tumour specimens ($p<0.01$), but no correlation to membranous KIT was detected.

Correlation to tumour stage, grade and size. In the correlation to clinicopathological data, patients with ccRCC with a higher tumour stage (pT3) exhibited a significantly lower expression of cytoplasmic KIT compared to patients with pT1 tumours (T1: cIRS=1.75±0.34 *vs*. T3: cIRS=0.59±0.17, $p<0.05$). There were no further significant clinicopathological correlations between KIT or SCF expression.

Discussion

Several studies have examined the expression of KIT in renal tumors (15-18, 26). Although the results are not totally consistent due to the different antibodies used in the immunohistochemical detection systems, the majority of studies demonstrated an increased expression of KIT in chRCC and oncocytoma compared to ccRCC and pRCC. This result is confirmed by the present study, which further differentiated KIT expression into cytoplasmic and membranous localization with positive cytoplasmic staining in all tumour subtypes and with a significantly higher expression in oncocytomas and chRCCs compared to ccRCCs and pRCCs. Whereas some authors described no expression of KIT in ccRCCs and pRCCs (16-18) the present study revealed at least a weak membranous and also cytoplasmic KIT staining reaction in these tumours. Similar results were described by Huo *et al*., Miliaras *et al*. and Ahmed *et al*. who also described a weak, but still present KIT expression in ccRCC and pRCC (15, 26, 27). Interpreting these differences, one has to take into account that different antibody staining procedures and staining interpretations were used. Some authors interpreted a weak staining as a negative result, or doubted the biological significance of cytoplasmic staining since active KIT is located on the cell membranes.

SCF expression was detected in all tumours analysed and expression was significantly higher in chRCC and oncocytoma compared to ccRCC and pRCC. The positive correlation of high SCF expression with the cytoplasmic expression of KIT suggests the release of SCF by the tumour cells themselves, which then in turn activates KIT *via* an autocrine or paracrine feedback-loop within the tumour microenvironment.

In normal renal tissues, the expression of KIT and SCF was predominately cytoplasmic, with low to no membranous expression. This might describe a ready-to-use system in which cytoplasmic SCF is released and binds in an autocrine or paracrine way to KIT, which is then shifted from the cytoplasm to the cell membrane. There, KIT can be activated by SCF in its growth receptor function in order to drive cell proliferation and thereby preserve tissue integration. In the renal tumors of oncocytoma and chRCC, cytoplasmic KIT expression was lower in benign tissue, but membranous expression in these tumour entities was higher than that in benign kidney parenchyma. The shift of KIT from the cytoplasm to the cell membrane might enhance cellular proliferation of these tumours. On the other hand, ccRCC and pRCC expressed less KIT and SCF than chRCC and oncocytoma. Additionally the loss of KIT expression correlated with a higher tumour stage (pT1 *vs*. pT3) in ccRCC. Obviously there are differences between the analysed tumour entities. Interestingly, in melanoma, loss of KIT expression seems to be associated with the progression of certain forms (28) and approximately 70% of melanoma metastases and human cell lines do not express KIT (28-30). In addition, exposure of KIT positive cells to SCF did not solely trigger cell proliferation, but in contrast, induced apoptosis (28). Therefore loss of KIT expression in higher ccRCC stages could serve as an escape mechanism to allow for tumour growth and subsequent metastasis.

Compared to other tumour entities, the expression of KIT cannot directly be regarded as a functional parameter of renal tumour progression and tumourigenesis as there are loss-of-function or gain-of-function mutations (28, 31). For example, in gastrointestinal stromal tumours, which are regarded as a perfect solid tumour model for KIT-driven tumour cell proliferation, a variety of activating KIT mutations has been described (28). In chRCC, such activating mutations have not been described (32, 33), only in pRCC was a specific KIT *intron 17* point mutation detected (34). Therefore a higher KIT expression in chRCC and oncocytoma cannot be seen automatically as a marker of increased cell activation and enhanced tumourigenesis.

Growth factor activated tyrosine kinases are the primary target in the treatment of metastatic RCC (mRCC) inhibited by tyrosine kinase inhibitors (35). With the development of the latter, KIT has also become a potential target in RCC and several phase I/II studies with the inhibitor imatinib have been performed in mRCC. None of theses studies showed any patient benefit, and imatinib is no longer applied for mRCC (36-37). One important reason for its inefficacy is the low expression of KIT in patients with ccRCC, as shown in this study, who were the main study population examined. Another reason is that imatinib inhibits gain-of-function mutations in the juxtamembrane domaine region (JMD) of KIT, which are lacking in RCC, but are present in gastrointestinal stromal tumours. The tyrosine kinase inhibitors sunitinib, pazopanib and sorafenib, which are recommended in the first- and second-line treatment of mRCC, include KIT in addition to the commonly known targets vascular endothelial growth factor receptor 1-3

(VEGFR1-3), PDGF receptors α/β and Fms-like tyrosine kinase 3 (Flt-3) in their multitarget panel. Here, sunitinib has the lowest inhibitory concentrations (IC_{50}) for KIT compared to pazopanib and sorafenib (38). The higher expression of KIT in chRCC might be one reason for the promising results of sunitinib in metastatic chRCC (39).

In conclusion, the simultaneous expression of SCF and KIT in renal tumours suggests paracrine and autocrine mechanisms in KIT activation, which seems to undergo a shift from the cytoplasm to the cell membrane. On the other hand, the different expression levels of KIT in benign renal epithelium, oncocytoma and chRCC compared to ccRCC and pRCC suggest different effector functions of KIT with regard to cellular proliferation and apoptosis, which are currently not totally known and need to be further elucidated.

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