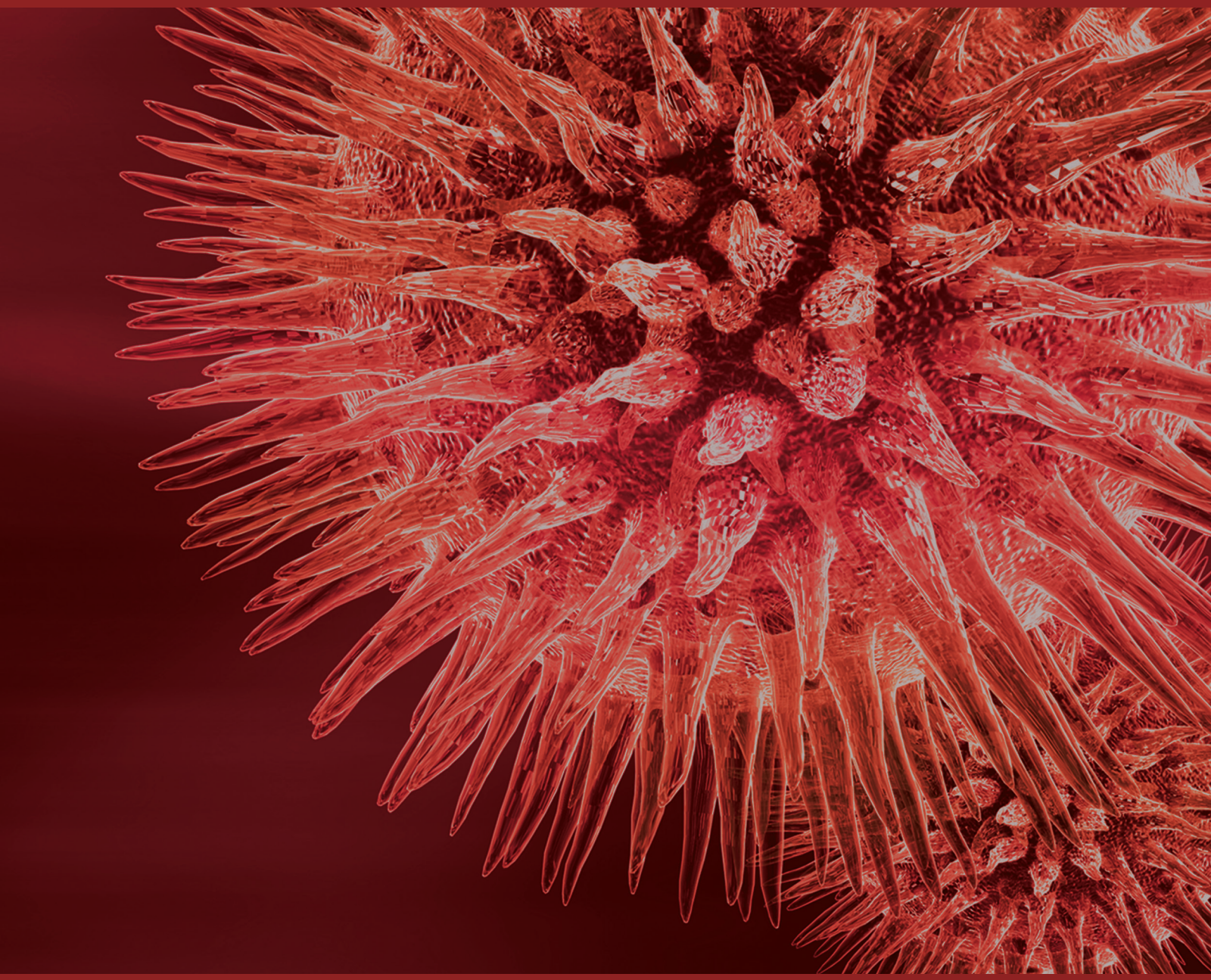
BioMed Research International

Recent Developments in Kidney Cancer: Biomarkers, Mechanism, and Treatment

Guest Editors: Xiaoping Zhang, Haifeng Yang, Adam S. Feldman, and Kong Chuize



Recent Developments in Kidney Cancer: Biomarkers, Mechanism, and Treatment

BioMed Research International

Recent Developments in Kidney Cancer: Biomarkers, Mechanism, and Treatment

Guest Editors: Xiaoping Zhang, Haifeng Yang, Adam S. Feldman, and Kong Chuize

Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 730390, 8 pages
http://dx.doi.org/10.1155/2015/730390

Research Article

Expression of Translationally Controlled Tumor Protein in Human Kidney and in Renal Cell Carcinoma

Maria R. Ambrosio,[1] Bruno J. Rocca,[1,2] Aurora Barone,[1] Monica Onorati,[1,3] Lucia Mundo,[1] Filippo Crivelli,[2] Franca Di Nuovo,[3] Giulia De Falco,[1,4] Maria T. del Vecchio,[5] Sergio A. Tripodi,[6] and Piero Tosi[1]

[1]*Section of Pathology, Department of Medical Biotechnology, University of Siena, Via delle Scotte 6, 53100 Siena, Italy*
[2]*Section of Pathology, Ospedale di Circolo di Busto Arsizio, Presidio Ospedaliero di Saronno, Piazzale Borella 1, 21047 Saronno, Italy*
[3]*Section of Pathology, Azienda Ospedaliera, "G. Salvini", Viale C. Forlanini 121, 20024 Garbagnate Milanese, Italy*
[4]*School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E14NS, UK*
[5]*Department of Medicine, Science and Neurosciences, University of Siena, Via delle Scotte 6, 53100 Siena, Italy*
[6]*Section of Pathology, Azienda Ospedaliera Universitaria Senese, Viale Bracci 16, 53100 Siena, Italy*

Correspondence should be addressed to Maria R. Ambrosio; maradot@libero.it

Received 25 February 2015; Revised 1 June 2015; Accepted 10 June 2015

Academic Editor: Kong Chuize

Translationally controlled tumor protein is a multifaceted protein involved in several physiological and biological functions. Its expression in normal kidney and in renal carcinomas, once corroborated by functional data, may add elements to elucidate renal physiology and carcinogenesis. In this study, translationally controlled tumor protein expression was evaluated by quantitative real time polymerase chain reaction and western blotting, and its localization was examined by immunohistochemistry on 84 nephrectomies for cancer. In normal kidney protein expression was found in the cytoplasm of proximal and distal tubular cells, in cells of the thick segment of the loop of Henle, and in urothelial cells of the pelvis. It was also detectable in cells of renal carcinoma with different pattern of localization (membranous and cytoplasmic) depending on tumor histotype. Our data may suggest an involvement of translationally controlled tumor protein in normal physiology and carcinogenesis. However, functional *in vitro* and *in vivo* studies are needed to verify this hypothesis.

1. Introduction

Translationally controlled tumor protein (TCTP) is a gene product ubiquitously expressed in all eukaryotes [1]. Its expression varies, depending on the cell type and developmental stage, with high level in mitotically active tissues and low level in resting cells [2]. In numerous experimental settings and biological systems, it has been demonstrated that TCTP expression is regulated by a wide range of extracellular signals and cellular conditions (growth factors, cytokines, proapoptotic/cytotoxic signals, and others), resulting in either upregulation or downregulation [3, 4]. Although the high degree of preservation and the abundance and the ubiquity of TCTP underline its critical role in the cell, the physiological functions of the protein are still poorly understood [1]. There is growing evidence that TCTP is a multifaceted protein associated with several different biological functions, such as development [5], cell cycle and division [6], cell proliferation and growth [7], cytoskeleton activity [8], chaperone-like activity [9], calcium binding [10], histamine release, and immune response [11]. In recent years attention has been focused on the possible role of TCTP in cancerogenesis [5]. Uncontrolled or promoted proliferation, loss of cell death and apoptosis, cell growth, and gene expression dysregulation are properties of tumor cells and all are influenced by TCTP activity [12–14]. Furthermore, TCTP has a crucial role in tumor reversion, a process by which some cancer cells lose their malignant phenotype [12]. TCTP

is expressed in more than 500 tissues and cell types [15–17]. It has been found in whole kidney lysates of embryonic mouse [2] and in rat urinary organs [18]. In contrast, the protein has never been previously detected in human kidney and in renal cell carcinomas, as also reported by the Swiss-Prot proteomic bank (http://www.ebi.ac.uk/swissprot) [19]. For this reason, in a previous study performed to evaluate the expression of TCTP in prostate gland, human kidney tissue was used as negative control [20]. Surprisingly, we observed TCTP expression in normal renal structures.

Based on this preliminary data the present study is aimed at

(i) confirming TCTP expression on a larger series of samples of normal kidneys and evaluating its pattern of expression in the different compartments of the kidney,

(ii) assessing TCTP expression in renal tumors.

2. Material and Methods

2.1. Patients. 84 nephrectomies for cancer were collected at the Section of Pathology of Siena University Hospital (Siena, Italy). Preliminarily, neoplastic and nonneoplastic areas were selected from each sample and examined by frozen section procedure to confirm the presence or absence of the tumor. One neoplastic and one nonneoplastic area from each nephrectomy were snap-frozen in liquid nitrogen and stored at −80°C until being used for evaluation of TCTP expression protein level by western blotting (WB). The remaining of each specimen followed the standard procedure for histological and immunohistochemical analysis and were used for TCTP mRNA detection by quantitative real-time polymerase chain reaction (RT-qPCR) after microdissection.

2.2. Ethics Statement. Ethical approval for this study was obtained by the Institutional Review Board of the University of Siena (Italy). Informed written consent was obtained in all cases.

2.3. Histology. Representative samples of tumors and of normal renal parenchyma were taken, fixed in 10% buffered formalin, and embedded in paraffin. From each block, 4 μm thick sections were cut and stained with haematoxylin and eosin. Tumor histotype, grade, and stage were established according to the last World Health Classification of tumors of urinary system and male genital organs [21] and to the updated International Society of Urologic Pathology (ISUP) grading system [22].

2.4. Detection of TCTP mRNA. Total RNA was extracted from cells isolated by microdissection from formalin-fixed, paraffin-embedded (FFPE) tissue blocks of normal (whole, medullary, and cortical regions) and both neoplastic benign and neoplastic malignant samples (oncocytoma, clear cell renal cell carcinoma, papillary carcinoma, chromophobe renal cell carcinoma, collecting duct carcinoma, and Wilm's tumor) using the RNA easy FFPE kit (Qiagen), following manufacturer's instructions. Reverse transcription was carried out using the QuantiTect Reverse Transcription Kit (Qiagen, CA). For each RNA specimen, a negative control was prepared by omitting the reverse transcriptase. The positive control was represented by placenta [20]. TCTP expression was analyzed in both normal and neoplastic samples by RT-qPCR, using TaqMan probes (Life Sciences, Applied Biosystems, CA) for TCTP and HPRT, used as housekeeping gene, according to the manufacturer's instructions. Relative quantification was calculated by the ΔΔCt method [23].

2.5. Western Blot Analysis. Frozen samples of renal tissue were reduced to small pieces with a razor blade and homogenized on ice three times (20 sec each) by a Polytron blender (Kinematica Lucerne, Switzerland) in lysis buffer (50 mM Tris-HCl, 5 mM magnesium acetate, 0.2% mM EDTA, 0.5 mM dithiothreitol, 10% (vol/vol) glycerol, and 0.2% (vol/vol) Triton X-100 (pH 7.5)) supplemented with a protease-inhibitor cocktail containing 4-(2-aminoethyl)benzenesulfonyl fluoride, pepstatin A, E64, bestatin, leupeptin, and aprotinin (Sigma Chemical Co.). Tissue homogenates were centrifuged at 750 ×g for 10 min at 4°C and the supernatant was assayed for total protein content and stored at −80°C. Extracts were separated by a 12% SDS-polyacrylamide gel (SDS-PAGE). After electrophoresis, proteins were transferred to nitrocellulose filters (Hybond-C, Amersham Biosciences Corp., Piscataway, NJ) for 1 h on ice in transfer buffer (20 mM Tris (pH 8.3), 0.15 M NaCl, and 0.1% Triton X-100). Filter was incubated in blocking solution for a specific site saturation for 1 h, followed by ON incubation with primary antibody (1 : 500), at 4°C. Filter was washed with TBST 6 × 5′, and then secondary antibody conjugated to HRP was incubated for 1 h at RT. After 30′ washing, detection was then obtained by chemiluminescence using the ECL kit (Amersham Biosciences Corp.), according to the manufacturer's instructions. The differences were normalized with an anti-actin antibody (1 : 1000). Densitometric analysis was carried out using the ImageMaster TotalLab software (Amersham Biosciences Corp.). The positive control was represented by placenta, as in other similar studies [20].

2.6. Immunohistochemistry. The most representative tumor blocks were selected on the basis of the morphological features. Immunohistochemical staining was performed on 4 ± 0.5 μm thick sections of each block employing the Ultravision Detection System Antipolyvalent HRP (Ultra V Block) (LabVision, Fremont, CA, USA, Bio-Optica). All the procedures were carried out automatically by using the Bond-III machine. Slides were incubated with an anti-TCTP antibody (dilution: 1 : 25) and the reaction revealed using fuchsine (Dako, Milan, Italy) as chromogen. Sections were weakly counterstained with Harris' haematoxylin and examined under a light microscope. Nonimmune serum immunoglobulins were used as negative control, whereas the positive control was represented by placental tissue [20].

2.7. Staining Assessment. All of the samples were independently evaluated and scored by two investigators

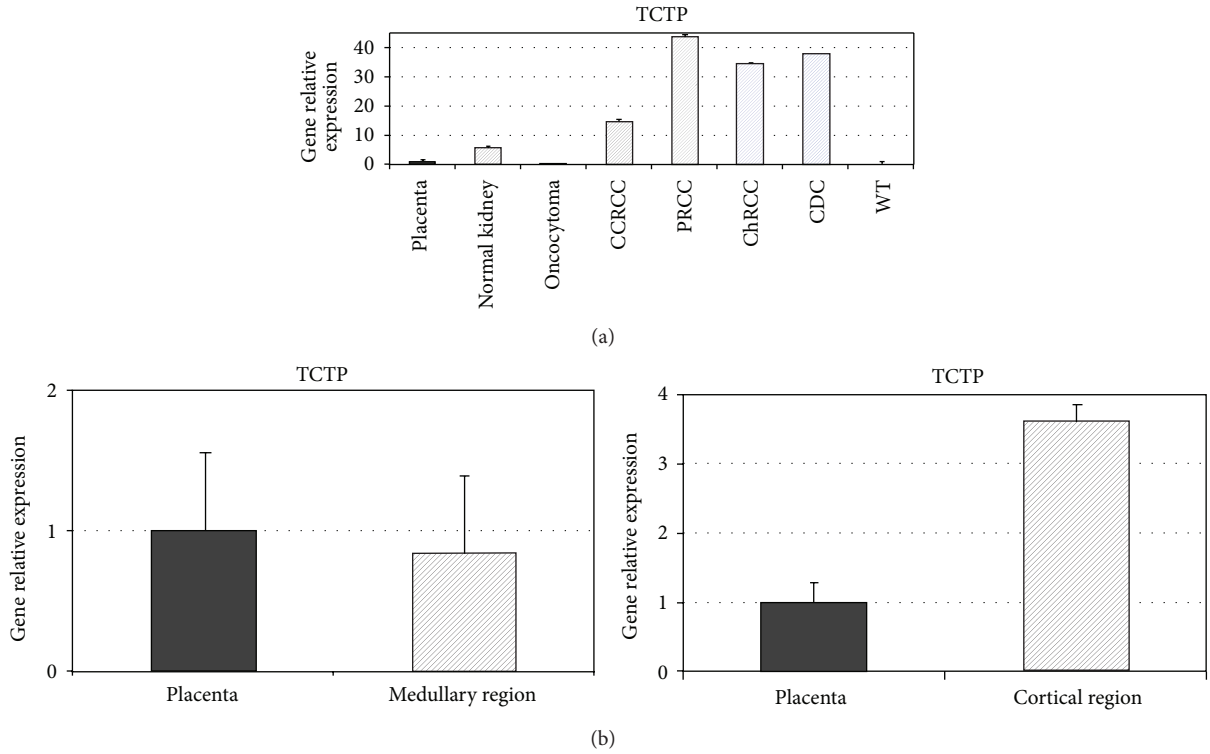


Figure 1: RT-qPCR analysis of TCTP in normal kidney and neoplastic specimens. Relative expression of TCTP mRNA was detected in normal kidney and in tumor samples. Values are expressed as the result of normalization using placenta samples. The graphs are representative of three different RT-qPCR experiments. Error bars represent standard deviation between duplicates. (a) TCTP mRNA was detected in normal kidney and, at higher level, in malignant tumor samples. (b) In normal kidney, a higher TCTP mRNA level was detected in the cortical region with respect to medullary region (CCRCC: clear cell renal cell carcinoma; PRCC: papillary renal cell carcinoma; ChRCC: chromophobe renal cell carcinoma; CDC: collecting duct carcinoma; WT: Wilm's tumour).

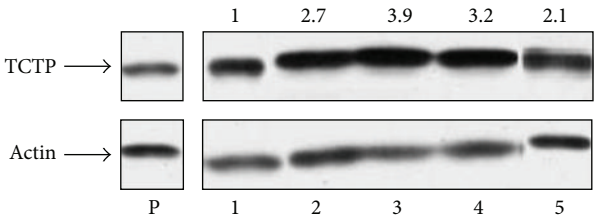
(Maria R. Ambrosio and Bruno J. Rocca), who were blinded to the clinicopathological information of the patients. TCTP protein expression levels were classified semiquantitatively combining the proportion and intensity of positively stained cells [24]. The percentage of positive-staining tumor cells was scored as follows: (1) <5% positive cells, (2) 5–50% positive cell, and (3) >50% positive cells. Staining intensity was scored as follows: (1) weak or not detectable staining, (2) moderate staining, and (3) strong staining [24]. Three different fields (at least 100 cells/field) were evaluated at ×200 magnification. In neoplastic samples TCTP protein expression level was evaluated and defined only in tumoural cells. The sum of the staining intensity score and the percentage score was used to define the TCTP protein expression level, low: 0–2; high: 3-4. The agreement between the two pathologists was about 90%. Cases with discrepancies were reviewed and discussed to reach the 100% of concordance.

2.8. Statistics. Statistical analysis was performed using a statistical software package (SigmaPlot 12.0, Systat Software), by employing Student's t-test, with p value <0.05 being considered significant.

3. Results

3.1. TCTP Is Present in Normal Kidney and in Renal Carcinomas. To evaluate the expression of TCTP, total RNA extracted from normal and neoplastic tissues was examined by RT-qPCR. We detected its expression at the mRNA level in normal kidney, being significantly higher in the cortical region ($p < 0.05$, $p = 0.01$) (Figures 1(a) and 1(b)). We then analyzed its expression level in neoplastic samples of renal tumors, representative of different histotypes. Relative quantification indicated that the expression of TCTP is significantly higher in renal cell cancers than in normal tissue and benign tumors (oncocytoma) ($p < 0.05$, $p = 0.03$); Wilm's tumors showed no TCTP expression (Figure 1(a)). TCTP protein expression was then confirmed in the specimens by western blotting. A specific band of the approximate molecular weight of 22 kDa was detected in all the neoplastic and nonneoplastic specimens (Figure 2). Higher expression of the protein was detected in tumor tissues, confirming the RT-qPCR results (Figure 2).

3.2. TCTP Staining Is Different in the Different Sites of Human Kidney and in the Different Tumor Histotypes. The different pattern of expression of TCTP was evaluated by



P: placenta

1: normal kidney

2: ChRCC: chromophobe renal cell carcinoma

3: PRCC: papillary renal cell carcinoma

4: CDC: collecting duct carcinoma

5: clear cell renal cell carcinoma

Figure 2: Western blotting analysis of TCTP protein expression in normal kidney and neoplastic specimens. WB was performed to confirm TCTP protein expression in normal kidney and in different renal tumors. A specific band of the MW of 22 kDa was detected in all the neoplastic and nonneoplastic specimens. Placenta was used as positive control. Relative expression of TCTP was measured with respect to actin, used as control. WB was carried out in triplicate. Densitometric analysis revealed higher expression of TCTP in tumor samples, with respect to normal kidney. (1) Normal kidney; ((2)–(5)) tumor samples: (2) ChRCC; (3) PRCC; (4) CDC; (5) CCRCC (WB: western blotting; MW: molecular weight; ChRCC: chromophobe renal cell carcinoma; PRCC: papillary renal cell carcinoma; CDC: collecting duct carcinoma; CCRCC: clear cell renal cell carcinoma).

immunohistochemistry in all the 84 FFPE nephrectomies. For each surgical specimen the most representative neoplastic and nonneoplastic area were selected. In normal tissue TCTP was found within the kidney cortex (both glomeruli and tubules) (Figures 3(a) and 3(b)). In glomeruli, a low level of TCTP expression was detectable in podocytes with variations from cell to cell and in endothelial and mesangial cells (Figures 3(c) and 3(d)). The protein expression was higher in the proximal tubules and lower in the distal ones (Figures 3(e) and 3(f)). In the medulla, TCTP immunoreactivity was very low or absent and present only in the thick segment of Henle's loop (Figures 3(g) and 3(h)). TCTP expression was also observed in the transitional epithelium of the renal pelvis (Figure 3(f), inset), being mostly concentrated in cells of the basal layer. In all these compartments TCTP expression was cytoplasmic. The negative control is shown in the inset of Figure 3(b).

Among the tumoural specimens, 4 were oncocytomas, 36 were clear cell carcinoma, 25 were papillary carcinoma, 12 were chromophobe carcinoma, 4 were collecting duct carcinoma, and 3 were Wilm's tumors. All the specimens, with the exception of oncocytomas and Wilm's tumors, expressed TCTP. The immunoreactivity of neoplastic cells was always intense; in clear cell carcinoma it was membranous (Figures 4(a) and 4(b), inset), whereas it was cytoplasmatic in papillary carcinoma (Figures 4(c) and 4(d)), in collecting duct carcinoma (Figures 4(e) and 4(f)), and in chromophobe carcinoma. The comparison with a nonneoplastic region of the same sites is demonstrated in Figure 4(g), whereas the negative control is shown in Figure 4(h). No differences were found among the various grades and stages.

The results on the expression of TCTP in both nonneoplastic and neoplastic samples are summarized in Table 1.

4. Discussion

TCTP is a protein already known to be expressed in almost all the human organs but not in the human kidney ([2, 6, 19], http://www.ebi.ac.uk/swissprot). To the best of our knowledge, only four previous studies addressing TCTP expression in the kidney have been published so far; however, they were performed on rat and mouse and mainly focused on TCTP content in cell lysates of the whole organ [2, 4, 18, 25]. All these studies suggested a potential role of TCTP in renal physiology. In our study we demonstrate for the first time the expression of the protein in human kidney. We detect that TCTP is mainly expressed in the cortex and in particular in the cytoplasm of the proximal tubular cells, opening to the possibility to link this expression to urine formation, as suggested by Sheverdin et al. [18], and, for instance, to calcium absorption, the proximal tubule being one of the major sites of calcium absorption. Our findings seem to be in contrast to what has been previously reported in the literature and in proteomic banks, that is, the absence of the protein in human kidney. We hypothesize that the lack of expression in detecting the protein might be related to a sampling error for which TCTP mRNA and protein were analyzed only in medullary region, in which the protein is exactly not present. However, functional data should be collected to try to validate this hypothesis. Many cellular processes which are regulated by TCTP (cell proliferation, cell death, apoptosis, stress and heat shock response, gene regulation, immune system activity, and pluripotency) could, if dysregulated, lead to cancer [5–11]. It has been found that TCTP expression increases in some types of cancer (colon, lung, prostate, breast, and melanoma) and decreases after tumor reversion, as demonstrated in different cell lines and by knockdown experiments that showed a suppression of the malignant phenotype following inhibition of TCTP expression [12, 13]. A large-scale screening analysis of genes expressed by parental tumor cells in comparison with revertants showed that TCTP had the most striking different expression, significantly higher in neoplastic cells than in nonneoplastic ones [12]. Two studies confirmed these findings in several different tumor cell lines and demonstrated that the mechanism of neoplastic reversion determined by TCTP relies on a reorganization of the process of cell cycle arrest, apoptosis, and terminal differentiation as a form of rerouting and trigger of the whole machinery that enables the tumor cells to quit the malignant pathway [12, 13]. Such a system could override the genetic changes in cancer not necessarily correcting the genetic alterations but instead bypassing them and reprogramming the cancer cells to recover some of their normal functions. In the present study, we describe the expression of TCTP in clear cell, chromophobe, papillary, and collecting duct carcinomas

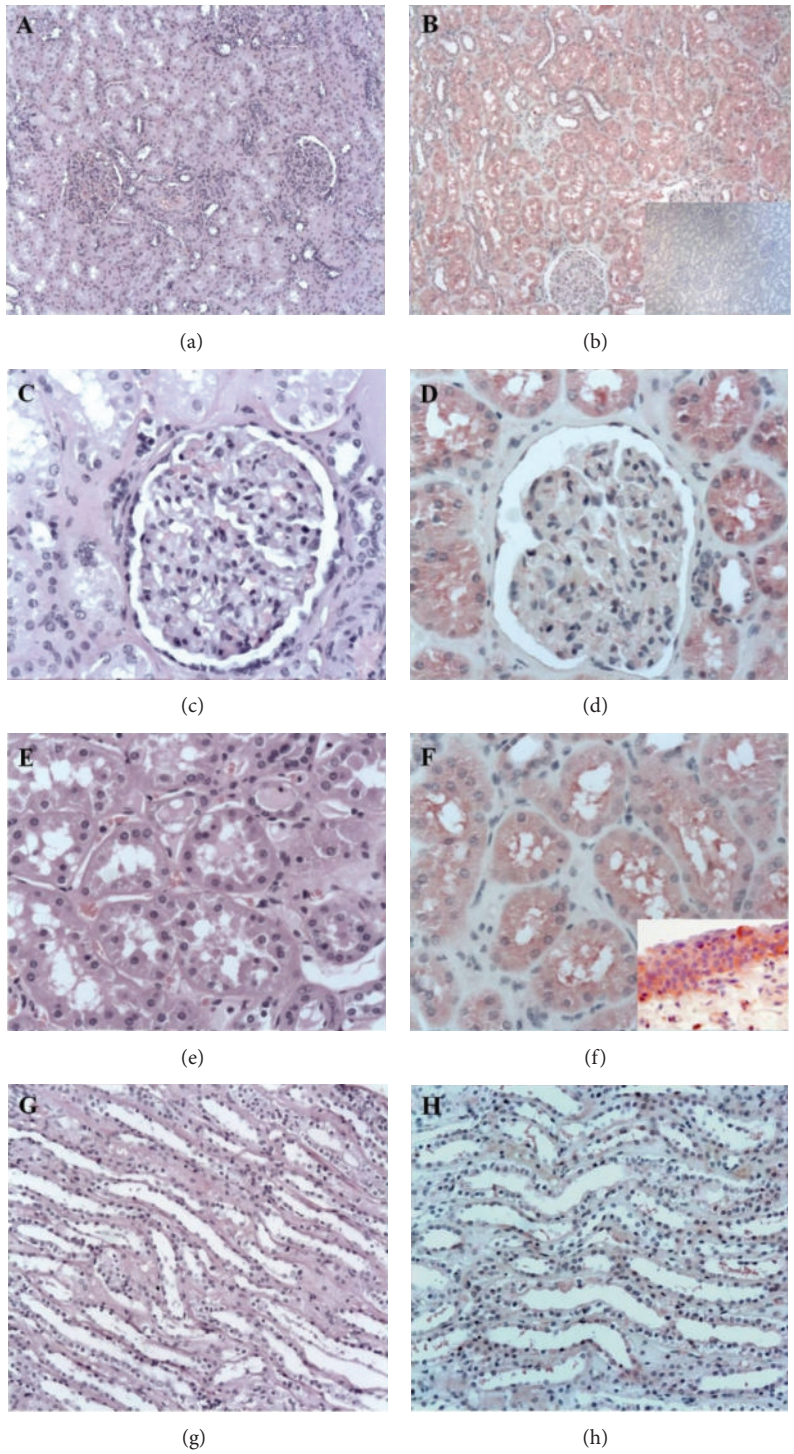


Figure 3: Immunohistochemical evaluation in normal kidney region. At low power, TCTP staining is present in all the structures belonging to the cortical region ((a) haematoxylin and eosin (H&E); (b) TCTP staining; ((a)-(b)) ×50; (b) inset, negative control, ×50); at higher power, weak TCTP expression is observed in glomeruli ((c) H&E; (d) TCTP staining; ((c)-(d)) ×200) and strong positivity is shown by proximal tubules ((e) H&E; (f) TCTP staining; ((e)-(f)) ×200). In the medullary region, TCTP immunoreactivity was very low or absent ((g) H&E; (h) TCTP staining, ×200). TCTP expression is also present in the transitional epithelium of the renal pelvis ((f) inset, TCTP staining, ×200).

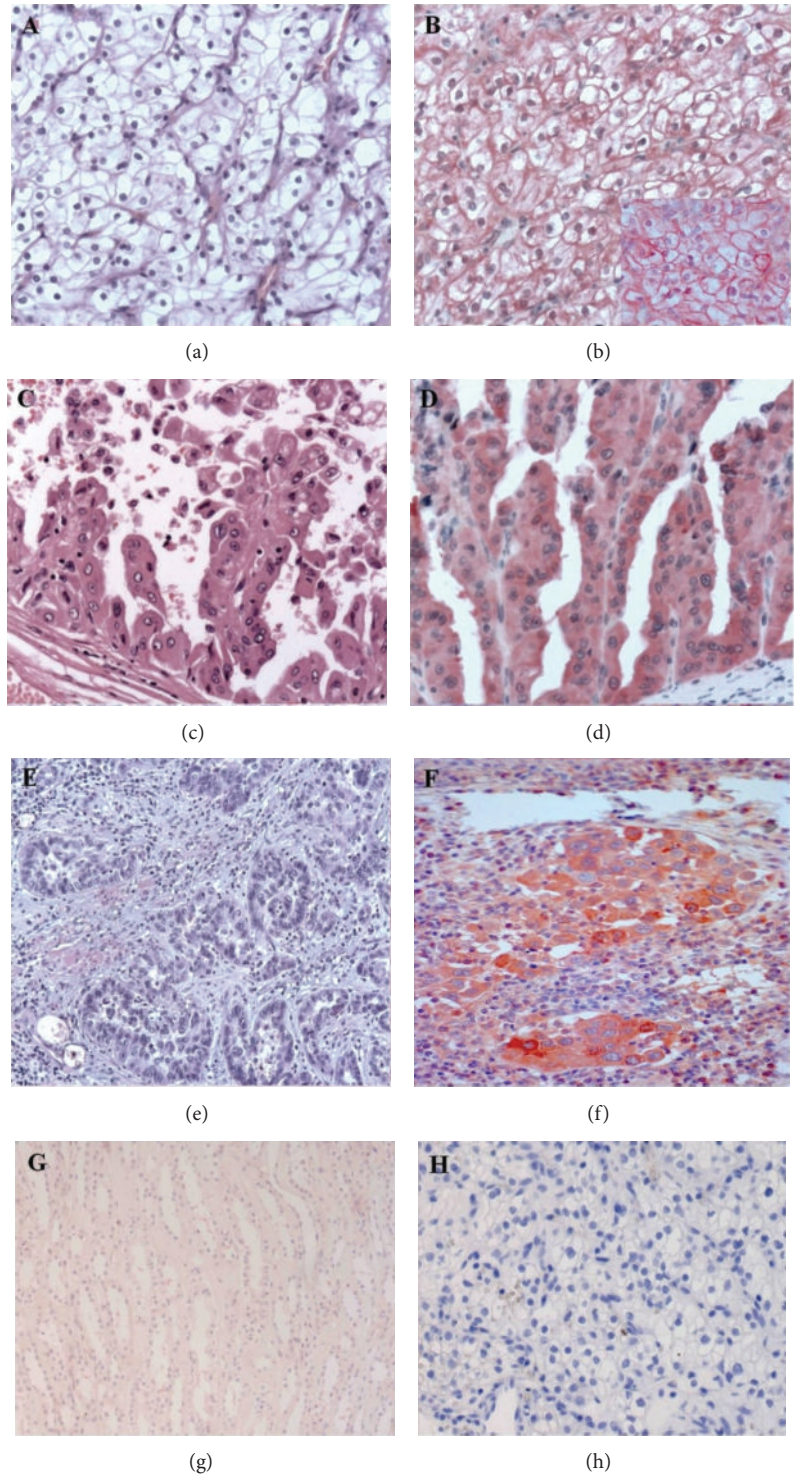


FIGURE 4: Immunohistochemical evaluation in cancer specimens. In clear cell carcinoma a membrane positivity is observed ((a) H&E; (b), (b) inset, TCTP staining; ((a)-(b)) ×200; (b) inset ×400); in papillary carcinoma ((c) H&E; (d) TCTP staining; ((c)-(d)) ×100) and in collecting duct carcinoma, the staining is cytoplasmic ((e) H&E; (f) TCTP staining; ((e)-(f)) ×200). The comparison with nonneoplastic tissue from the same samples is shown ((g) TCTP stain, ×200) as well as the negative control ((h) ×200).

Table 1: Immunohistochemical results in normal and neoplastic kidney samples. The staining for TCTP is listed, with higher protein expression in cortical regional and malignant tumours.

Cell tissue	*N*	TCTP protein expression		Pattern of expression
		High	Low/absent	
Cortical region	84	80	4	Cytoplasmatic
Proximal tubules				Strong
Glomeruli				Weak
Medullary region	84	5	79	Cytoplasmatic
Clear cell carcinoma	36	34	2	Membranous
Papillary carcinoma	25	24	1	Cytoplasmatic
Chromophobe carcinoma	12	11	1	Cytoplasmatic
Collecting duct carcinoma	4	4	0	Cytoplasmatic
Wilm's tumors	3	0	3	nd
Oncocytomas	4	0	4	nd

N: number of cases; nd: not detectable.

which is much higher than in normal tissue and benign tumors (i.e., oncocytoma). The negativity for TCTP in Wilm's tumor may be related to the different histogenesis of the neoplasm (i.e., abnormally persistent metanephric blastemal cells). We also observed a different pattern of localization of TCTP in the various tumor histotypes: membranous in clear cell carcinomas, cytoplasmatic in papillary, chromophobe, and collecting duct carcinoma. Membranous expression of TCTP has never been described so far [26] and hence it is possible to hypothesize that the membranous localization of the protein in clear cell carcinoma may be related to the content of the cytoplasm of the neoplastic cells (i.e., abundant glycogen and lipid) that may displace the TCTP towards the plasma membrane. No significant differences of expression were found in the various histological grades and stages of the carcinomas.

Both the hypothetical involvement in renal physiology and the eventual implication of TCTP in renal carcinogenesis are at moment only speculative, since functional *in vitro* and *in vivo* data are still lacking. Moreover, it is known that protein expression in neoplasia can also be an effect of transformation rather than a cause. A study is currently ongoing in our laboratory to verify the hypotheses arising from the present data. Whether confirmed, since recent studies have provided the preclinical proof of principle of the therapeutic role of an antisense oligonucleotide-mediated TCTP knockdown in those cancers in which the protein is expressed [27], TCTP might be considered in the setting of a more tailored cancer therapy.

5. Conclusions

The findings of this study let us hypothesize that TCTP is involved in renal tubular functions, since tubular cells express the protein at the highest level, and that it might be implicated in renal carcinogenesis since it is expressed in all the histotypes of renal cell carcinoma and not in benign tumors. However, functional *in vivo* and *in vitro* studies are needed to confirm these hypotheses.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank Dr. JC Sanchez (Geneva Proteomic Center, Central Clinical Chemistry Laboratory, Geneva University Hospital, Geneva, Switzerland) for providing TCTP antibody and Dr. S. Mannucci (Section of Pathology, Azienda Ospedaliera Universitaria Senese, Siena, Italy) and Dr. S. Lazzi (Section of Pathology, Azienda Ospedaliera Universitaria Senese, Siena, Italy) for their support.

References

[1] U.-A. Bommer and B.-J. Thiele, "The translationally controlled tumour protein (TCTP)," *International Journal of Biochemistry and Cell Biology*, vol. 36, no. 3, pp. 379–385, 2004.

[2] L. Susini, S. Besse, D. Duflaut et al., "TCTP protects from apoptotic cell death by antagonizing bax function," *Cell Death and Differentiation*, vol. 15, no. 8, pp. 1211–1220, 2008.

[3] H. Thiele, M. Berger, A. Skalweit, and B.-J. Thiele, "Expression of the gene and processed pseudogenes encoding the human and rabbit translationally controlled turnout protein (TCTP)," *European Journal of Biochemistry*, vol. 267, no. 17, pp. 5473–5481, 2000.

[4] E. Guillaume, C. Pineau, B. Evrard et al., "Cellular distribution of translationally controlled tumor protein in rat and human testes," *Proteomics*, vol. 1, no. 7, pp. 880–889, 2001.

[5] M. J. Koziol and J. B. Gurdon, "TCTP in development and cancer," *Biochemistry Research International*, vol. 2012, Article ID 105203, 9 pages, 2012.

[6] M. Nagano-Ito and S. Ichikawa, "Biological effects of mammalian translationally controlled tumor protein (TCTP) on cell death, proliferation, and tumorigenesis," *Biochemistry Research International*, vol. 2012, Article ID 204960, 7 pages, 2012.

[7] Z.-M. Ma, Q. Hong, Y.-G. Wang et al., "siRNA-mediated silencing of translationally controlled tumor protein induces

apoptosis and inhibits cell growth," *Journal of Infectious Diseases and Immunity*, vol. 3, no. 1, pp. 6–13, 2011.

[8] F. Bazile, A. Pascal, I. Arnal, C. le Clainche, F. Chesnel, and J. Z. Kubiak, "Complex relationship between TCTP, microtubules and actin microfilaments regulates cell shape in normal and cancer cells," *Carcinogenesis*, vol. 30, no. 4, pp. 555–565, 2009.

[9] M. Gnanasekar, G. Dakshinamoorthy, and K. Ramaswamy, "Translationally controlled tumor protein is a novel heat shock protein with chaperone-like activity," *Biochemical and Biophysical Research Communications*, vol. 386, no. 2, pp. 333–337, 2009.

[10] M. Kim, Y. Jung, K. Lee, and C. Kim, "Identification of the calcium binding sites in translationally controlled tumor protein," *Archives of Pharmacal Research*, vol. 23, no. 6, pp. 633–636, 2000.

[11] T. Yoon, J. Jung, M. Kim, K. Lee, E. C. Choi, and K. Lee, "Identification of the self-interaction of rat TCTP/IgE-dependent histamine-releasing factor using yeast two-hybrid system," *Archives of Biochemistry and Biophysics*, vol. 384, no. 2, pp. 379–382, 2000.

[12] M. Tuynder, G. Fiucci, S. Prieur et al., "Translationally controlled tumor protein is a target of tumor reversion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 43, pp. 15364–15369, 2004.

[13] M. Tuynder, L. Susini, S. Prieur et al., "Biological models and genes of tumor reversion: cellular reprogramming through tpt1/TCTP and SIAH-1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 23, pp. 14976–14981, 2002.

[14] S. B. Rho, J. H. Lee, M. S. Park et al., "Anti-apoptotic protein TCTP controls the stability of the tumor suppressor p53," *FEBS Letters*, vol. 585, no. 1, pp. 29–35, 2011.

[15] M. Gnanasekar, K. V. N. Rao, L. Chen et al., "Molecular characterization of a calcium binding translationally controlled tumor protein homologue from the filarial parasites *Brugia malayi* and *Wuchereria bancrofti*," *Molecular and Biochemical Parasitology*, vol. 121, no. 1, pp. 107–118, 2002.

[16] K. V. N. Rao, L. Chen, M. Gnanasekar, and K. Ramaswamy, "Cloning and characterization of a calcium-binding, histamine-releasing protein from *Schistosoma mansoni*," *The Journal of Biological Chemistry*, vol. 277, no. 34, pp. 31207–31213, 2002.

[17] H. Thiele, M. Berger, A. Skalweit, and B. J. Thiele, "Expression of the gene and processed pseudogenes encoding the human and rabbit translationally controlled turnout protein (TCTP)," *European Journal of Biochemistry*, vol. 267, no. 17, pp. 5473–5481, 2000.

[18] V. Sheverdin, S.-Y. Bae, D. H. Shin, and K. Lee, "Expression and localization of translationally controlled tumor protein in rat urinary organs," *Microscopy Research and Technique*, vol. 75, no. 11, pp. 1576–1581, 2012.

[19] J.-C. Sanchez, D. Schaller, F. Ravier et al., "Translationally condrolled tumor protein: a protein identified in several nontumoral cells including erythrocytes," *Electrophoresis*, vol. 18, no. 1, pp. 150–155, 1997.

[20] F. Arcuri, S. Papa, A. Carducci et al., "Translationally controlled tumor protein (TCTP) in the human prostate and prostate cancer cells: expression, distribution, and calcium binding activity," *Prostate*, vol. 60, no. 2, pp. 130–140, 2004.

[21] J. N. Eble, G. Sauter, J. J. Epstein, and I. A. Sesterhenn, *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs*, IARC Press, Lyon, France, 2004.

[22] B. Delahunt, J. C. Cheville, G. Martignoni et al., "The international society of urological pathology (ISUP) grading system for renal cell carcinoma and other prognostic parameters," *American Journal of Surgical Pathology*, vol. 37, no. 10, pp. 1490–1504, 2013.

[23] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method," *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[24] M. R. Ambrosio, M. Navari, L. Di Lisio et al., "The Epstein Barr-encoded BART-6-3p microRNA affects regulation of cell growth and immuno response in Burkitt lymphoma," *Infectious Agents and Cancer*, vol. 9, no. 1, article 12, 2014.

[25] S. Li, X. Chen, Y. Ding, X. Liu, Y. Wang, and J. He, "Expression of translationally controlled tumor protein (TCTP) in the uterus of mice of early pregnancy and its possible significance during embryo implantation," *Human Reproduction*, vol. 26, no. 11, pp. 2972–2980, 2011.

[26] Y.-P. Ma and W.-L. Zhu, "Cytoplasmic and nuclear localization of TCTP in normal and cancer cells," *Biochemistry Research International*, vol. 2012, Article ID 871728, 4 pages, 2012.

[27] B. J. Rocca, A. Ginori, A. Barone et al., "Translationally controlled tumor protein in prostatic adenocarcinoma: correlation with tumor grading and treatment-related changes," *BioMed Research International*, vol. 2015, Article ID 985950, 8 pages, 2015.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 891045, 9 pages
http://dx.doi.org/10.1155/2015/891045

Research Article

Preoperative Neutrophil-to-Lymphocyte Ratio and Neutrophilia Are Independent Predictors of Recurrence in Patients with Localized Papillary Renal Cell Carcinoma

Jiwei Huang,[1] Douglas M. Dahl,[2] Liang Dong,[1] Qiang Liu,[3] Kristine Cornejo,[4] Qi Wang,[5] Shulin Wu,[4] Adam S. Feldman,[2] Yiran Huang,[1] Wei Xue,[1] and Chin-Lee Wu[2,4]

[1]*Department of Urology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200123, China*
[2]*Department of Urology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA*
[3]*Department of Pathology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200123, China*
[4]*Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA*
[5]*Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115, USA*

Correspondence should be addressed to Wei Xue; uroxuewei@163.com and Chin-Lee Wu; cwu2@mgh.harvard.edu

Received 8 February 2015; Accepted 19 April 2015

Academic Editor: Giovanni Lughezzani

Objective. To evaluate the role of preoperative neutrophil-to-lymphocyte ratio (NLR) and absolute neutrophil count (ANC) in patients' prognosis with localized papillary renal cell carcinoma (pRCC). *Methods*. Data from 218 localized pRCC patients (T1-3 N0/+ M0), operated between 1991 and 2011 at two centers, were evaluated retrospectively. Univariable and multivariable analyses using the Cox regression model were performed to determine the associations of NLR and ANC with recurrence-free survival (RFS). Prognostic accuracy was evaluated with the Harrell concordance index. *Results*. The 5-year RFS rate was 87.0%. Multivariable analysis identified increased NLR (≥3.6) and ANC (≥5300/μL) as independent prognostic factors for RFS (hazard ratio (HR) = 4.01, $P = 0.018$) and (HR = 4.71, $P = 0.045$). The final model built by the addition of NLR or ANC improved predictive accuracy (c-index: 0.824, 0.842) compared with the clinicopathological base model (c-index: 0.800), which included TNM stage and tumor necrosis. *Conclusions*. The NLR and ANC appear to be independent prognostic factors for RFS after surgery for localized pRCC. They significantly increase the accuracy of established prognostic factors. Therefore, we recommend adding NLR and ANC to traditional prognostic model, which may improve its predictive accuracy.

1. Introduction

Papillary renal cell carcinoma (pRCC) accounts for approximately 10% to 15% of all renal cell carcinoma (RCC) and is the second most frequent histologic form after clear cell RCC (ccRCC) [1, 2]. Because clear cell histology accounts for approximately 70–80% of all RCCs, most data regarding prognostic factors have been served for this subtype. It is important to note that a particular histologic subtype is characterized by distinct morphological features, clinical behaviors, and genetic changes [1–3]. Patients with pRCC have a significantly better prognosis than those with clear cell RCC but some patients with pRCC die of metastatic disease [1, 2]. Because of the relatively lower frequency of recurrence, it is also complicated to identify prognostic factors in pRCC compared with ccRCC. It is accurately crucial to determine the risk of disease recurrence in the postoperative phase since it impacts the frequency and extent of surveillance imaging and possible inclusion in adjuvant clinical trial [4]. Therefore, particular prognostic factors and models should be independently validated for pRCC.

Several prognostic factors and models were proposed to determine the risk of disease recurrence of RCC [5–7]. The systemic inflammatory response, which is usually measured by surrogate blood-based parameters, such as neutrophil or platelet count, C-reactive protein, has been shown to independently predict the clinical outcome of various human cancer types [8]. Neutrophil-to-lymphocyte

ratio (NLR) could serve as independent predictors of survival [9–12] in ccRCC patients or in RCC patients, which were composed of overwhelming majority of ccRCCs. Only one study investigated NLR in nonclear cell RCC, but they did not analyze it in separate histologic subtype [13]. One recent study focused on ALC in pRCC [14].

To our knowledge, the prognostic value of absolute neutrophil count (ANC), NLR in pRCC has not been investigated. Compared to ccRCC, pRCC involves different biological pathways, has distinct prognostic factors, and is associated with a more favorable prognosis [2]. We therefore evaluated the prognostic value of preoperative ANC, absolute lymphocyte count (ALC), and NLR, which are routinely measured, in localized pRCC patients treated with curative intent surgery.

2. Materials and Methods

2.1. Study Population. After obtaining the institutional review board (IRB) approval from both hospitals, we retrospectively reviewed 415 consecutive patients with pRCC who underwent radical or partial nephrectomy for pRCC between 1991 and 2011 at two academic centers. We finally included 218 patients who underwent full resection of unilateral, sporadic pRCC (stage T1-3N0M0 or T1-3N+M0) by radical or partial nephrectomy with available data on ALC and ANC within 4 weeks before surgery. We excluded patients with tumor measuring 5 mm or less (papillary adenoma), those with chronic leukemia or lymphoma, inflammatory disease, and autoimmune disease, those receiving neoadjuvant or adjuvant therapy, those with coexisting other subtypes of RCC, and those with a prior history of RCC. Figure 1 shows a flow chart of patients who met inclusion criteria.

2.2. Clinical and Pathological Evaluation. Patients' data including age, gender, symptoms at presentation, hypertension, diabetes mellitus, pathological features, ANC, ALC, and follow-up information were collected. All clinical data were retrieved from medical records at both institutions. The pathologic slides were rereviewed by the urologic pathologists at the individual institution (Kristine Cornejo and Chin-Lee Wu from Massachusetts General Hospital, and Qiang Liu from Renji Hospital). In total, 6 pathological slides could not be found in two institutions. Their pathological data were retrieved from pathology records at two hospitals. Patients with a palpable flank or abdominal mass, discomfort, gross hematuria, acute onset varicocele, or constitutional symptoms, including sweating, weight loss, fatigue, early satiety, rash, and anorexia, were considered symptomatic at presentation. For preoperative staging patients underwent computed tomography (CT) or magnetic resonance imaging (MRI) of the abdomen and CT or X-ray of the chest. Radionuclide bone scan and cranial CT were performed as clinically indicated. TNM staging was performed according to 2010 criteria [15], T, N stages were assigned pathologically and M stage was assigned clinically. Patients with pathological nodes on imaging underwent lymph node dissection. Pathological confirmation was done in all study patients with positive nodes. Histological differentiation was graded according to Fuhrman's nuclear grading system [16]. Each lesion was also classified as type 1 or 2 based on the features used to describe pRCC in the original study by Delahunt and Eble [17]. Any tumor with type 2 areas was classified as type 2.

2.3. Follow-Up. The postoperative surveillance strategy was institution and physician dependent. Recurrence was defined as local relapse, lymph-node metastasis, and distant metastasis, as determined by imaging (mainly CT, MRI, and bone scan) and most of which was confirmed by pathology.

2.4. Laboratory Assays. Venous blood samples were collected as part of routine clinical procedures before surgery at both institutions and laboratory parameters for this study were obtained from medical records. The cutoff points to stratify ANC, ALC, and NLR were using ANC < 5,300 cells per μL to identify low ANC, ALC < 1,300 cells per μL to identify low ALC, and NLR < 3.6 to identify low NLR [13].

2.5. Statistical Analysis. As to the clinicopathological outcomes according to the ANC, ALC, and NLR, for continuous variables, Student's t-test was used for those variables reported as mean (plus or minus standard deviation [SD]) and Wilcoxon rank-sum test was used for the variables reported as median with interquartile ranges (IQRs); for categorical variables, the chi-square and continuity corrected chi-square tests were used. The primary outcome of this study, recurrence-free survival (RFS), was estimated by the Kaplan-Meier method and compared by a log-rank test and calculated from the date of surgery to the date of disease recurrence or the time of the last visit. The effect of ANC, ALC, and NLR on RFS was examined by using a Cox proportional hazard regression model. All variables including ANC, NLR with a P less than 0.05 on univariable analyses were entered into multivariable stepwise Cox regression analysis. To prevent against overfitting by decreasing the number of variables, pT stage and pN stage were summarized as the TNM group in multivariable analysis. Hazard ratio (HR) and 95% confidence interval (CI) were computed. The predictive accuracy was evaluated using the Harrell concordance index (c-index) and given as a percentage [18]. A clinicopathological base model was built, consisting of all variables that demonstrated a significant independent prognostic value in the multivariable Cox proportional hazard regression model. To examine whether ANC or NLR data can provide additional prognostic power when used with basic clinical variables, we built predictive models by integrating clinical variables with ANC or/and NLR data using the statistical method described in [19]. For each core set, we randomly split the samples into two groups: 80% as the training set and 20% as the test set. The multivariate Cox models were built based on training set with the R package "survival." We then applied the models thereby obtained to the test set for prediction and calculated the c-index from test set using the R package "survival." For each core set, the above procedure was repeated 100 times to generate 100 c-indexes. Then, we used the Wilcoxon signed rank test to calculate the P value (using 0.05 as the significance cutoff). The differences were

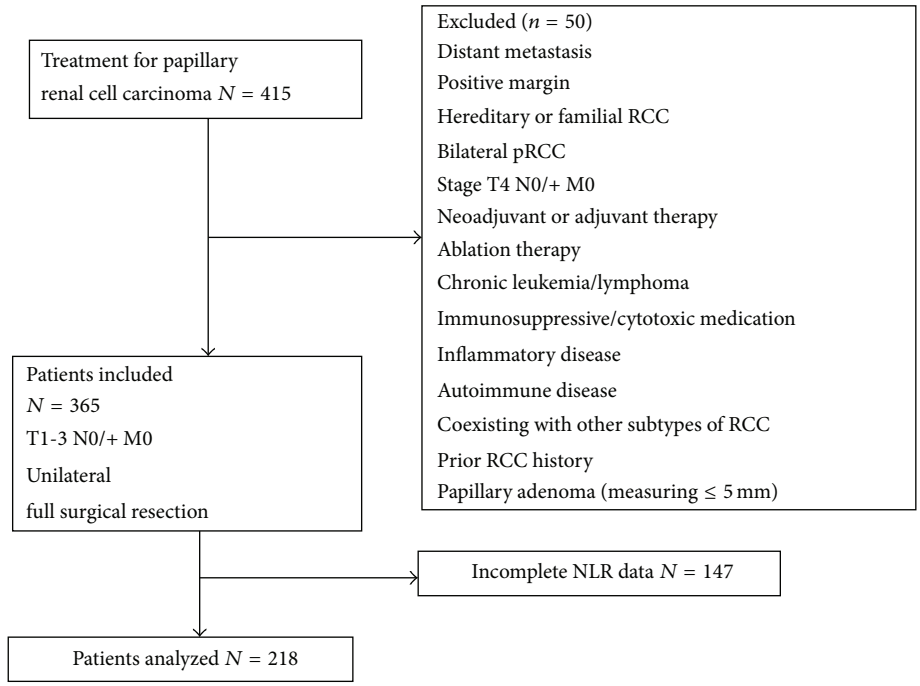


Figure 1: Flow chart of patients who met study inclusion/exclusion criteria.

considered to be statistically significant if $P < 0.05$. Statistical analysis was carried out using SPSS, version 22.0.

3. Results

3.1. Clinical Characteristics. Our final cohort included 169 men (77.5%) and 49 women (22.5%). Mean age at surgery was 58.9 years (Table 1). Radical and partial nephrectomy was performed in 129 (59.2%) and 89 patients (40.8%), respectively.

3.2. Associations

3.2.1. With Clinical and Pathological Characteristics. The median preoperative ANC, ALC, and NLR was 5.3/nL (IQR: 4.2, 7.0), 1.7/nL (IQR: 1.4, 2.1), and 3.1 (IQR: 2.4, 4.2). Compared with patients with lower ANC (<5.3/nL) and NLR (<3.6), patients with higher ANC and NLR were more likely to have larger tumor size ($P = 0.044$, $P = 0.005$) (Tables 1–3). There were no significant differences between the groups according to ANC, ALC, and NLR with regard to other established prognostic factors, such as pathological stage, symptoms at diagnosis. Of host related factors, neutrophilia was only associated with gender ($P = 0.038$).

3.2.2. With Prognosis of pRCC. At a median follow-up period of 43.0 months (IQR 17.8–67.5, mean 52.8), disease recurrence occurred in 20 patients; the 5-year disease-free survival rate was 87.0%.

The patients with preoperative neutrophilia and high NLR had a significantly worse rate of survival than those without neutrophilia and high NLR with regard to RFS (Log-rank test, with each $P < 0.001$, Figure 2). Univariable and multivariable analyses (stepwise analysis) of the factors influencing RFS are presented in Table 4. Univariable analyses demonstrated that pT stage, pN stage, TNM stage, Fuhrman grade, pRCC type, tumor necrosis, neutrophilia, and high NLR were significant predictors of RFS. Multivariable analyses showed that neutrophilia (HR 4.71, $P = 0.045$) and high NLR (HR 4.01, $P = 0.018$) were independent predictors of RFS, along with the presence of TNM stage (HR 2.19, $P = 0.003$) and tumor necrosis (HR 2.55, $P = 0.057$).

The predictive accuracy was calculated with and without the inclusion of ANC and NLR. In the base model, including the traditional predictor variables of TNM stage and tumor necrosis, predictive accuracy was 80.0%; with the addition of ANC 5.3/nL, predictive accuracy was 84.2%; with the addition of NLR 3.6, predictive accuracy was 82.4%. In a model including all four variables, predictive accuracy was 87.8% (Table 5). Notably, the integrated models resulted in statistically significantly improved predictive power compared to the base model (one-sided Wilcoxon signed rank test, TNM stage + tumor necrosis + ANC: $P < 7.5 \times 10^{-4}$; TNM stage + tumor necrosis + NLR: $P < 2.5 \times 10^{-3}$; TNM stage + tumor necrosis + NLR: $P < 5.3 \times 10^{-6}$) (Figure 3).

4. Discussion

This study showed that ANC and NLR were independent prognostic factors after surgery with curative intent for localized pRCC and found that both of them, especially NLR, could significantly increase the accuracy of established prognostic model. Therefore, NLR and ANC may improve the predictive accuracy of traditional prognostic model.

Table 1: Clinical and pathological characteristics of 218 pRCC patients stratified according to NLR.

Variables	Number of Pts (%)	NLR ≥ 3.6	NLR < 3.6	*P* value
Patients, *n* (%)	218	76 (34.9)	142 (65.1)	
Age (years), mean ± SD	58.9 ± 12.2	59.7 ± 11.6	58.5 ± 12.5	0.460
Gender				0.712
Male	169 (77.5)	60 (78.9)	109 (76.8)	
Female	49 (22.5)	16 (21.1)	33 (23.2)	
Symptoms at presentation				0.140
Symptom	37 (17.0)	9 (11.8)	28 (19.7)	
Asymptomatic	181 (83.0)	67 (88.2)	114 (80.3)	
Hypertension				0.053
Yes	91 (41.7)	25 (32.9)	66 (46.5)	
No	127 (58.3)	51 (67.1)	76 (53.5)	
Diabetes mellitus				0.431
Yes	22 (10.1)	6 (7.9)	16 (11.3)	
No	196 (89.9)	70 (92.1)	126 (88.7)	
Tumor size (cm), median (IQR)	3.5 (2.5–6.0)	4.0 (3.0–7.0)	3.2 (2.1–5.0)	0.005
T stage				0.290
T1	160 (73.4)	52 (68.4)	108 (76.1)	
T2	20 (9.2)	10 (13.2)	10 (7.0)	
T3	38 (19.5)	14 (18.4)	24 (16.9)	
N stage				0.097
N1	7 (3.2)	5 (6.6)	2 (1.4)	
N0	211 (96.8)	71 (93.4)	140 (98.6)	
Fuhrman grade				0.763
1-2	155 (71.1)	55 (72.4)	100 (70.4)	
3-4	63 (28.9)	21 (27.6)	42 (29.6)	
pRCC type				0.079
1 type	126 (57.8)	38 (51.4)	88 (63.8)	
2 type	86 (39.4)	36 (48.6)	50 (36.2)	
Unknown	6 (2.8)			
Tumor necrosis				0.653
Yes	34 (15.6)	13 (17.1)	21 (14.8)	
No	184 (84.4)	63 (82.9)	121 (85.2)	

To our knowledge, this is the first study of NLR focusing on pRCC. In published studies to date, only patients with ccRCC were included or the subtypes were predominantly clear cell. Only one study investigated NLR in nonclear cell RCC and did not access it in papillary cell subtype. In a cohort of 678 patients with nonmetastatic ccRCC, Pichler et al. [10] found that preoperative NLR was an independent prognostic factor for overall survival (HR 1.59; $P = 0.014$). de Martino et al. [13] showed that patients with increased preoperative NLR, evaluated as a continuous variable, had a high risk of disease recurrence in nonclear cell RCC. Mehrazin et al. [14] demonstrated that patients with lymphopenia had an inferior overall survival and a trend significance for worse cancer-specific survival ($P = 0.071$) in pRCC.

In the present study, patients who had both pRCC and high NLR or neutrophilia were more likely to have larger tumor size. de Martino et al. reported that patients with increasing ANC and NLR were associated with lymph node metastasis. We found a nonsignificant trend in favor of increasing NLR associated with lymph node metastasis ($P = 0.097$). These differences of tumor characteristics may partly explain why the patients with high NLR or neutrophilia in our cohort had more aggressive disease.

Despite the fact that recent progress in the identification of genetic and common molecular alterations in RCC has been made [20], the most widely used routine prognostic assessment of RCC currently still relies on traditional clinicopathological prognostic variables [5–7]. The predictive accuracy of prognostic model can be improved by these molecular markers, but the high costs of analysis, the time-consuming preparation, and the lack of evidence together turn them into clinical practice. At the meantime, several inflammatory hematological indexes, such as C-reactive protein [21], have been proved to improve the accuracy of these models in patients with RCC. In our study, we also showed that adding NLR or ANC was able to raise the predictive accuracy in this cohort of patients. The base model, which included the traditional predictor variables of TNM stage and

Table 2: Clinical and pathological characteristics of 218 pRCC patients stratified according to ANC.

Variables	ANC ≥ 5.3/nL	ANC < 5.3/nL	*P* value
Patients, *n* (%)	113 (51.8)	105 (48.2)	
Age (years), mean ± SD	59.3 ± 12.2	58.5 ± 12.3	0.614
Gender			0.038
Male	94 (83.2)	75 (71.4)	
Female	19 (16.8)	30 (28.6)	
Symptoms at presentation			0.131
Symptom	15 (13.3)	22 (21.0)	
Asymptomatic	98 (86.7)	83 (79.0)	
Hypertension			0.090
Yes	41 (36.3)	50 (47.6)	
No	72 (63.7)	55 (52.4)	
Diabetes mellitus			0.279
Yes	9 (8.0)	13 (12.4)	
No	104 (92.0)	92 (87.6)	
Tumor size (cm), median (IQR)	4.0 (2.5–6.6)	3.5 (2.5–4.8)	0.044
T stage			0.130
T1	77 (68.1)	83 (79.0)	
T2	14 (12.4)	6 (5.7)	
T3	22 (19.5)	16 (15.3)	
N stage			0.150
N1	6 (5.3)	1 (1.0)	
N0	107 (94.7)	104 (99.0)	
Fuhrman grade			0.918
1-2	80 (70.8)	75 (71.4)	
3-4	33 (29.2)	30 (28.6)	
pRCC type			0.119
1 type	61 (54.5)	65 (65.0)	
2 type	51 (45.5)	35 (35.0)	
Tumor necrosis			0.888
Yes	18 (15.9)	16 (15.2)	
No	95 (84.1)	89 (84.8)	

Table 3: Clinical and pathological characteristics of 218 pRCC patients stratified according to ALC.

Variables	ALC ≥ 1.3/nL	ANC < 1.3/nL	*P* value
Patients, *n* (%)	171 (78.4)	47 (21.6)	
Age (years), mean ± SD	58.1 ± 11.9	62.0 ± 12.9	0.053
Gender			0.863
Male	133 (77.8)	36 (76.6)	
Female	38 (22.2)	11 (23.4)	
Symptoms at presentation			0.992
Symptom	29 (17.0)	8 (17.0)	
Asymptomatic	142 (83.0)	39 (83.0)	
Hypertension			0.227
Yes	75 (43.9)	16 (34.0)	
No	96 (56.1)	31 (66.0)	
Diabetes mellitus			1.000
Yes	17 (9.9)	5 (10.6)	
No	154 (90.1)	42 (89.4)	
Tumor size (cm), median (IQR)	3.5 (2.3–6.0)	4.0 (3.0–5.5)	0.050
T stage			0.831
T1	125 (73.1)	35 (74.5)	
T2	15 (8.8)	5 (10.6)	
T3	31 (18.1)	7 (14.9)	
N stage			1.000
N1	5 (2.9)	2 (4.3)	
N0	166 (97.1)	45 (95.7)	
Fuhrman grade			0.565
1-2	120 (70.2)	35 (74.5)	
3-4	51 (29.8)	12 (25.5)	
pRCC type			0.551
1 type	101 (60.5)	25 (55.6)	
2 type	66 (39.5)	20 (44.4)	
Tumor necrosis			0.761
Yes	26 (15.2)	8 (17.0)	
No	145 (84.8)	39 (83.0)	

tumor necrosis, was of a predictive accuracy (80.0%), which could be further improved by the addition of NLR (82.4%) or ANC (84.2%). Our findings are in agreement with the study of de Martino et al. [13], who also found that adding NLR to their base model (TNM stage, grade and microvascular invasion) could improve the predictive accuracy from 78.8% to 80.8% in 281 nonclear cell RCC patients with regard to RFS. Considering NLR that is widely available and relatively easy to assess even before surgery, they may become attractive variables for patients counseling and clinical trial entry.

Increasing evidence supports the involvement of systemic inflammation in cancer development and progression. On one hand, inflammation and activation of the immune system enable antitumor activity; on the other hand, they contribute to carcinogenesis, tumor growth, and progression in human cancers [22]. Local inflammation, which is reflected by intratumoral infiltration of neutrophils, macrophages, and leucocytes, was also identified as an independent factor for reduced survival in clear cell RCC patients [23]. In our opinion, inflammation no matter systemic or local contributing to carcinogenesis may be the underlying reason why NLR, neutrophil count, and local intratumoral neutrophil presence are significantly associated with the poor outcome of the RCC patients.

The present study has several limitations, including the retrospective nature of the data collection and data limited to 2 centers. There was no standard for postoperative surveillance, which may have impact on the outcome measurement and subsequent statistical evaluation. Although pathology was rereviewed by urologic pathologists at individual institution, we did not provide a central pathology review. Also, some other prognostic factors, such as performance status

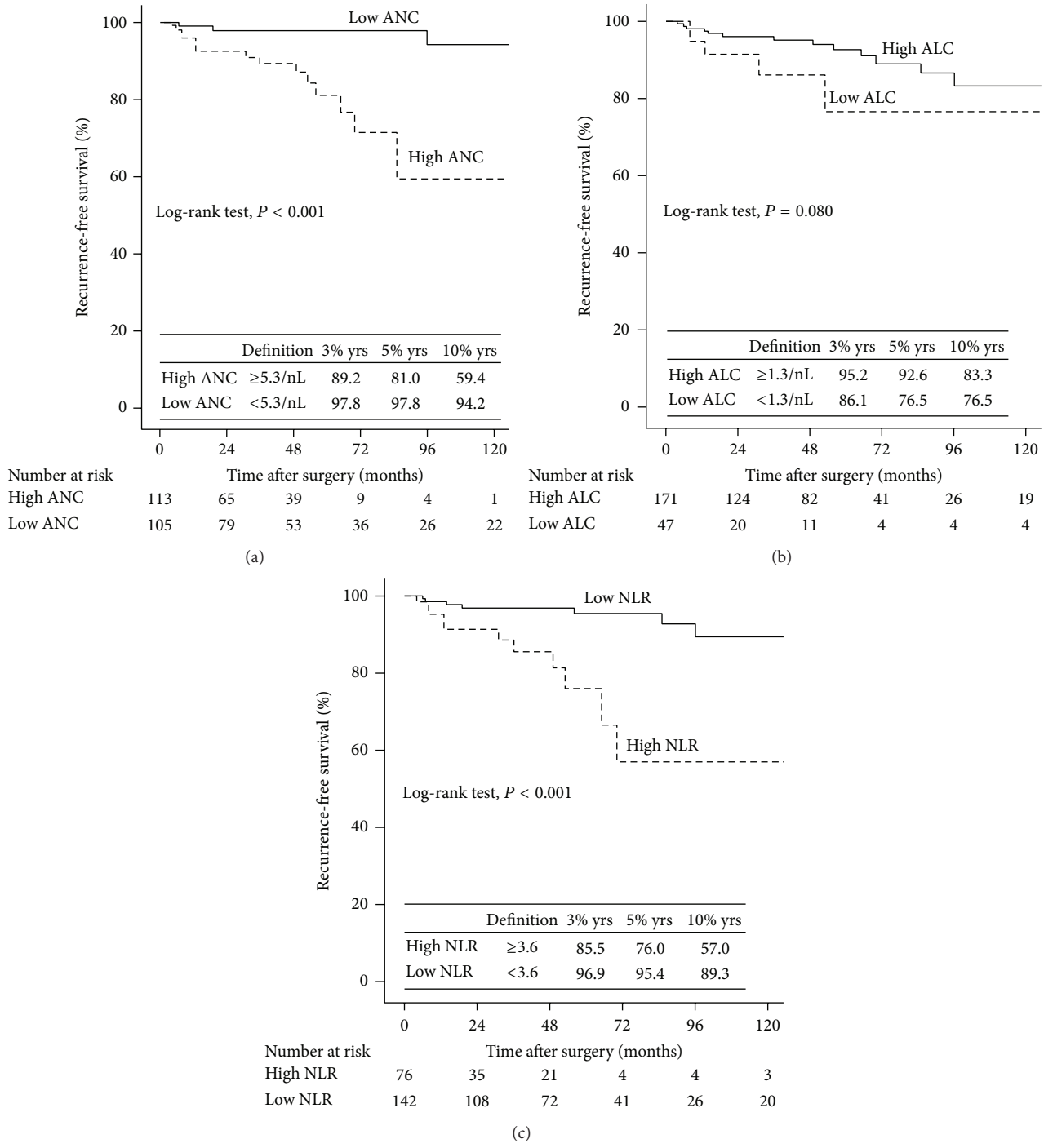


Figure 2: Kaplan-Meier curves for pRCC patients RFS groups categorized (a) by ANC, (b) ALC, and (c) NLR.

score were not evaluated in this study. In addition to the evaluation of the integration of the NLR and ANC to our base model, other prognostic models such as the Kattan nomogram [5] or the UISS model [6] should be evaluated. Nonetheless, even considering these limitations, our data clearly indicate that an increased pretreatment of NLR and ANC might represent an independent prognostic factor for RFS in localized pRCC patients.

5. Conclusions

In conclusion, an increased NLR and ANC are independent prognostic factors for RFS after surgery with curative intent for localized pRCC. They significantly increase the predictive accuracy of established prognostic factors. Therefore, we recommend adding NLR and ANC to traditional prognostic model, which may improve its predictive accuracy.

Table 4: Univariable and multivariable Cox regression models to predict RFS in 218 patients treated with nephrectomy with curative intent for pRCC.

Variables	Univariable HR (95% CI)	*P* value	Stepwise analyses HR (95% CI)	*P* value
Age greater than 60	0.74 (0.31–1.79)	0.507		
Gender (male versus female)	0.92 (0.34–2.54)	0.875		
Symptoms at presentation (yes versus no)	2.07 (0.79–5.41)	0.138		
Hypertension (yes versus no)	1.40 (0.56–3.46)	0.471		
Diabetes mellitus (yes versus no)	0.43 (0.06–3.24)	0.414		
pTNM stage				
T (III versus II versus I)	2.43 (1.50–3.92)	<0.001		
N1 versus pNx/0	5.82 (1.29–26.28)	0.022		
TNM group (III versus II versus I)	2.62 (1.62–4.26)	<0.001	2.19 (1.31–3.64)	0.003
Grade (G3-4 versus G1-2)	3.24 (1.31–7.99)	0.011	—	—
pRCC type (type 2 versus 1)	3.07 (1.21–7.77)	0.018	—	—
Tumor necrosis (yes versus no)	3.10 (1.23–7.80)	0.016	2.55 (0.97–6.70)	0.057
ANC (≥5.3/nL versus <5.3/nL)	8.64 (2.39–31.15)	0.001	4.71 (1.04–21.35)	0.045
ALC (≥1.3/nL versus <1.3/nL)	0.43 (0.16–1.14)	0.089		
NLR (≥3.6 versus <3.6)	5.27 (2.11–13.17)	<0.001	4.01 (1.26–12.73)	0.018

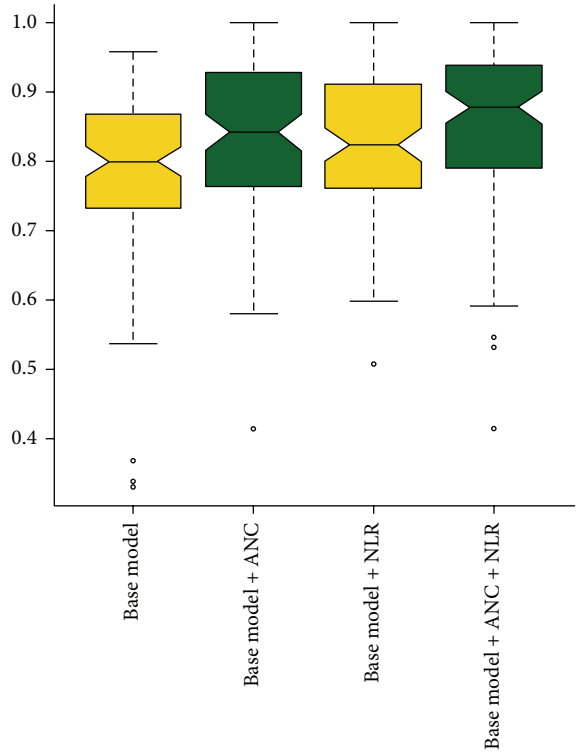


Figure 3: Comparison of the survival predictive power of base model (TNM stage + tumor necrosis) and integrated model combined with ANC, NLR.

6. Clinical Practice Points

Papillary renal cell carcinoma (pRCC) is the second most frequent histologic form of RCC and is characterized by distinct clinical behaviors and better outcome compared with ccRCC. Although neutrophil-to-lymphocyte ratio (NLR) was showed to serve as independent predictor of survival in ccRCC or RCC patients, which were composed of overwhelming majority of patients, the prognostic value of absolute neutrophil count (ANC), NLR in pRCC has not been investigated.

The findings of this study indicate that compared with patients with lower NLR and ANC, patients with higher NLR and ANC were more likely to have larger tumor size. NLR and ANC were independent prognostic factors

Table 5: Multivariable model of possible independent prognostic variables in pRCC patients.

Covariable	Categories	Multivariable HR (95% CI)	*P* value	Multivariable HR (95% CI)	*P* value	Multivariable HR (95% CI)	*P* value	Multivariable HR (95% CI)	*P* value
TNM stage	III versus II versus I	2.43 (1.49–3.98)	<0.001	2.34 (1.41–3.89)	0.001	2.39 (1.44–3.96)	0.001	2.21 (1.33–3.68)	0.002
Tumor necrosis	Yes versus no	2.19 (0.85–5.64)	0.103	2.72 (1.04–7.14)	0.042	2.68 (1.03–7.01)	0.044	2.83 (1.08–7.39)	0.034
ANC	≥5.3 versus <5.3			9.64 (2.46–37.76)	0.001			6.04 (1.38–26.40)	0.017
NLR	≥3.6 versus <3.6					5.98 (2.24–15.98)	<0.001	3.18 (1.12–9.05)	0.030
Predictive accuracy (%)		80.0		84.2		82.4		87.8	

for RFS of localized pRCC. Adding each factor into the clinicopathological base model could improve the predictive accuracy.

We recommend adding NLR and ANC to traditional prognostic model, which may improve its predictive accuracy. However, until now, NLR and ANC have not been yet included in well-known prognostic models for localized ccRCC, such as UISS, SSIGN models. So, there is still a long way to go to include preoperative NLR and ANC in prognostic models for pRCC patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Jiwei Huang and Douglas M. Dahl contributed equally to the paper.

Acknowledgment

The design and conduct of the project described were supported by Award no. 81402084 from the National Natural Science Foundation of China.

References

[1] J. C. Cheville, C. M. Lohse, H. Zincke, A. L. Weaver, and M. L. Blute, "Comparisons of outcome and prognostic features among histologic subtypes of renal cell carcinoma," *American Journal of Surgical Pathology*, vol. 27, no. 5, pp. 612–624, 2003.

[2] P. E. Teloken, R. H. Thompson, S. K. Tickoo et al., "Prognostic impact of histological subtype on surgically treated localized renal cell carcinoma," *The Journal of Urology*, vol. 182, no. 5, pp. 2132–2136, 2009.

[3] T. Klatte and A. J. Pantuck, "Molecular biology of renal cortical tumors," *Urologic Clinics of North America*, vol. 35, no. 4, pp. 573–580, 2008.

[4] T. Klatte, J. S. Lam, B. Shuch, A. S. Belldegrun, and A. J. Pantuck, "Surveillance for renal cell carcinoma: why and how? When and how often?" *Urologic Oncology: Seminars and Original Investigations*, vol. 26, no. 5, pp. 550–554, 2008.

[5] M. W. Kattan, V. Reuter, R. J. Motzer, J. Katz, and P. Russo, "A postoperative prognostic nomogram for renal cell carcinoma," *The Journal of Urology*, vol. 166, no. 1, pp. 63–67, 2001.

[6] A. Zisman, A. J. Pantuck, F. Dorey et al., "Improved prognostication of renal cell carcinoma using an integrated staging system," *Journal of Clinical Oncology*, vol. 19, no. 6, pp. 1649–1657, 2001.

[7] B. C. Leibovich, M. L. Blute, J. C. Cheville et al., "Prediction of progression after radical nephrectomy for patients with clear cell renal cell carcinoma: a stratification tool for prospective clinical trials," *Cancer*, vol. 97, no. 7, pp. 1663–1671, 2003.

[8] C. S. D. Roxburgh and D. C. McMillan, "Role of systemic inflammatory response in predicting survival in patients with primary operable cancer," *Future Oncology*, vol. 6, no. 1, pp. 149–163, 2010.

[9] Y. Ohno, J. Nakashima, M. Ohori, T. Hatano, and M. Tachibana, "Pretreatment neutrophil-to-lymphocyte ratio as an independent predictor of recurrence in patients with nonmetastatic renal cell carcinoma," *Journal of Urology*, vol. 184, no. 3, pp. 873–878, 2010.

[10] M. Pichler, G. C. Hutterer, C. Stoeckigt et al., "Validation of the pre-treatment neutrophil-lymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients," *British Journal of Cancer*, vol. 108, no. 4, pp. 901–907, 2013.

[11] S. Saroha, R. G. Uzzo, E. R. Plimack, K. Ruth, and T. Al-Saleem, "Lymphopenia is an independent predictor of inferior outcome in clear cell renal carcinoma," *Journal of Urology*, vol. 189, no. 2, pp. 454–460, 2013.

[12] Y. Ohno, J. Nakashima, M. Ohori, T. Gondo, T. Hatano, and M. Tachibana, "Followup of neutrophil-to-lymphocyte ratio and recurrence of clear cell renal cell carcinoma," *Journal of Urology*, vol. 187, no. 2, pp. 411–417, 2012.

[13] M. de Martino, A. J. Pantuck, S. Hofbauer et al., "Prognostic impact of preoperative neutrophil-to-lymphocyte ratio in localized nonclear cell renal cell carcinoma," *The Journal of Urology*, vol. 190, no. 6, pp. 1999–2004, 2013.

[14] R. Mehrazin, R. G. Uzzo, A. Kutikov et al., "Lymphopenia is an independent predictor of inferior outcome in papillary renal cell carcinoma," *Urologic Oncology*, 2014.

[15] G. Novara, V. Ficarra, A. Antonelli et al., "Validation of the 2009 TNM version in a large multi-institutional cohort of patients treated for renal cell carcinoma: are further improvements needed?" *European Urology*, vol. 58, pp. 588–595, 2010.

[16] S. A. Fuhrman, L. C. Lasky, and C. Limas, "Prognostic significance of morphologic parameters in renal cell carcinoma," *American Journal of Surgical Pathology*, vol. 6, no. 7, pp. 655–663, 1982.

[17] B. Delahunt and J. N. Eble, "Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors," *Modern Pathology*, vol. 10, no. 6, pp. 537–544, 1997.

[18] F. E. Harrell Jr., R. M. Califf, D. B. Pryor, K. L. Lee, and R. A. Rosati, "Evaluating the yield of medical tests," *The Journal of the American Medical Association*, vol. 247, no. 18, pp. 2543–2546, 1982.

[19] Y. Yuan, E. M. Van Allen, L. Omberg et al., "Assessing the clinical utility of cancer genomic and proteomic data across tumor types," *Nature Biotechnology*, vol. 32, no. 7, pp. 644–652, 2014.

[20] M. Gerlinger, A. J. Rowan, S. Horswell et al., "Intratumor heterogeneity and branched evolution revealed by multiregion sequencing," *The New England Journal of Medicine*, vol. 366, no. 10, pp. 883–892, 2012.

[21] M. de Martino, T. Klatte, C. Seemann et al., "Validation of serum C-reactive protein (CRP) as an independent prognostic factor for disease-free survival in patients with localised renal cell carcinoma (RCC)," *BJU International*, vol. 111, no. 8, pp. E348–E353, 2013.

[22] L. M. Coussens and Z. Werb, "Inflammation and cancer," *Nature*, vol. 420, no. 6917, pp. 860–867, 2002.

[23] H. K. Jensen, F. Donskov, N. Marcussen, M. Nordsmark, F. Lundbeck, and H. von der Maase, "Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma," *Journal of Clinical Oncology*, vol. 27, no. 28, pp. 4709–4717, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 802153, 6 pages
http://dx.doi.org/10.1155/2015/802153

Research Article

iTRAQ-Based Quantitative Proteomic Analysis Identified HSC71 as a Novel Serum Biomarker for Renal Cell Carcinoma

Yushi Zhang, Yi Cai, Hongyan Yu, and Hanzhong Li

Department of Urology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

Correspondence should be addressed to Hanzhong Li; lihanzhongdoc@126.com

Received 27 October 2014; Revised 14 February 2015; Accepted 2 March 2015

Academic Editor: Haifeng Yang

Renal cell carcinoma (RCC) is one of the most lethal urologic cancers and about 80% of RCC are of the clear-cell type (ccRCC). However, there are no serum biomarkers for the accurate diagnosis of RCC. In this study, we performed a quantitative proteomic analysis on serum samples from ccRCC patients and control group by using isobaric tag for relative and absolute quantitation (iTRAQ) labeling and LC-MS/MS analysis to access differentially expressed proteins. Overall, 16 proteins were significantly upregulated (ratio > 1.5) and 14 proteins were significantly downregulated (ratio < 0.67) in early-stage ccRCC compared to control group. HSC71 was selected and subsequently validated by Western blot in six independent sets of patients. ELISA subsequently confirmed HSC71 as a potential serum biomarker for distinguishing RCC from benign urologic disease with an operating characteristic curve (ROC) area under the curve (AUC) of 0.86 (95% confidence interval (CI), 0.76~0.96), achieving sensitivity of 87% (95% CI 69%~96%) at a specificity of 80% (95% CI 61~92%) with a threshold of 15 ng/mL. iTRAQ-based quantitative proteomic analysis led to identification of serum HSC71 as a novel serum biomarker of RCC, particularly useful in early diagnosis of ccRCC.

1. Introduction

Renal cell carcinoma (RCC) is the most frequent form of kidney cancer, with an increasing incidence over the past decades [1]. The majority of RCC are of the clear-cell type (ccRCC), which accounts for approximately 80% of kidney cancer [2]. Early diagnosis of RCC is one of the most important factors contributing to the successful treatment and favorable prognosis. The diagnosis of RCC is mainly based on imaging findings, which however has limited accuracy and cannot be used reliably to confirm the nature of the lesion [3]. Due to the increase of disease rates, together with the fact that there are no serum biomarkers available, inexpensive and noninvasive test of prediction for RCC would be urgently required for the early detection of RCC. Serum and plasma, containing proteins both secreted and shed from tumor cells, are ideal fluids for the detection of cancer biomarkers since they are characterized by ease of sampling and storing. However, their variable composition and vast dynamic range of proteins present in serum pose tremendous technical challenges in identifying clinically relevant biomarkers [4]. A recently novel proteomics named isobaric tags for relative and absolute quantification (iTRAQ) combined with mass spectrometry technology (LC-MS/MS) now represents a powerful tool for identification of cancer biomarkers [5]. The iTRAQ technology has been successfully applied to biomarker screening of multiple tumors and diseases in both tissue and serum samples [6].

In this study, we performed quantitative proteomic analysis using the iTRAQ and LC-MS/MS to identify proteins dysregulated in serum of early-stage ccRCC patients compared to healthy people. We revealed differential expression of a number of proteins in serum of ccRCC patients compared with control group. In addition, we confirmed the most dysregulated expression of heat shock cognate 71 kDa protein (HSC71) on six independent sets of serum by Western blot. ELISA subsequently confirmed HSC71 as a potential serum biomarker for diagnosis of RCC.

Table 1: Description and comparison of clinical and laboratory characteristics of the study subjects.

Characteristic	Discovery samples by iTRAQ		Validation samples by ELISA	
	RCC ($n = 10$)	Control ($n = 10$)	RCC ($n = 30$)	Control ($n = 30$)
Age (years)	54.80 ± 13.32	52.80 ± 9.13	53.10 ± 13.33	55.63 ± 13.98
Gender (male/female)	7/3	7/3	18/12	16/14
BMI	22.86 ± 2.57	22.55 ± 2.55	21.78 ± 2.83	22.16 ± 3.02
Hypertension	4 (40%)	2 (20%)	6 (20%)	4 (13.3%)
Diabetes mellitus	2 (20%)	1 (10%)	5 (16.7%)	3 (10%)
Smoking	3 (30%)	3 (30%)	7 (23.3%)	6 (20%)
Pathological stage				
pT1	10 (100%)		16 (53.3%)	
pT2			11 (36.7%)	
pT3			2 (6.7%)	
pT4			1 (3.3%)	
M0	10 (100%)		28 (93.3%)	
M1			2 (6.7%)	
Fuhrman grades				
G1-2	10 (100%)		21 (70%)	
G3-4			9 (30%)	
Histological subtype				
Clear-cell	10 (100%)		23 (76.7%)	
Papillary			3 (10%)	
Chromophobe			4 (13.3%)	

Data are expressed as n (%), or mean ± SD. RCC: renal cell carcinoma.

2. Materials and Methods

2.1. Patients and Serum Collection. This study was approved by the Human Ethics Committee of Peking Union Medical College Hospital. Serum samples from healthy volunteers and patients were all collected from the Department of Urology at Peking Union Medical College Hospital between October 2013 and October 2014. The control group consisted of 10 healthy controls and 20 patients diagnosed with other urologic diseases such as angiomyolipoma of kidney (10 patients), benign prostatic hyperplasia (4 patients), urinary tract infection (4 patients), and urolithiasis (2 patients). The detailed demographic profiles of the participants are provided in Table 1. All samples were collected before breakfast, then centrifuged at 3000 g for 15 min at 4°C, and subsequently stored at −80°C prior to further processing. All serum samples were collected before any treatment or surgery.

2.2. Affinity Depletion of Serum Samples. To reduce the individual differences, sera collected were pooled into two groups, each containing sera from 10 RCC patients or 10 healthy people. Pooled serum samples were depleted of the 14 high-abundance proteins using a high capacity 4.6 × 100 mm multiple affinity removal column (Agilent Technologies, CA). Approximately 35 μL of serum was processed per sample and analyzed with the Agilent 1260 HPLC system according to the manufacturer's protocol. Protein concentrations were determined using a Bradford protein assay kit (Pierce). All measurements were performed in duplicate.

2.3. iTRAQ Labeling and Strong Cation Exchange Liquid Chromatography. Prior to iTRAQ labeling, the samples were concentrated and desalted using 10 kDa molecular weight cutoff spin concentrators (Millipore). Then, 100 μg peptides from each sample were labeled with the iTRAQ reagents according to the manufacturer's instructions and as previously described [7]. Peptides were labeled individually with iTRAQ tag (Applied Biosystems, USA) as follows: 114.1 for ccRCC and 115.1 for control group, respectively. To reduce sample complexity, labeled samples were pooled and fractionated by strong cation exchange (SCX) using a Polysulfoethyl A column (PolyLC Inc., USA) as previously described [7].

2.4. Reverse-Phase LC-MS/MS Analysis. Dried SCX fractions were reconstituted in 100 mL of buffer A (5% acetonitrile; 95% H_2O; 0.1% formic acid) and loaded into a reverse-phase C18 Peptide Captrap (Agilent Technologies, USA). After desalting, peptides were eluted by running a 5% to 80% buffer B gradient (95% acetonitrile; 5% H_2O; 0.1% formic acid) at a flow rate of 0.4 μL/min for 65 min. The LC elution was subjected to positive-ion nanoflow electrospray analysis using a Qstar XL MS/MS system (Applied Biosystems Inc., USA). A survey scan was acquired from m/z 400–1800 for

0.5 s with up to four precursor ions selected from m/z 100–2000 for MS/MS. Each fraction from SCX chromatography was analyzed in duplicate.

2.5. Western Blot Analysis. The 14 most abundant proteins were depleted from sera from 6 RCC patients and 6 healthy people using a high capacity 4.6 × 100 mm multiple affinity removal column. Total protein concentration was determined with Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, USA). Protein samples (10 μg) were separated on a 10% SDS-PAGE gel, transferred to PVDF membranes, and probed with rabbit polyclonal antibodies to HSC71 (R&D, USA) overnight at 4°C. As there are no reliable internal control proteins for Western blot analysis of the serum samples, a loading control sample was generated by pooling all of the samples from all groups in equal volumes (20 μL) for each gel. The optical intensity of each protein staining was determined using Quantity One. The loading control sample in each gel was used as the standard for quantification.

2.6. ELISA Analysis. Human heat shock cognate 71 kDa protein ELISA kit from Huamei Biological Inc. (Wuhan, China) was purchased and used according to the manufacturer's instruction. The serum levels of HSC71 were assayed in the 30 RCC patients, 10 healthy controls, and 20 other urologic diseases patients. All serum samples and the standards were run in duplicate.

2.7. Statistical Data and Graphics. We performed the entire statistical analysis with SPSS 19.0. Student's t-test was applied for comparisons of quantitative data. We performed operating characteristic curve (ROC) analysis to quantify serum HSC71 positivity and statistical uncertainty. Data are expressed as the mean ± standard deviation (M ± SD). For all analysis, a P value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Quantitative Proteomic Analysis of Serum Proteins by iTRAQ. To identify dysregulated serum proteins between ccRCC patients and control group, we compared 10 ccRCC patients' sera and 10 healthy people's sera by iTRAQ quantitative proteomic analysis. Among 375 identified proteins, 16 proteins were increased more than 1.5-fold and 14 proteins were decreased less than 0.67-fold in the serum of early-stage ccRCC patients compared to that of healthy controls (Table 2). Furthermore, serum HSC71 was highly elevated compared to control group (3.07-fold upregulated expression) and was evaluated further for its potential as a serum biomarker for ccRCC.

3.2. Validation of Serum HSC71 Upregulation by Western Blot. To confirm the dysregulated expression of HSC71 in ccRCC patients and healthy controls sera, we performed Western blot analysis in six sets of discovery cohort individually (Figure 1(a)). Quantitative analysis showed that HSC71 was significantly upregulated in sera of ccRCC ($P = 0.0037$) (Figure 1(b)), in agreement with our iTRAQ quantitative proteomic findings.

3.3. Validation of Serum HSC71 as a Novel Biomarker for ccRCC by ELISA. ELISA, performed in 30 ccRCC patients, 10 healthy people, and 20 patients with other urologic diseases (Table 1), showed significantly higher serum HSC71 levels in ccRCC patients versus the control group (10 healthy people and 20 other urologic diseases patients) (Figure 2(a)). These observations were consistent with the results obtained by iTRAQ analysis and Western blot, and the HSC71 concentrations obtained by ELISA were used to assess the clinical performances of the serum HSC71. The diagnostic values of serum HSC71 were evaluated by ROC curve analysis. The area under the receiver-operating characteristic curve (AUC) for differentiating between ccRCC and control group was 0.86 (95% confidence interval (CI), 0.76~0.96), achieving sensitivity of 87% (95% CI 69%~96%) at a specificity of 80% (95% CI 61~92%) with a threshold of 15 ng/mL (Figure 2(b)).

4. Discussion

In this study, we initially used two pooled samples for proteomic studies to identify the biomarker candidates using iTRAQ and LC–MS/MS. HSC71 was strongly increased in the sera samples from the RCC patients compared to control group. Then, we examined serum HSC71 levels of six sets of screening samples by Western blotting individually and confirmed that the data were comparable with quantitative proteomic analysis. Finally, we examined the clinical significance of HSC71 by ELISA in 30 RCC patients, 20 other urologic disease patients, and 10 healthy people to evaluate the clinical utilities of the candidate with ROC curve analysis. We demonstrated for the first time that serum HSC71 concentration is a biomarker for the diagnosis of RCC.

HSC71, also known as heat shock 70 kDa protein 8 (HSPA8), is a member of the heat shock protein 70 family, located on chromosome 11q23.3. It is constitutively expressed under nonstressful conditions and also participated in protein folding as well as differentiation procedures [8, 9]. Previous studies showed that upregulation of HSC71 in leukemic cells contributes to cell cycle disruption, and HSC71 binding to cyclin D1 in nuclei leads to the stabilization of the cyclin D1/CDK4 complex, promoting cell proliferation [10]. High levels of HSP70 family expression have been demonstrated in human cancers, which appear at least of prognostic value in osteosarcoma [11, 12]. In the previous studies, heat shock proteins (HSPs) family expression is induced in a variety of kidney diseases suggesting that specific HSPs may have distinct functions in renal malignancies [13]. However, to our knowledge, no studies have evaluated the value of serum HSC71 in the potential diagnosis role for RCC.

Here we report raised HSC71 concentrations in sera from RCC patients compared with healthy people and other urologic diseases. HSPs have gained interest as a promising anticancer drug target, due to its importance in maintaining the stability, integrity, conformation, and function of key oncogenic proteins, including HSP40, HSP70, and HSP90

Table 2: List of differentially expressed proteins in ccRCC compared to control group.

Accession	Description	Score	Coverage	Ratio
	Proteins upregulated in ccRCC compared with control group			
P11142	Heat shock cognate 71 kDa protein	31.11	4.80%	3.07
P02763	Alpha-1-acid glycoprotein 1	251.48	40.80%	2.087
Q16777	Histone H2A type 2-C	164.19	49.61%	1.984
P68871	Hemoglobin subunit beta	320.13	60.54%	1.942
Q00532	Cyclin-dependent kinase-like 1	20.04	1.96%	1.828
P02748	Complement C9	984.63	41.86%	1.815
P02787	Serotransferrin	1689.45	61.46%	1.775
P07360	Complement component C8 gamma chain	466.53	61.39%	1.753
P02768	Serum albumin	2997	82.76%	1.705
P69905	Hemoglobin subunit alpha	140.62	26.76%	1.697
P25311	Zinc-alpha-2-glycoprotein	275.61	35.91%	1.673
P01031	Complement C5	2686.11	49.16%	1.646
P68363	Tubulin alpha-1B chain	127.08	6.21%	1.605
P00738	Haptoglobin 1 alpha	922.06	59.61%	1.58
Q12931	Heat shock protein 75 kDa	43.68	1.99%	1.546
Q8NG11	Tetraspanin-14	34.11	2.96%	1.518
	Proteins downregulated in ccRCC compared with control group			
Q96KN2	Beta-Ala-His dipeptidase	142.84	12.62%	0.651
Q5T686	Arginine vasopressin-induced protein 1	45.27	4.08%	0.643
P01614	Ig kappa chain V-II region Cum	83.17	33.04%	0.627
P55056	Apolipoprotein C-IV	189.85	40.94%	0.622
Q16610	Extracellular matrix protein 1	643.87	34.44%	0.614
Q96KK5	Histone H2A type 1-H	177.42	50.00%	0.606
P14618	Pyruvate kinase PKM	47.37	3.95%	0.582
P33908	Mannosyl-oligosaccharide	60.74	4.75%	0.495
P01019	Angiotensinogen	620.93	28.45%	0.426
O60814	Histone H2B type 1-K	67.21	15.87%	0.419
Q15166	Serum paraoxonase/lactonase 3	138.75	11.86%	0.415
P68431	Histone H3.1	24.05	5.15%	0.377
P01815	Ig heavy chain V-II region COR	12.87	5.83%	0.37
P62805	Histone H4	171.24	45.63%	0.353

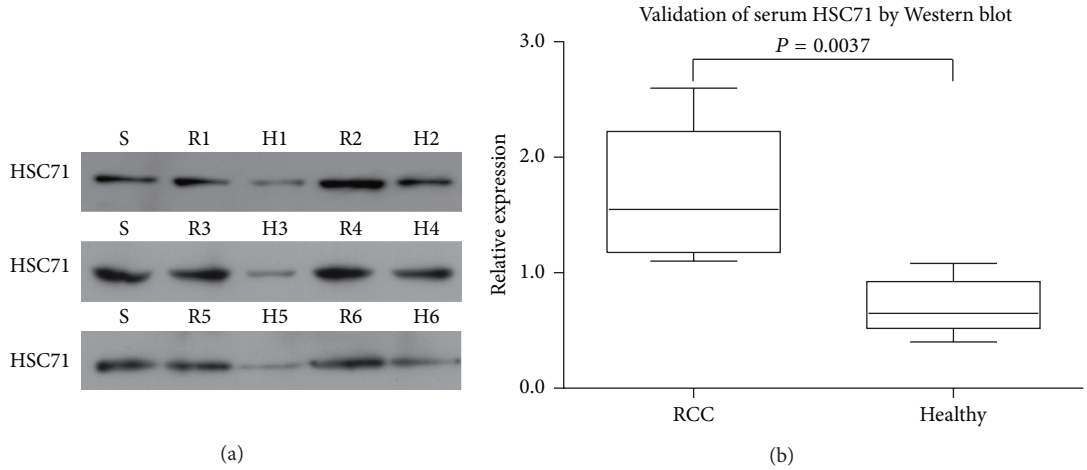


Figure 1: Validation of the serum HSC71 by Western blotting. (a) Serum HSC71 level was further examined using Western blotting in 6 additional serum samples from ccRCC patients (R1–R6) and healthy people (H1–H6). (b) Quantification of the densitometric analysis of the Western blotting bands was performed. The average concentration of HSC71 was 2.39-fold ($P = 0.0037$) higher in the RCC groups than in the Healthy group.

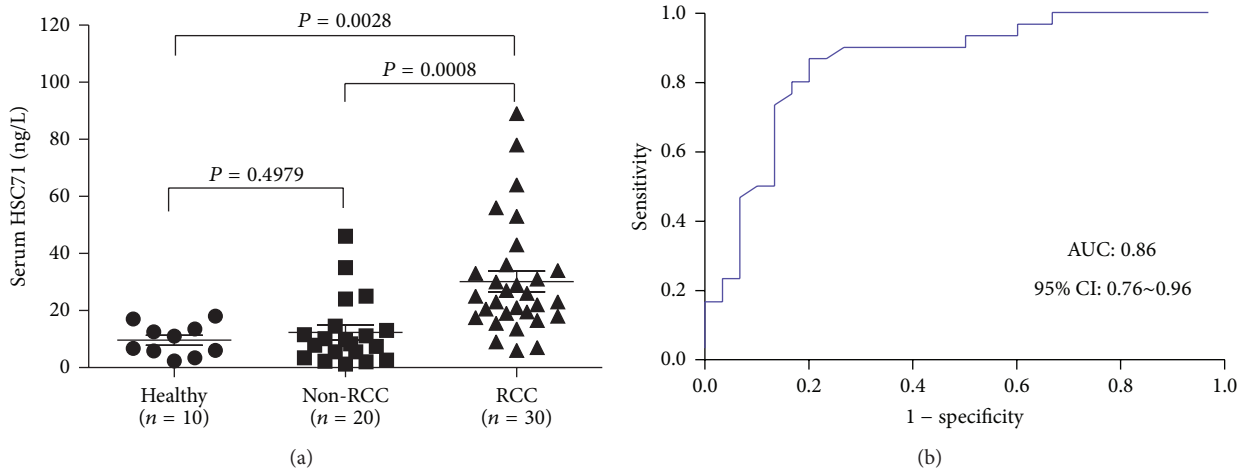


Figure 2: Validation of the diagnostic value of serum HSC71 concentration in RCC by ELISA. (a) The serum HSC71 concentration in 30 RCC patients, 10 healthy people, and 20 other urologic diseases (Non-RCC) was determined using ELISA. The mean concentrations of serum HSC71 in Healthy, Non-RCC, and RCC groups were 9.64, 12.34, and 30.17 ng/L, respectively. Both Healthy and Non-RCC groups showed significantly lower levels of HSC71 than the RCC group ($P = 0.0028$, $P = 0.0008$). (b) The receiver-operating characteristic curve (ROC curve) for the serum levels of HSC71 in patients with RCC compared with control group. The area under each curve is 0.86 (95% CI, 0.76~0.96).

family members [14, 15]. In addition, regarding the differentially expressed proteins, some of these were previously reported to be involved in RCC such as serum albumin [16], haptoglobin [17], and alpha-1-acid glycoprotein 1 [18], which provides confidence to our dataset and provides an independent confirmation of these candidates. In addition, previous studies have reported that prostate cancer, colon cancer, and squamous cell lung cancer may secrete common plasma proteins such as zinc-alpha-2-glycoprotein [19, 20] and complement C9 [21]. However, those candidate biomarkers need further research to confirm the clinical utility of as biomarker for diagnosis of RCC.

Clinical applications of serum HSC71 may require additional investigation. First, our results were obtained only for pT1-pT3 RCC patients; therefore, more pT4 and metastasis RCC patients should be included in the further validation studies. In addition, we could not show statistically significant correlation between serum HSC71 levels and different stages of RCC patients because of small patient numbers. The potential association of serum HSC71 with progression of RCC or tumor load requires further investigation using a larger sample. Second, the optimal cutoff value should be determined in a larger number of serum samples. Any single marker may not be sufficient for the final decision about therapeutic strategy, and all clinical and pathological information should be considered when using biomarker data. We do not consider that HSC71 is the only and best biomarker protein for RCC and the additional biomarker candidates should be further investigated using the other proteomic modalities.

5. Conclusion

In conclusion, using iTRAQ-based comparative proteomic analysis and validating by Western blotting and ELISA analysis, we identified serum HSC71 concentration as a novel biomarker for the diagnosis of RCC. Further investigation of the origin of serum HSC71 and the mechanisms underlying the correlation between serum HSC71 and carcinogenesis will allow greater insights into RCC biology.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yushi Zhang and Yi Cai contributed equally to this work.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant 30772165) and the Wu Jieping Medical Foundation (Grant 320.6750.13257).

References

[1] R. Siegel, J. Ma, Z. Zou, and A. Jemal, "Cancer statistics, 2014," *CA: Cancer Journal for Clinicians*, vol. 64, no. 1, pp. 9–29, 2014.

[2] A. Lopez-Beltran, J. C. Carrasco, L. Cheng, M. Scarpelli, Z. Kirkali, and R. Montironi, "2009 update on the classification of renal epithelial tumors in adults," *International Journal of Urology*, vol. 16, no. 5, pp. 432–443, 2009.

[3] M. S. Feldstein, D. J. Rhodes, A. S. Parker, R. R. Orford, and E. P. Castle, "The haphazard approach to the early detection of asymptomatic renal cancer: results from a contemporary executive health programme," *BJU International*, vol. 104, no. 1, pp. 53–56, 2009.

[4] L. A. Echan, H.-Y. Tang, N. Ali-Khan, K. Lee, and D. W. Speicher, "Depletion of multiple high-abundance proteins improves protein profiling capacities of human serum and plasma," *Proteomics*, vol. 5, no. 13, pp. 3292–3303, 2005.

[5] S. Tonack, M. Aspinall-O'Dea, R. E. Jenkins et al., "A technically detailed and pragmatic protocol for quantitative serum proteomics using iTRAQ," *Journal of Proteomics*, vol. 73, no. 2, pp. 352–356, 2009.

[6] D. Xu, Y. Li, X. Li et al., "Serum protein S100A9, SOD3, and MMP9 as new diagnostic biomarkers for pulmonary tuberculosis by iTRAQ-coupled two-dimensional LC-MS/MS," *Proteomics*, vol. 15, no. 1, pp. 58–67, 2015.

[7] L.-R. Yan, D.-X. Wang, H. Liu et al., "A pro-atherogenic HDL profile in coronary heart disease patients: an iTRAQ labelling-based proteomic approach," *PLoS ONE*, vol. 9, no. 5, Article ID e98368, 2014.

[8] B. Bukau, E. Deuerling, C. Pfund, and E. A. Craig, "Getting newly synthesized proteins into shape," *Cell*, vol. 101, no. 2, pp. 119–122, 2000.

[9] Y. Matsuda, T. Ishiwata, H. Yoshimura, M. Hagio, and T. Arai, "Inhibition of nestin suppresses stem cell phenotype of glioblastomas through the alteration of post-translational modification of heat shock protein HSPA8/HSC71," *Cancer Letters*, vol. 357, no. 2, pp. 602–611, 2015.

[10] E. S. José-Enériz, J. Román-Gómez, L. Cordeu et al., "BCR-ABL1-induced expression of HSPA8 promotes cell survival in chronic myeloid leukaemia," *British Journal of Haematology*, vol. 142, no. 4, pp. 571–582, 2008.

[11] C. Sarto, P.-A. Binz, and P. Mocarelli, "Heat shock proteins in human cancer," *Electrophoresis*, vol. 21, no. 6, pp. 1218–1226, 2000.

[12] K. Trieb and R. Kotz, "Proteins expressed in osteosarcoma and serum levels as prognostic factors," *International Journal of Biochemistry and Cell Biology*, vol. 33, no. 1, pp. 11–17, 2001.

[13] F.-X. Beck, W. Neuhofer, and E. Müller, "Molecular chaperones in the kidney: distribution, putative roles, and regulation," *American Journal of Physiology: Renal Physiology*, vol. 279, no. 2, pp. F203–F215, 2000.

[14] R. E. Wang, "Targeting heat shock proteins 70/90 and proteasome for cancer therapy," *Current Medicinal Chemistry*, vol. 18, no. 27, pp. 4250–4264, 2011.

[15] W. Wen, W. Liu, Y. Shao, and L. Chen, "VER-155008, a small molecule inhibitor of HSP70 with potent anti-cancer activity on lung cancer cell lines," *Experimental Biology and Medicine*, vol. 239, no. 5, pp. 638–645, 2014.

[16] M. Stenman, A. Laurell, and M. Lindskog, "Prognostic significance of serum albumin in patients with metastatic renal cell carcinoma," *Medical Oncology*, vol. 31, no. 3, article 841, 2014.

[17] J. Tolson, R. Bogumil, E. Brunst et al., "Serum protein profiling by SELDI mass spectrometry: detection of multiple variants of serum amyloid alpha in renal cancer patients," *Laboratory Investigation*, vol. 84, no. 7, pp. 845–856, 2004.

[18] B. Ljungberg, K. Grankvist, and T. Rasmuson, "Serum acute phase reactants and prognosis in renal cell carcinoma," *Cancer*, vol. 76, no. 8, pp. 1435–1439, 1995.

[19] I. Katafigiotis, S. I. Tyritzis, K. G. Stravodimos et al., "Zinc α2-glycoprotein as a potential novel urine biomarker for the early diagnosis of prostate cancer," *BJU International*, vol. 110, no. 11, pp. E688–E693, 2012.

[20] Y. Xue, F. Yu, D. Yan et al., "Zinc-alpha-2-glycoprotein: a candidate biomarker for colon cancer diagnosis in Chinese population," *International Journal of Molecular Sciences*, vol. 16, no. 1, pp. 691–703, 2015.

[21] A. Narayanasamy, J.-M. Ahn, H.-J. Sung et al., "Fucosylated glycoproteomic approach to identify a complement component 9 associated with squamous cell lung cancer (SQLC)," *Journal of Proteomics*, vol. 74, no. 12, pp. 2948–2958, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 598134, 8 pages
http://dx.doi.org/10.1155/2015/598134

Research Article

Growth Pattern of Clear Cell Renal Cell Carcinoma in Patients with Delayed Surgical Intervention: Fast Growth Rate Correlates with High Grade and May Result in Poor Prognosis

Lei Zhang,[1] Wenshi Yin,[1] Lin Yao,[1] Xuesong Li,[1] Dong Fang,[1] Da Ren,[1] Zhongyuan Zhang,[1] Yu Fan,[1] Qun He,[1] Weimin Ci,[2] Zhisong He,[1] and Liqun Zhou[1]

[1]*Department of Urology, Peking University First Hospital, Institute of Urology, Peking University, National Urological Cancer Center, Beijing 100034, China*
[2]*Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, China*

Correspondence should be addressed to Xuesong Li; pineneedle@sina.com and Liqun Zhou; zhouliqunmail@sina.com

Received 19 October 2014; Accepted 8 March 2015

Academic Editor: Paul L. Crispen

Objectives. Previous studies revealed an unclear correlation between the growth rate of renal cell carcinoma (RCC) and tumor grade and did not focus on certain histological subtype. This report investigated the correlation between the growth rate and tumor grade in clear cell RCC (ccRCC). *Methods*. We reviewed 60 patients with 61 ccRCC confirmed by delayed surgeries after at least 12 months of active surveillance. The linear growth rate (LGR), volumetric growth rate (VGR), and volume doubling time (VDT) were calculated, and their correlations with clinicopathologic characteristics were analyzed. *Results*. The mean LGR, VGR, and VDT were 0.86 (range 0–4.74) cm/year, 20.96 (range 0.31–211.93) cm^3/year, and 667 (range 33–3321) days, respectively. ccRCCs with high grade had greater LGR ($P < 0.001$) and VGR ($P = 0.001$) and lower VDT ($P = 0.017$) than ccRCCs with low grade. Grade (OR = 5.185, $P = 0.004$) was the only independent risk factor of LGR >0.5 cm/year, and grade (OR = 3.006, $P = 0.046$) and initial size (OR = 0.392, $P = 0.004$) were independent risk factors of VDT <1 year. Five patients developed metastasis after surgery with LGR >0.5 cm/yr altogether; of them, four had cancer-related death by the last follow-up. *Conclusions*. Fast growth rate of ccRCC is significantly correlated with high tumor grade and may result in poor prognosis, especially for those with LGR >0.5 cm/yr.

1. Introduction

Due to high surgical comorbidity or short life expectancy for certain patients, active surveillance (AS) for renal tumors is being applied selectively by urologists in clinical practice. Although the risk of metastasis progression during AS is only approximately 2% [1], there is no effective systemic therapy for RCC. Inability of identifying lethal RCC is the most major problem while performing AS at present. Growth rate of renal tumors is believed to be the main trigger for intervention during AS. Anyway, there is no evidence supporting that the growth rate of RCC during AS is related to its prognosis so far. Because of the limitation of small sample size, the lack of pathological diagnosis, the generally favorable prognosis of RCC, and the follow-up not long enough until the cancer-related death occurred, it is hard to directly figure out whether fast growth rate under AS is related to poor prognosis of RCC.

Tumor grade is one of the most powerful prognostic factors for RCC [2]. The median 5-year RCC-specific survival is 94%, 86%, 59%, and 31% in patients with Fuhrman grades I, II, III, and IV RCC, respectively [3]. Hence, if the fast growth rate of RCC during AS is correlated with high tumor grade, we could indirectly believe that fast growth rate of RCC during AS could result in poor prognosis.

Although a prospective study with biopsy before AS is appropriate to investigate the correlation between the growth rate of RCC and grade intuitively, biopsy has the weakness in grading renal tumors comparing with surgical specimens. Hence, a retrospective study enrolling patients receiving delayed surgeries after AS is the unique opportunity to resolve this problem. However, only a few articles on this subject are available [4, 5]. All the available studies included small sample size and did not focus on a certain histological subtype.

The biological behavior of RCC is different by histological subtypes. Grade 1 clear cell RCC may grow faster than grade 2 papillary RCC. Hence, the correlation between growth rate and grade of RCC is still unclear. For example, our previous studies confirmed a significantly higher growth rate in grade 2 RCC compared with grade 1 RCC; however, the growth rate of grade 3 was not significantly different from that of grade 1 or 2 RCC [4].

Clear cell RCC (ccRCC) accounts for 70–80% of all RCCs and is characterized as the common aggressive behavior compared to other subtypes [6]. Our previous study demonstrated that the growth rate of RCC tended to correlate with the histologic subtype and that ccRCC tended to grow faster than papillary cell carcinoma [4]. Hence, understanding the growth behavior of ccRCC is the most beneficial among all the subtypes.

On basis of these thoughts, we expand sample size and further focused on ccRCC to investigate the correlation between growth rate and tumor grade by reviewing 61 patients who had received delayed surgeries after at least 12 months of AS for renal tumors that later were confirmed to be ccRCC pathologically. In addition, we report our experience of AS with long-term of follow-up after surgery and characterize the ccRCC with failure of cancer control after delayed surgery.

2. Patients and Methods

2.1. Patient Selection. We retrospectively reviewed the kidney cancer databases at the Institute of Urology, Peking University, to identify patients with renal masses treated by AS initially for at least 12 months between January 1990 and August 2014. A total of 90 patients with 91 renal tumors were included. Patients without delayed surgical treatment until the last follow-up were excluded, and only ccRCC cases confirmed by surgical pathology were included. Patients with Von Hippel-Lindau syndrome or history of hereditary RCC were excluded. A total of 60 patients with 61 ccRCCs were included in the analysis.

2.2. Imaging Examination and Measurement of Lesions. During the period of AS, CT or MRI was performed at least every 6 months. When possible, the measurements were performed based on the same technique. All the images were reviewed by a professional radiologist and a urologic oncologist. The tumor size was defined as the maximal diameter of the tumor recorded during each imaging procedure. The linear growth rate (LGR) of a tumor was defined as the mean growth rate of the maximal diameter on a series of 2-dimensional images. The tumor volume was calculated as described in a previous study [7]: if 3 dimensions were present, the formula $0.5326xyz$ was employed; if 2 dimensions were available, the formula $0.5326xy(x + y/2)$ was used; if only 1 dimension was reported, the formula for volume of a sphere 0.5236×3 was employed. The volumetric growth rate (VGR) was defined as the average change in tumor volume per year. In addition, the volume doubling time (VDT) was also calculated based on the Schwartz equation, as previously described [8]: $\text{VDT} = (T - T_0) \times \log 2 / \log(V/V_0)$ (T: the date of the final imaging procedure, T_0: the date of the initial imaging procedure, V: the volume at the final imaging evaluation, and V_0: the volume at the initial imaging evaluation).

2.3. Pathological Examinations. Due to tumor growth, obvious enhancement on CT, or metastatic lesion, delayed surgical intervention was performed on all patients at Peking University First Hospital after a mean of 39.5 months of AS. All surgical specimens were reviewed by two senior pathologists who were blinded to the patients' personal data. The pathological results confirmed ccRCC for all tumors. The histological classification was determined by the Heidelberg typing system. The tumor stage was assessed according to the 2002 American Joint Committee on Cancer TNM staging system, and tumor grading was performed according to the Fuhrman grade system.

2.4. Statistical Analysis. The chi-squared test was used to test the distribution of categorical variables. The correlations between two continuous variables were assessed by calculating Pearson's correlation coefficient. The Mann-Whitney U or Kruskal-Wallis H test was used to compare two or three groups of continuous variables. A logistic regression analysis was used to identify the independent risk factors of an LGR <0.5 cm/year and a VDT <1 year. The SPSS v.14.0 software package (SPSS Inc., Chicago, IL, USA) was used for data processing. $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. AS of ccRCC. A total of 60 patients with 61 ccRCCs treated by delayed treatment after at least 12 months of AS were identified for analysis. The clinical, demographic, and tumor characteristics are summarized in Table 1. Of the 61 tumors, 49 (80.3%) were asymptomatic for the entire clinical course and diagnosed incidentally during imaging procedures for physical examination; the other 12 cases had complaints of flank pain or occasional hematuria at presentation. The reasons that patients initially choose AS included patient preference (41 of 60, 68.3%), a benign diagnosis considered at presentation (15 of 60, 25.0%), the existence of bilateral disease (2 of 60, 3.3%), and concomitant malignancy (2 of 60, 3.3%). Of the 60 patients, 48 (78.7%) were male, and 12 (21.3%) were female. The mean patient age was 55 years (range, 26–81).

After a mean AS of 39.5 months, the mean tumor size increased from 2.32 cm (range, 0.10–6.70) at presentation to 4.44 cm (range, 1.40–11.80). The distribution of tumor size is shown in Figure 1; of 52 (85.2%) tumors, the initial tumor size was ≤4 cm at presentation. Stage progression was documented in 22 tumors: 15 tumors progressed from T1a to T1b, 6 tumors progressed from T1a to T2, and 1 tumor progressed from T1b to T2. No disparity between pT stage and cT stage at operation was found for any tumor. Only one patient (1.7%) developed metastatic disease during AS. The patient presented with a biopsy-proven metastasis ccRCC in the lung at the 155th month of AS; during this period, the primary tumor size increased from 1.6 cm to 4.4 cm.

Table 1: Patient demographics and tumor characteristics.

Sex	
Men (%)	48 (78.7)
Women (%)	13 (21.3)
Age, yr	
Median	56
Mean	55
Range	26–81
Side	
Left (%)	32 (52.5)
Right (%)	29 (47.5)
Initial tumor size	
Maximal diameter, cm	
Median	1.90
Mean	2.32
Range	0.10–6.70
Volume, cm^3	
Median	3.591
Mean	11.186
Range	0.001–82.967
Final tumor size	
Maximal diameter, cm	
Median	4.00
Mean	4.44
Range	1.40–11.8
Volume, cm^3	
Median	28.595
Mean	66.992
Range	0.982–560.017
Duration of AS, mo	
Median	27.00
Mean	39.5
Range	12–155
Grade	
1	13
2	38
3	10
Pathological stage	
T1a	30
T1b	20
T2	6
T3	5

AS: active surveillance; LGR: linear growth rate; DT: doubling time.

Although this tumor did not grow fast, the mean LGR was 0.20 cm/year.

The indication of surgical intervention included tumor growth, the presence of obvious enhancement on CT, or metastatic lesion (palliative excision of the primary lesion). Forty-four of the 61 tumors (72.1%) were treated by radical nephrectomy; the other 17 (27.9%) tumors were treated by partial nephrectomy. The pathological results confirmed ccRCC in all 61 tumors. Thirteen tumors (21.3%) were grade 1, 38 tumors (62.3%) were grade 2, and 10 tumors (16.4%) were grade 3.

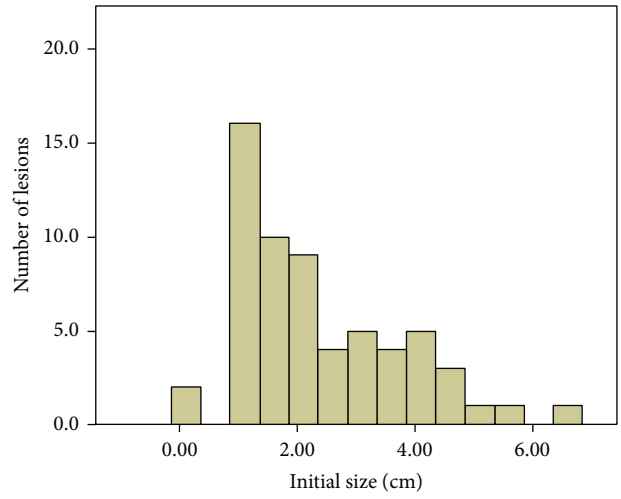


Figure 1: Distribution of initial tumor sizes of ccRCCs.

3.2. Growth Kinetics, the Correlation between Growth Rate and Tumor Grade. The results of LGR, VGR, and VDT and their association with clinicopathologic variables are summarized in Table 2. The mean of LGR, VGR, and VDT was 0.86 (range, 0–4.74) cm/year, 20.96 (range, 0.31–211.93) cm^3/year, and 667 (range, 33–3321) days, respectively. ccRCC with a high grade had a greater LGR ($P < 0.001$) and VGR ($P = 0.001$) and a lower VDT ($P = 0.017$) than ccRCC with a low grade. High-grade ccRCC showed significantly aggressive growth kinetics compared to low-grade ccRCC.

The distribution of LGR and VGR for ccRCC is shown in Figures 2(a)-2(b). Thirty-four (55.7%) ccRCCs presented an LGR >0.5 cm/year, and the other 27 ccRCCs showed slow growth, with an LGR ≤ 0.5 cm/year; only one (1.6%) ccRCC showed zero growth in maximal diameter during 17 months of AS. According to the patients' age, sex, and initial tumor size, no correlations with LGR or VGR were found ($P > 0.05$). A logistic regression analysis revealed that tumor grade (OR = 5.185, $P = 0.004$) was the only independent risk factor of an LGR >0.5 cm/year for ccRCC.

The distribution of reciprocal of VDT (calculated as 365 divided by VDT) is shown in Figure 2(c). For 22 (36.1%) ccRCCs, VDT was less than 1 year, and 10 ccRCCs revealed a VDT <0.5 years. VDT was weakly positively correlated with the initial tumor size ($r = 0.335$, $P = 0.008$): ccRCC with a smaller initial size had a shorter VDT than ccRCC with a larger initial size. No correlation between the patients' age or sex and VDT was found. The logistic regression analysis revealed that tumor grade (OR = 3.006, $P = 0.046$) and initial size (OR = 0.392, $P = 0.004$) were independent risk factors of a VDT <1 year for ccRCC.

3.3. Postoperative Follow-Up. Six patients (9.8%) were lost during follow-up after surgery. Regarding the remaining 55 patients, the median follow-up after surgery was 50 months.

Table 2: Growth rate for ccRCC: overall and correlation with clinicopathologic variables.

	LGR (cm/yr)	VGR (cm^3/yr)	VDT (days)
Overall growth per year, $n = 61$			
Median	0.61	7.49	561
Mean ± SD	0.86	20.96	667
Range	0.00–4.74	0.31–211.93	33–3321
Growth rates and clinicopathologic variables			
Grade			
1, $n = 13$	0.32 ± 0.06	6.10 ± 3.15	885.69 ± 169.00
2, $n = 38$	0.74 ± 0.11	12.14 ± 2.27	684.08 ± 107.79
3, $n = 10$	2.03 ± 0.50	73.79 ± 22.79	319.60 ± 87.29
P value	<0.001*	0.001*	0.017*
Age			
R	−0.061	0.001	−0.045
P value	0.638	0.991	0.733
Initial size			
R	0.207	0.027	0.335
P value	0.110	0.836	0.008*
Sex			
Men, $n = 48$ (mean ± SD)	0.83 ± 0.14	20.15 ± 6.05	661.15 ± 91.29
Women, $n = 13$ (mean ± SD)	1.00 ± 0.28	23.95 ± 6.47	690.00 ± 169.95
P value	0.355	0.098	0.673

LGR: linear growth rate; VGR: volumetric growth rate; VDT: volume doubling time.
*Statistically significant.

Of them, 5 patients developed metastasis after surgery: 1 case of lung metastasis, 1 case of brain metastasis, 1 case of pleural and pulmonary metastasis, 1 case of metastasis in the neck, and 1 case of metastasis in the head of the pancreas. The data of the 5 patients were summarized in Table 4. The LGR values were all greater than 0.5 cm/year in the 5 patients. The tumors sizes were larger than 5 cm at operation for 4 patients of them. In the entire cohort, 5 deaths were found after a mean of 25.8 months of follow-up after surgery; of them, 4 were cancer-related deaths, and the remaining one death was related to cardiovascular events.

3.4. Discussion. As most renal masses are removed surgically soon after detection, it is difficult to characterize the natural history of RCC. However, AS is becoming gradually accepted, especially for patients with a high risk of surgery and limited life expectancy, providing a unique opportunity for understanding the natural history of RCC. Consistent with the aggressive features of ccRCC, previous studies [4, 9] have demonstrated that ccRCC shows a trend of rapid growth compared with other subtypes of RCC. A pooled analysis demonstrated that renal tumors that progressed during AS were predominantly ccRCC [1]. It would be expected that a complete understanding of ccRCC growth pattern during AS may help in selecting optimal patients for AS in consideration of the risk of fast-growing tumors in such cases.

We also reviewed past reports about the natural history of renal masses and performed a pooled analysis (Table 3) involving 1171 patients with 1271 renal tumors [4, 5, 7, 8, 10–27]. Based on this pooled analysis, we found that the renal masses generally grew slowly and seldom metastasized. The initial tumor size ranged from 1.73 to 7.2 cm. The mean age ranged from 52.2 to 80.4 years. The means of LGR and VGR were 0.33 (range, 0.06–0.8) cm/year and 11.0 cm^3/year, respectively. Compared with LGR, VGR is rarely used to describe the growth kinetics of renal masses. Only 19 (1.6%) patients developed metastatic disease during AS. Although a lack of pathological result is a major limitation for previous studies, of the 1271 renal tumors in the present study, only 444 (34.9%) had pathological results, and 380 (29.9%) were RCC. No work has reported the growth kinetics of ccRCC in detail, with the rarity of samples and lack of pathological results being the major reason for scarce knowledge about the growth pattern of ccRCC.

Based on the pooled analysis, the current study revealed a larger LGR (0.86 cm/yr versus 0.33 cm/yr) and VGR (20.96 cm^3/yr versus 9.48 cm^3/yr) and a younger age (55 years versus 69.5 years). Although Kouba and colleagues reported that younger patients show a faster growth rate than older patients [17], we did not find a significant correlation between age and growth rate in ccRCC. Only inclusion of cases with delayed surgical treatment might bring bias of growth rate, because the tumors that need delayed surgeries after AS usually have rapid growth rate. Anyway, to eliminate the bias, we further focused on the studies where all the cases were treated by delayed surgery and confirmed to be RCC pathologically, four studies with 96 cases were enrolled (Table 3) [4, 5, 8, 12], and the LGR of our cohort is still greater (0.86 cm/yr versus 0.62 cm/yr). However, all the four studies did not focus on certain pathological subtype to discuss the

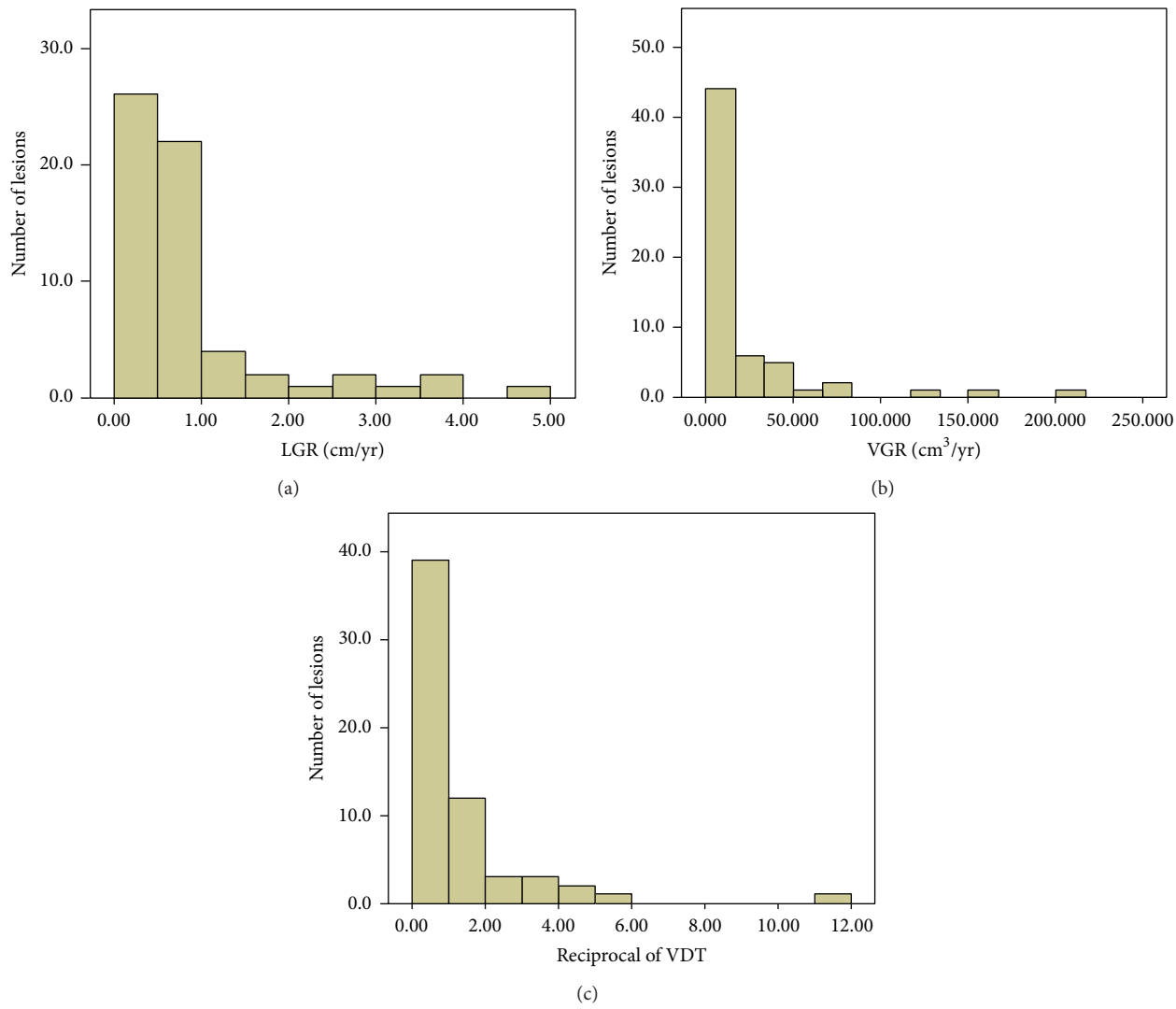


Figure 2: (a) Distribution of linear growth rate (LGR, cm/year) of ccRCCs. (b) Distribution of the volumetric growth rate (VGR, cm^3/year) of ccRCCs. (c) Distribution of the reciprocal of the volume doubling time (VDT) (calculated as 365 divided by VDT) of ccRCCs.

growth rate. These results suggested that ccRCC might have a rapid growing potential among the whole of RCC. Hence, the fast growth kinetics in the present study is partly due to the presence of ccRCC pathology for all cases.

Inability of identifying the lethal RCC is the most concern when applying AS. The growth rate of renal tumors is the most common observational index during AS, and renal tumors would be excised if showing rapid growth during AS. Although a pooled analysis on small renal masses progressing to metastases under AS revealed that renal masses with metastatic progression have a relatively rapid growth compared to those without metastasis during AS [1], there is no definitive evidence demonstrating that a rapid growth rate is an independent risk factor of poor prognosis. It is hard to make clear the correlation between the growth rate of RCC during AS and prognosis, because it needs a large number of samples, pathological diagnosis, and the long enough follow-up until the cancer-related death occurs; however, RCC has a favorable prognosis generally. It is widely accepted that tumor grade is correlated with the prognosis of RCC, and high tumor grade of RCC indicates a poor prognosis [3]. In a compromise way, if it could be proved that fast growth rate of RCC during AS correlates with high tumor grade, we believe that fast growth rate of RCC during AS may result in poor prognosis. So it is essential to make clear the correlation between the growth rate of RCC during AS and tumor grade.

As percutaneous renal biopsy is unreliable for small tumors [28] and may underestimate tumor grade of RCC [29], reviewing the patients with renal tumors which received delayed surgery and hence got the pathological diagnosis with grade and histological type is the only opportunity to investigate the correlation between the growth rate of RCC during AS and tumor grade. At present, the correlation between tumor grade and the growth rate of RCC is still controversial. Kato and colleagues demonstrated a significantly higher LGR in RCCs with grade 3 compared with RCCs with grade 2

Table 3: Published series on the natural history of renal masses.

	Year	Patients/lesions (n)	Mean age (years)	Mean initial MTD (cm)	Mean follow-up (months)	Mean LGR (cm/year)	Mean VGR (cm^3/year)	Progression to metastasis, n (%)	Pathologic RCC
Fujimoto et al. [10]	1995	6/6	59.7	2.47	24	0.47	9.7	0 (0)	5/5
Bosniak et al. [11]	1995	37/40	65.5	1.73	39	0.36	5.26	0 (0)	22/26
Oda et al. [12]	2003	16/16	54[a]	2.0[a]	25	0.54[a]	—	0 (0)	16/16[*]
Volpe et al. [7]	2004	29/32	71[a]	2.48	27.9	0.1	3.8	0 (0)	8/9
Wehle et al. [13]	2004	29/29	70	1.83	32	0.12	—	0 (0)	3/4
Kato et al. [5]	2004	18/18	56.5	2.0	27	0.42	4.4	0 (0)	18/18[*]
Lamb et al. [14]	2004	36/36	76.1	7.2	27.7	0.39	—	1 (2.8)	23/24
Chawla et al. [15]	2006	49/61	71	2.97	36	0.2	—	1 (1.6)	16/21
Youssif et al. [16]	2007	35/44	71.8	2.2	47.6	0.21	2.7	2 (5.7)	6/8
Kouba et al. [17]	2007	43/46	67	2.92	32.8	0.7	—	0 (0)	12/14
Siu et al. [18]	2007	41/47	68	2.0	29	0.27	—	1 (2.4)	10/16
Fernando et al. [19]	2007	13/13	80.4	5.01	38.38	0.17	11.97	1 (7.7)	0
Matsuzaki et al. [20]	2007	15/15	67	2.2	38	0.06	0.67	0 (0)	3/3
Lee et al. [8]	2008	30/30	65.5	2.6	12.6	0.59	19.1	3 (10.0)	30/30[*]
Beisland et al. [21]	2009	63/65	76.3	4.3	33	0.66	—	2 (3.2)	15/18
Crispen et al. [22]	2009	154/173	69	2.45	31	0.285	17.0	2 (1.3)	52/61
Rosales et al. [23]	2010	212/223	71[a]	2.8[a]	35[a]	0.34[a]	—	4 (1.9)	32/40
Hwang et al. [24]	2010	56/58	64.3	2.1	22	0.21	1.9	0 (0)	10/15
Jewett et al. [25]	2011	127/151	73	2.1	28	0.13	—	1 (0.7)	37/46
Li et al. [4]	2012	32/32	52.2	2.14	46	0.8	—	0 (0)	32/32[*]
Mehrazin et al. [26]	2014	68/72	68.9	5.3	38.9	0.44	—	0 (0)	16/23
Brunocilla et al. [27]	2014	62/64	75	2.0	91.5	0.4	4.6	1 (1.6)	14/16
Total		1171/1271	69.5	2.82	34.6	0.33	9.48	19 (1.6)	380/444
This study		60/61	55	2.32	39.46	0.86	20.96	1 (1.6)	61/61

[a]Median.

—: not stated

[*]All cases received delayed surgical intervention and were confirmed to be renal cell carcinoma (RCC) pathologically.

Table 4: Clinical and pathological characteristics of SRMs that progressed to metastasis after delayed treatment.

Cases	Sex	Age (years)	Grade	ITS (cm)	UTS (cm)	LGR (cm/year)	Duration of AS (months)	Surgical treatment	Time to metastasis after surgery	Site of metastasis	Outcome
1	Male	63	3	6.7	8.8	0.9	28	RN	9	Pleura and lung	Mortality at 57 mo. after surgery
2	Male	61	2	4.5	5.4	1.08	10	RN	16	Neck	Mortality at 24 mo. after surgery
3	Female	65	1	1.9	2.81	0.61	18	RN	14	Lung	Alive at 20 mo. after surgery
4	Male	58	3	0.1	8.0	4.74	20	RN	12	Brain	Mortality at 19 mo. after surgery
5	Female	59	2	3.6	7.0	1.28	32	PN	66	Head of pancreas	Alive at 101 mo. after surgery

SRMs: small renal masses; ITS: initial tumor size; UTS: ultimate tumor size; LGR: linear growth rate; RN: radical nephrectomy; PN: partial nephrectomy.

($P = 0.01$); however, they did not observe a significant difference in growth rate between grades 1 and 2 and between grades 1 and 3 [5]. Our previous studies also confirmed a significantly higher LGR in grade 2 RCC compared with grade 1 RCC, and the LGR of grade 3 tended to be faster than that of grades 1 and 2 RCC; however, the difference was not significant [4]. These studies suggested a correlation between RCC grade and growth rate, but the correlation remains unclear. It should be noted that these studies did not focus on certain histological subtype when discussing the correlation between grade and growth rate in RCC. In the current study, when focusing on ccRCC, we found a clear and strong significant correlation that the growth rate of ccRCC with a higher grade was faster than that of ccRCC with a lower grade, regardless of the measurement index used (i.e., LGR, VGR, or VDT). Additionally, tumor grade was found to be an independent risk factor of an LGR <0.5 and a VDT <1 year in ccRCC. Our findings more precisely reflect the correlation between grade and growth rate in ccRCC.

In the present study, we did not find that the initial tumor size correlated with LGR or VGR; however, we did find a positive correlation between initial tumor size and VDT: ccRCC with a smaller tumor size has a shorter VDT compared with ccRCC with a larger tumor size. Consistent with our results, Crispen and colleagues demonstrated that smaller renal tumors grew faster than larger renal tumors [22]. These results suggest Gompertzian growth kinetics in ccRCC, which theorizes that the growth rate of tumors is exponential initially and decreases with an increase in tumor size.

There were 5 patients who developed metastatic disease after surgery; 4 of them have died of RCC by the last follow-up. The ccRCC in these patients showed rapid growth kinetics that the LGR were all greater than 0.5 cm/year. In addition, the tumor sizes exceed 5 cm at operation for 4 of the 5 patients. Up to now, there is no definite indication of surgical treatment during AS. Jewett et al. had recommended treatment if the tumor grew rapidly or reached 4 cm in maximal diameter [25]. Based on our results, we recommend treatment for patients with renal masses during AS if the tumor size reaches 4 cm in maximal diameter or the LGR reaches 0.5 cm/year. However, these criteria still need to be validated.

4. Conclusions

We discovered a strongly significant correlation between the growth rate of ccRCC during AS and tumor grade. Fast growth rate during AS for ccRCC correlates with high tumor grade and may result in a poor prognosis, especially for ccRCCs with LGR >0.5 cm/yr. AS should be used cautiously for ccRCC with a fast growth rate. Further investigation on the natural history of non-ccRCC subtypes is needed. More attention should be paid to identify the lethal RCC for early intervention.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Lei Zhang and Wenshi Yin contributed equally.

Acknowledgment

The authors thank the entire staff of the Department of Urology, Peking University First Hospital.

References

[1] M. C. Smaldone, A. Kutikov, B. L. Egleston et al., "Small renal masses progressing to metastases under active surveillance: a systematic review and pooled analysis," *Cancer*, vol. 118, no. 4, pp. 997–1006, 2012.

[2] G. Novara, G. Martignoni, W. Artibani, and V. Ficarra, "Grading systems in renal cell carcinoma," *Journal of Urology*, vol. 177, no. 2, pp. 430–436, 2007.

[3] N. Rioux-Leclercq, P. I. Karakiewicz, Q.-D. Trinh et al., "Prognostic ability of simplified nuclear grading of renal cell carcinoma," *Cancer*, vol. 109, no. 5, pp. 868–874, 2007.

[4] X.-S. Li, L. Yao, K. Gong et al., "Growth pattern of renal cell carcinoma (RCC) in patients with delayed surgical intervention," *Journal of Cancer Research and Clinical Oncology*, vol. 138, no. 2, pp. 269–274, 2012.

[5] M. Kato, T. Suzuki, Y. Suzuki, Y. Terasawa, H. Sasano, and Y. Arai, "Natural history of small renal cell carcinoma: evaluation of growth rate, histological grade, cell proliferation and apoptosis," *Journal of Urology*, vol. 172, no. 3, pp. 863–866, 2004.

[6] A. J. Wein, L. R. Kavoussi, A. C. Novick, A. W. Partin, and C. A. Peters, *Campbell-Walsh Urology*, Saunders Elsevier, Philadelphia, Pa, USA, 10th edition, 2012.

[7] A. Volpe, T. Panzarella, R. A. Rendon, M. A. Haider, F. I. Kondylis, and M. A. S. Jewett, "The natural history of incidentally detected small renal masses," *Cancer*, vol. 100, no. 4, pp. 738–745, 2004.

[8] J. Y. Lee, C. K. Kim, D. Choi, and B. K. Park, "Volume doubling time and growth rate of renal cell carcinoma determined by helical CT: a single-institution experience," *European Radiology*, vol. 18, no. 4, pp. 731–737, 2008.

[9] J. Zhang, S. K. Kang, L. Wang, A. Touijer, and H. Hricak, "Distribution of renal tumor growth rates determined by using serial volumetric CT measurements," *Radiology*, vol. 250, no. 1, pp. 137–144, 2009.

[10] N. Fujimoto, A. Sugita, Y. Terasawa, and M. Kato, "Observations on the growth rate of renal cell carcinoma," *International Journal of Urology*, vol. 2, no. 2, pp. 71–76, 1995.

[11] M. A. Bosniak, B. A. Birnbaum, G. A. Krinsky, and J. Waisman, "Small renal parenchymal neoplasms: further observations on growth," *Radiology*, vol. 197, no. 3, pp. 589–597, 1995.

[12] T. Oda, A. Takahashi, N. Miyao et al., "Cell proliferation, apoptosis, angiogenesis and growth rate of incidentally found renal cell carcinoma," *International Journal of Urology*, vol. 10, no. 1, pp. 13–18, 2003.

[13] M. J. Wehle, D. D. Thiel, S. P. Petrou, P. R. Young, I. Frank, and N. Karsteadt, "Conservative management of incidental contrast-enhancing renal masses as safe alternative to invasive therapy," *Urology*, vol. 64, no. 1, pp. 49–52, 2004.

[14] G. W. A. Lamb, E. J. Bromwich, P. Vasey, and M. Aitchison, "Management of renal masses in patients medically unsuitable for nephrectomy–natural history, complications, and outcome," *Urology*, vol. 64, no. 5, pp. 909–913, 2004.

[15] S. N. Chawla, P. L. Crispen, A. L. Hanlon, R. E. Greenberg, D. Y. T. Chen, and R. G. Uzzo, "The natural history of observed enhancing renal masses: meta-analysis and review of the world literature," *Journal of Urology*, vol. 175, no. 2, pp. 425–431, 2006.

[16] T. A. Youssif, W. Kassouf, J. Steinberg, A. G. Aprikian, M. P. Laplante, and S. Tanguay, "Active surveillance for selected patients with renal masses: updated results with long-term follow-up," *Cancer*, vol. 110, no. 5, pp. 1010–1014, 2007.

[17] E. Kouba, A. Smith, D. McRackan, E. M. Wallen, and R. S. Pruthi, "Watchful waiting for solid renal masses: insight into the natural history and results of delayed intervention," *Journal of Urology*, vol. 177, no. 2, pp. 466–470, 2007.

[18] W. Siu, K. S. Hafez, W. K. Johnston III, and J. S. Wolf Jr., "Growth rates of renal cell carcinoma and oncocytoma under surveillance are similar," *Urologic Oncology*, vol. 25, no. 2, pp. 115–119, 2007.

[19] H. S. Fernando, S. Duvuru, and S. J. Hawkyard, "Conservative management of renal masses in the elderly: our experience," *International Urology and Nephrology*, vol. 39, no. 1, pp. 203–207, 2007.

[20] M. Matsuzaki, Y. Kawano, H. Morikawa, Y. Shiga, H. Murata, and H. Komatsu, "Conservative management of small renal tumors," *Acta Urologica Japonica*, vol. 53, no. 4, pp. 207–211, 2007.

[21] C. Beisland, K. M. Hjelle, L. A. R. Reisæter, and L. Bostad, "Observation should be considered as an alternative in management of renal masses in older and comorbid patients," *European Urology*, vol. 55, no. 6, pp. 1419–1429, 2009.

[22] P. L. Crispen, R. Viterbo, S. A. Boorjian, R. E. Greenberg, D. Y. T. Chen, and R. G. Uzzo, "Natural history, growth kinetics, and outcomes of untreated clinically localized renal tumors under active surveillance," *Cancer*, vol. 115, no. 13, pp. 2844–2852, 2009.

[23] J. C. Rosales, G. Haramis, J. Moreno et al., "Active surveillance for renal cortical neoplasms," *Journal of Urology*, vol. 183, no. 5, pp. 1698–1702, 2010.

[24] C. K. Hwang, K. Ogan, J. Pattaras, and V. A. Master, "Estimated volume growth characteristics of renal tumors undergoing active surveillance," *Canadian Journal of Urology*, vol. 17, no. 6, pp. 5459–5464, 2010.

[25] M. A. Jewett, K. Mattar, J. Basiuk et al., "Activesurveillance of small renal masses: progressionpatterns of early stage kidney cancer," *European Urology*, vol. 60, no. 1, pp. 39–44, 2011.

[26] R. Mehrazin, M. C. Smaldone, A. Kutikov et al., "Growth kinetics and short-term outcomes of cT1b and cT2 renal masses under active surveillance," *The Journal of Urology*, vol. 192, no. 3, pp. 659–664, 2014.

[27] E. Brunocilla, M. Borghesi, R. Schiavina et al., "Small renal masses initially managed using active surveillance: results from a retrospective study with long-term follow-up," *Clinical Genitourinary Cancer*, vol. 12, no. 3, pp. 178–181, 2014.

[28] E. Lechevallier, M. André, D. Barriol et al., "Fine-needle percutaneous biopsy of renal masses with helical CT guidance," *Radiology*, vol. 216, no. 2, pp. 506–510, 2000.

[29] A. J. Blumenfeld, K. Guru, G. J. Fuchs, and H. L. Kim, "Percutaneous biopsy of renal cell carcinoma underestimates nuclear grade," *Urology*, vol. 76, no. 3, pp. 610–613, 2010.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 163423, 8 pages
http://dx.doi.org/10.1155/2015/163423

Clinical Study

Prognostic Outcomes and Risk Factors for Patients with Renal Cell Carcinoma and Venous Tumor Thrombus after Radical Nephrectomy and Thrombectomy: The Prognostic Significance of Venous Tumor Thrombus Level

Qi Tang,[1] Yi Song,[1] Xuesong Li,[1] Maxwell Meng,[2] Qian Zhang,[1] Jin Wang,[3] Zhisong He,[1] and Liqun Zhou[1]

[1]*Department of Urology, Peking University First Hospital, Institute of Urology, Peking University, National Urological Cancer Center, Beijing 100034, China*
[2]*Department of Urology, University of California, San Francisco, CA 94143-0738, USA*
[3]*Department of Cardiac Surgery, Peking University First Hospital, Beijing 100034, China*

Correspondence should be addressed to Xuesong Li; pineneedle@sina.com and Liqun Zhou; zhoulqmail@sina.com

Received 22 November 2014; Accepted 24 April 2015

Academic Editor: Stefan Zastrow

Introduction. To evaluate the prognostic outcomes and risk factors for renal cell carcinoma (RCC) patients with venous tumor thrombus in China. *Materials and Methods*. We reviewed the clinical information of 169 patients who underwent radical nephrectomy and thrombectomy. Overall and cancer-specific survival rates were analyzed. Univariate and multivariate analyses were used to investigate the potential prognostic factors. *Results*. The median survival time was 63 months. The five-year overall survival and cancer-specific survival rate were 53.6% and 54.4% for all patients. For all patients, significant survival difference was only observed between early (below hepatic vein) and advanced (above hepatic vein) tumor thrombus. However, significant differences existed between both RV/IVC and early/advanced tumor thrombus groups in N0M0 patients. Multivariate analysis demonstrated that higher tumor thrombus level ($p = 0.016$, RR = 1.58), N ($p = 0.013$, RR = 2.60), and M ($p < 0.001$, RR = 4.14) stages and adrenal gland invasion ($p = 0.001$, RR = 4.91) were the most significant negative prognostic predictors. *Conclusions*. In this study, we reported most cases of RCC patients with venous extension in China. We proved that patients with RCC and venous tumor thrombus may have relative promising long-term survival rate, especially those with early tumor thrombus.

1. Introduction

Renal cell carcinoma (RCC) represents approximately 5% of new cancer cases in the United States and is the third most common cancer of the urinary system [1]. According to the 2012 Chinese Cancer Registry Annual Report, RCC accounts for 2.2% of cancer cases in China [2]. Tumor extension to the venous system, including the renal vein (RV) and the inferior vena cava (IVC), is a feature of locally advanced RCC and occurs in approximately 4–10% of cases [3].

The management of RCC patients with venous involvement has changed in recent decades. With advanced surgical techniques, particularly the development of cardiopulmonary bypass, more surgeons are able to surgically remove the kidney and attached tumor thrombus as the primary management approach [4–7]. Recently, even laparoscopic techniques have been introduced into surgical strategies for these patients because of their advantages in kidney mobilization and blood loss control [8, 9]. It is well documented that patients with RCC and venous tumor thrombus may significantly benefit from radical surgical resection, even those with distant metastasis [5]. Although there are data reporting the long-term outcomes of these patients worldwide, reliable statistics based on a large number of patients in China remain lacking.

Some studies have researched the prognostic significance of many factors in RCC patients with venous extension, such

as tumor size, tumor thrombus level, histological subtype, Fuhrman grade, nodal status, and distant metastasis [10–13]. However, the significance of venous involvement and tumor thrombus level still remain controversial. RCCs with venous tumor thrombus are aggressive tumors belonging to stage T3 and are associated with poor prognosis [14]. Some investigators reported that patients with higher tumor thrombus levels may have a poorer long-term outcome [7, 12]. However, other studies demonstrated no significant worsened survival between different thrombus levels [14, 15]. In addition to tumor size, thrombus level, and pathological features, the negative effect of tumor perinephric fat invasion on the prognosis of RCC patients with venous involvement has also been recognized recently [11, 16, 17].

The objectives of the present study were to report our experience in the surgical management of RCC patients with venous tumor thrombus, to assess the overall survival and cancer-specific survival rate of Chinese patients and to evaluate the significance of potential prognostic factors, especially the venous tumor thrombus level. To our knowledge, this is the largest report of patients with RCC and venous tumor extension in China.

2. Materials and Methods

2.1. Patient Selection. We retrospectively reviewed the records of all RCC patients treated in our hospital, from August 2000 to December 2012. Patients with RCC and venous tumor thrombus who were treated with radical nephrectomy and tumor thrombectomy were included. Patients with incomplete medical records or palliative surgical resection were excluded.

2.2. Clinical and Pathology Information. All patients had preoperative routine blood examinations, chest X-rays, electrocardiograms, and abdominal computerized tomography or magnetic resonance imaging evaluations. The venous tumor extension was indicated by preoperative radiological examination and confirmed during surgery procedure. To define the level of venous tumor thrombus extension, we followed the Neves classification system [18]. The first level of tumor thrombus includes those that are restricted to renal vein. Level I IVC thrombus refers to those that extend into inferior vena cava but below the hepatic veins. Level II IVC thrombus represents those that extend above the hepatic veins but below the diaphragm, and level III IVC thrombus is defined as a tumor thrombus that extends above the diaphragm or into the right atrium. Given the diversity of surgical strategies, we classified patients into early and advanced venous tumor thrombus, using the hepatic vein as the cut-off line. The venous tumor thrombus levels for all patients were decided comprehensively by preoperative radiologic information, including computerized tomography, magnetic resonance imaging, transesophageal echocardiography, and exploration during surgery. Preoperative distant metastasis status was routinely confirmed by chest X-rays, chest CT, and abdominal ultrasound. Cranial MRI or bone scans may also be performed if the patients had relevant symptoms. Postoperative immunotherapy or targeted molecular therapies were suggested if distant metastasis existed before surgery.

A multidisciplinary consultation, including specialists from urology, general surgery, cardiac surgery, anesthesiology, and radiology departments, will give a comprehensive assessment of the patient before surgery. For patients who cannot tolerate the surgery, palliative therapies may be suggested rather than radical resection.

To evaluate the potential prognostic factors for patients with RCC and venous tumor extension, clinical information such as age, gender, tumor size, first symptoms, operation time, blood loss, pathology subtype, Fuhrman grade, perinephric fat invasion, and 2009 TNM stage was also reviewed.

2.3. Surgery and Complications. A total of 169 patients completed radical nephrectomy and thrombectomy. Urologists, cardiac surgeons, and anesthetists jointly decided whether extracorporeal circulation was required based on the upper limit of tumor thrombus and the estimation of collateral circulation. Extracorporeal circulation was mainly used in patients with thrombus extension beyond the hepatic vein. Cardiopulmonary bypass with deep hypothermia circulatory arrest was seldom used in our institute because of high complication rate. Extended lymph node dissections were not routinely performed, but we performed lymphadenectomy for N staging if enlarged regional lymph nodes were observed in preoperative imaging or during the operation procedure. Gentle manipulations were always emphasized in our operations, especially around the renal vein and inferior vena cava. They would help decrease the risk of pulmonary embolism caused by tumor thrombus detachment. Recently, we introduced a laparoscopic technique for the surgical management of patients with venous involvement. We demonstrated that the combination of laparoscopic nephrectomy and open thrombectomy could decrease the operation time and blood loss.

The perioperative complications were reviewed. Complications which needed no intervention or only pharmacological treatment were defined as minor complications, while major complications referred to those that needed invasive intervention, life-threatening complications, or even perioperative death.

2.4. Monitoring and Follow-Up. Patients were required to complete routine laboratory tests and imaging assessment (including chest X-rays, urinary system ultrasound, and abdominal CT/MRI) every 3 months in the first two years, every 6 months in the subsequent year, and once a year in the following years. Appropriate treatments were provided in cases of local recurrence or distant metastasis. Follow-up information was obtained from phone interviews and outpatient records. The last follow-up was completed in December 2013. During follow-up, the cause of patient's death was confirmed by the death certificate offered by the hospital.

2.5. Statistical Analysis. Continuous parametric and non-parametric variables were reported differently as the mean

Table 1: Clinical and surgical characteristics of patients.

	RV	IVC level I	IVC level II	IVC level III	p
Patients (n)	93	49	21	6	
Male (%)	75.3 (70/93)	59.2 (29/49)	71.4 (15/21)	83.3 (5/6)	0.219
Age (year)	59.5 ± 9.7	54.4 ± 12.1	55.1 ± 10.2	54.7 ± 10.0	0.03
Symptoms					0.491
Yes	59	36	15	3	
No	34	13	6	3	
Tumor position					<0.001
Left	60	11	2	3	
Right	33	38	19	3	
Operation time (hour)					<0.001
Median	3.0	4.0	7.8	8.8	
Range	1.4–6.5	2.0–12.0	4.5–11.5	7.5–13.0	
Blood loss (mL)					<0.001
Median	400	700	3000	2700	
Range	30–6850	100–12800	500–12000	1800–4000	
Blood transfusion (mL)					<0.001
Median	0	350	1550	2700	
Range	0–4250	0–6500	0–7400	1900–10000	

value ± SD and median values. Continuous variables were analyzed using Student's t-test (normally distributed data) and Mann-Whitney U test (nonnormally distributed data). Categorical variables were compared using the Pearson $\chi 2$ test. The survival time was calculated from the date of operation to death or the date of last follow-up (when the patient was confirmed to be alive). The Kaplan-Meier method was used to analyze the survival curve for different tumor thrombus levels, and differences between groups were tested using the log-rank test. We used the Cox proportional hazard model to evaluate multiple predictors of outcome and to eliminate confounding factors. A two-sided p value <0.05 was considered to be statistically significant. All data were collected and analyzed by SPSS 20.0 software (IBM Corp, Armonk, NY, USA).

3. Results

3.1. Clinical and Pathological Characteristics. A total of 169 RCC patients with venous tumor thrombus and radical nephrectomy with thrombectomy were included in our study. The patients consisted of 119 men (70.4%) and 50 women (29.6%), with a median age of 58 years (range 15 to 80). The patients' clinical characteristics were summarized (Table 1). There were 93 (55.0%) patients with RV tumor thrombus and 76 (45.0%) with IVC tumor thrombus including 49 with level I, 13 with level II, and 6 with level III IVC tumor thrombus. The pathological features of all patients were listed in Table 2.

3.2. Surgical Strategy. The patients' surgical characteristics were also summarized in Table 1. There were 24 patients who required a cardiopulmonary bypass during surgery, including 1 with IVC level I, 17 with IVC level II, and 6 with IVC level III tumor thrombus. The laparoscopic technique was used for 19 patients all of whom had tumor thrombus below the hepatic vein, including 14 patients with pure laparoscopic surgery and 5 patients with combined laparoscopic and open surgery.

3.3. Perioperative Complications. The complication rate was 37.3% (63/169), including 47 minor complications and 16 major complications. The most common postoperative minor complication was transient renal insufficiency which needed no dialysis. One patient suffered from ileus and did not recover after conservative therapies and underwent a reoperation on the twenty-fifth day after the first surgery. Three patients had solitary kidney before surgery and required routine dialysis after the radical resection. Four patients experienced postoperative pulmonary embolism and one of them died because of heart failure and severe pulmonary infection.

3.4. Survival and Prognosis Factors. The median follow-up time was 45 months (2–114 months). The survival information of 143 patients was available, with a follow-up rate of 84.6% (143/169). At the last follow-up, 52 patients deceased, including 49 cancer-related deaths and 3 deaths unrelated to RCC (cerebral infarction, primary hepatic carcinoma, and interstitial pneumonia). Eleven patients were alive with tumor progression, while 80 patients were alive and disease-free at the last follow-up. The estimated median overall survival (OS) was 63.0 months for all patients. The 3-year and 5-year OS were 73.4% and 53.6%, while the 3-year and 5-year cancer-specific survival (CSS) were 74.4% and 54.4%. There were 96 patients (67.1%, 96/143) who had no evidence of initial nodal and distant metastasis (N0M0). For the N0M0 subgroup patients, the 5-year OS and CSS were 71.8% and 73.2%.

Table 2: Pathological features of patients.

	RV	IVC level I	IVC level II	IVC level III	p
Tumor size (cm)					0.499
Average	8.9 ± 2.8	8.5 ± 2.8	9.1 ± 2.9	9.6 ± 3.2	
Perinephric fat invasion					0.096
Yes	54	21	10	1	
No	39	28	11	5	
Adrenal gland invasion					0.161
Yes	8	0	2	0	
No	85	49	19	6	
Number of N classifications					0.849
0	82	42	17	5	
1	11	7	4	1	
Number of M classifications					0.132
0	69	42	16	6	
1	24	7	5	0	
Histological subtype					0.446
Clear cell	87	41	16	6	
Papillary	4	3	3	0	
Chromophobe	1	2	1	0	
Collecting duct	0	2	1	0	
Unclassified	1	1	0	0	
Sarcomatoid					0.942
Yes	23	12	6	1	
No	70	37	15	5	
Fuhrman grade					0.613
G2	36	15	6	3	
G3	53	31	12	3	
G4	4	3	3	0	

The median CSS of different tumor thrombus levels was 75 months (RV), 61 months (IVC level I), 58 months (IVC level II), and 45 months (IVC level III). For all patients, there was no significant difference of CSS between neither the four tumor thrombus levels ($p = 0.117$) nor the RV and IVC tumor thrombus ($p = 0.743$), while statistically significant CSS difference existed between the early and the advanced tumor thrombus groups ($p = 0.021$) (Figure 1). However, when considering the N0M0 subgroup, whether between the four different tumor thrombus levels, between RV and IVC tumor thrombus, or between the early and the advanced tumor thrombus, all CSS showed significant differences ($p = 0.011$, 0.036, and 0.004). According to the 2009 TNM classification, T3b and T3c patients were divided by the diaphragm. We compared the prognosis of T3b (IVC level I and level II) and T3c (IVC level III) patients, and no difference was found ($p = 0.284$) (Figure 2(a)). However, when taking the hepatic vein as the cut-off line, significant difference of prognosis was found between IVC level I and IVC level II + III patients ($p = 0.023$) (Figure 2(b)).

Univariate and multivariate analyses results were demonstrated (Tables 3 and 4). In the univariate analysis, higher T ($p = 0.001$), N ($p < 0.001$), and M ($p < 0.001$) stages, Fuhrman grade ($p = 0.045$), and adrenal gland invasion ($p < 0.001$) were negative prognostic predictors for all patients. While in the multivariate analysis, independent prognostic risk factors were higher tumor thrombus level ($p = 0.015$, HR = 1.58), N ($p = 0.013$, HR = 2.60) and M ($p < 0.001$, HR = 4.14) stages, and adrenal gland invasion ($p < 0.001$, HR = 4.91).

4. Discussion

Various publications have reported that the 5-year survival rate for RCC patients with venous tumor thrombus ranges from 18% to 57% [4–7, 10, 19–21]. However, this does not include data for Chinese RCC patients. To our knowledge, this study is the largest survey of Chinese RCC patients with venous involvement to report long-term outcomes after surgery. By summarizing the clinical and histological features, we analyzed the potential prognostic predictors of these patients and focused on the survival outcome of patients with different levels of venous tumor extension.

The concomitant involvement of the renal vein (RV) and the inferior vena cava (IVC) at RCC diagnosis is relatively rare. In previous years, because of the high surgical complication rate and mortality, patients were always conservatively

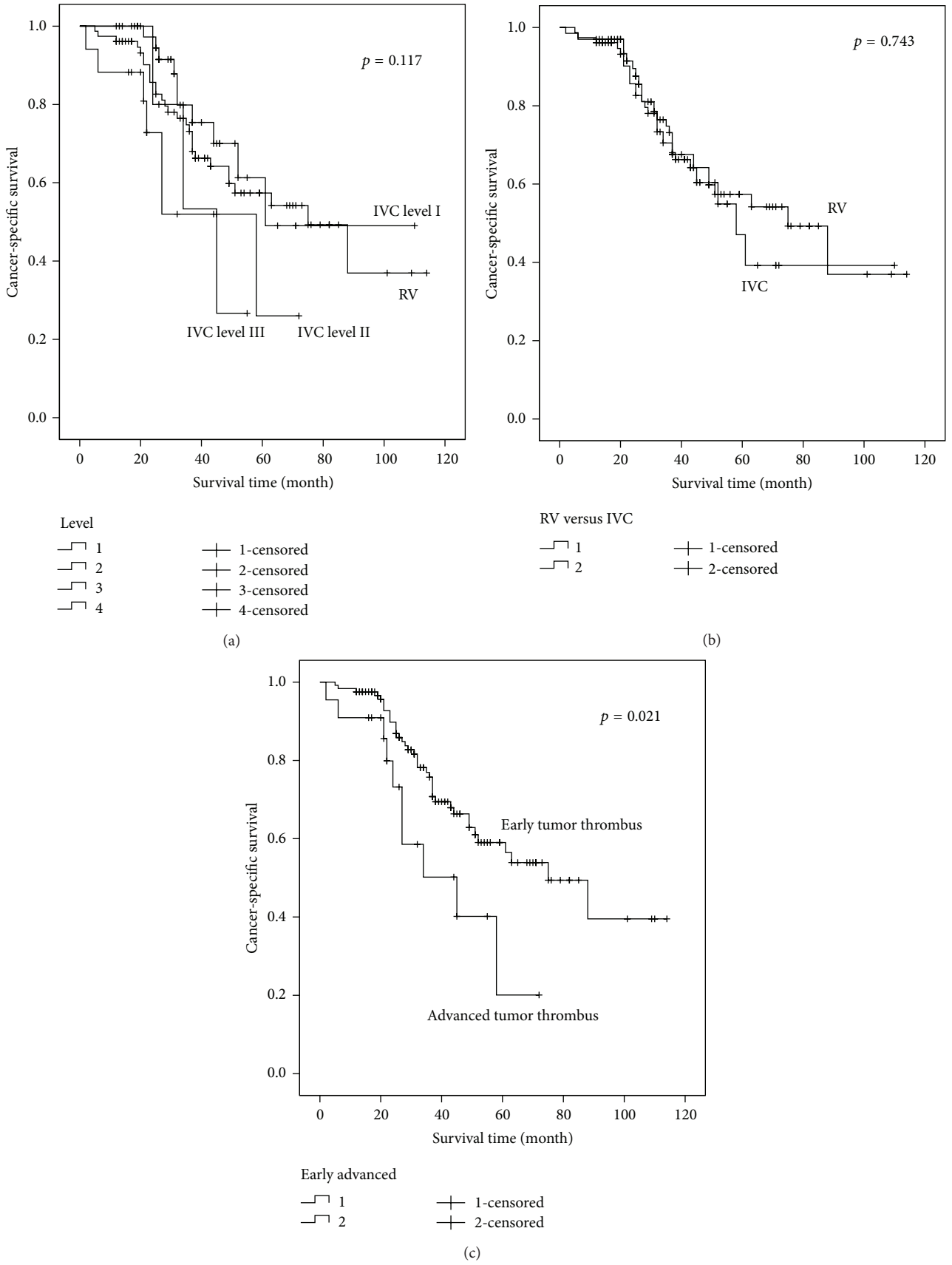


Figure 1: Prognosis comparison between patients with different tumor thrombus levels. (a) No significant difference between four tumor thrombus levels ($p = 0.117$). (b) No significant difference between RV and IVC tumor thrombus ($p = 0.743$). (c) Significantly better prognosis for the patients with early tumor thrombus, compared to the advanced patients ($p = 0.021$).

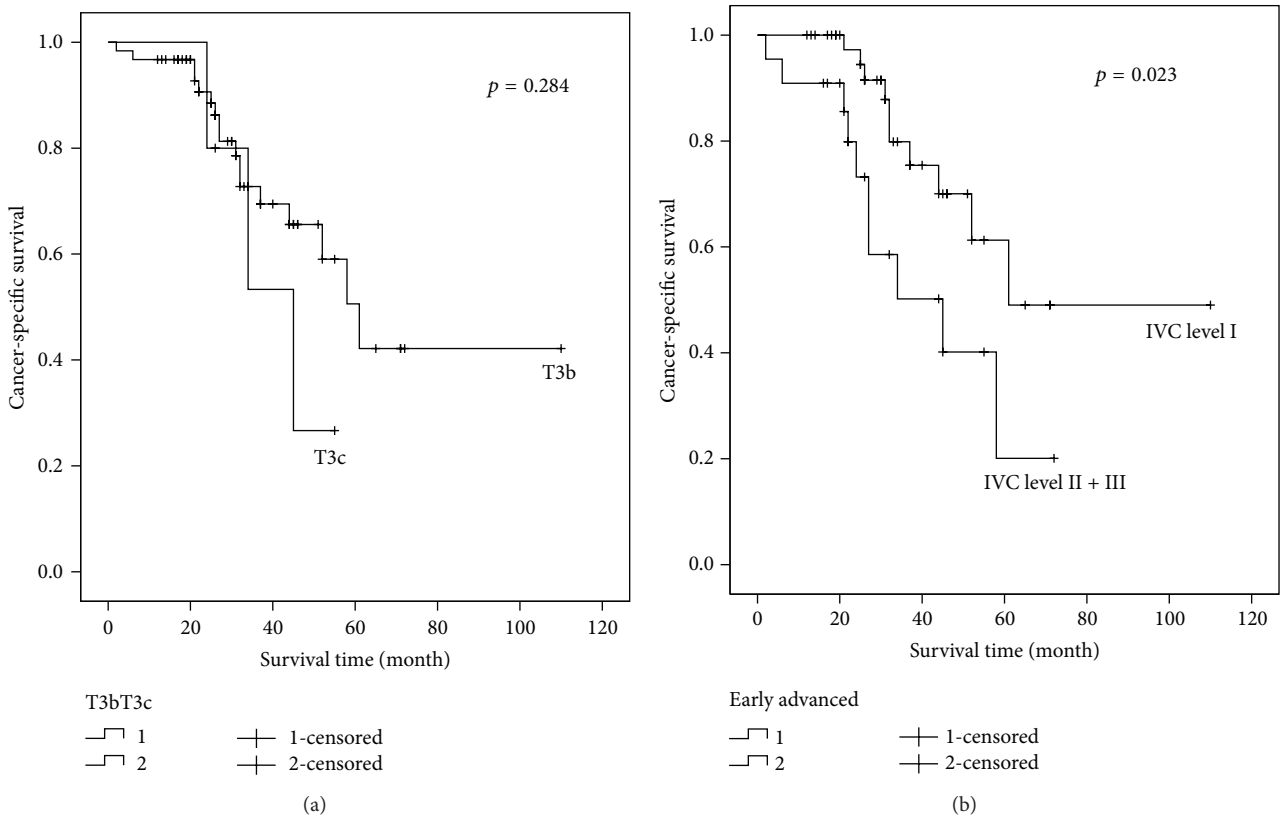


Figure 2: Prognosis comparison between T3 subgroups. (a) T3b and T3c patients had no significantly different long-term survival ($p = 0.284$). (b) Patients with IVC level II or level III tumor thrombus had significantly worse prognosis than those with IVC level I tumor thrombus ($p = 0.023$).

Table 3: Univariate analysis of prognostic risk factors.

Covariate	HR (95% CI)	p	HR (95% CI)	p
	All patients		N0M0 patients	
Gender	0.99 (0.54–1.80)	0.971	1.12 (0.45–2.82)	0.807
Age	1.00 (0.97–1.03)	0.949	0.98 (0.95–1.02)	0.373
Symptoms	1.73 (0.90–3.31)	0.102	1.45 (0.56–3.79)	0.446
Tumor position	0.81 (0.46–1.42)	0.461	0.90 (0.37–2.16)	0.806
Preoperative embolization	1.18 (0.65–2.11)	0.592	1.37 (0.54–3.43)	0.507
Tumor size	1.06 (0.96–1.17)	0.242	1.03 (0.88–1.21)	0.705
Tumor thrombus level	1.26 (0.91–1.75)	0.167	1.91 (1.24–2.95)	0.003
Perinephric fat invasion	1.08 (0.62–1.90)	0.783	0.59 (0.23–1.48)	0.259
Adrenal gland invasion	5.65 (2.59–12.35)	<0.001	3.14 (0.659–14.933)	0.151
T	1.65 (1.24–2.21)	0.001	1.92 (1.25–2.94)	0.003
N	4.94 (2.51–9.74)	<0.001		
M	4.35 (2.45–7.72)	<0.001		
Histological subtype	1.021 (0.87–1.21)	0.803	0.90 (0.67–1.22)	0.509
Fuhrman grade	1.74 (1.01–3.00)	0.045	1.54 (0.65–3.66)	0.327

treated but had poor outcomes [5]. Surgical resection has become a first-line treatment for these patients in recent decades due to the development of improved surgical techniques and medical facilities. The 3-year and 5-year overall survival rates of all patients in our study are 73.4% and 53.6%, which are comparable to the data from previous reports. For the N0M0 patients subgroup, the survival rates are even higher. Our study confirms the validity for using surgical resection in RCC patients with venous involvement at our institute.

The prognostic predictors for RCC patients with venous extension have been extensively analyzed, especially the level of tumor thrombus, which is still controversial. Some studies have demonstrated a decreased survival rate in patients with

Table 4: Multivariate analysis of prognostic risk factors.

Covariate	HR (95% CI)	p	HR (95% CI)	p
	All patients		N0M0 patients	
Gender	0.98 (0.49–1.95)	0.954	0.88 (0.28–2.76)	0.830
Age	0.99 (0.96–1.02)	0.391	0.99 (0.95–1.04)	0.714
Symptoms	1.22 (0.57–2.62)	0.611	2.28 (0.74–7.01)	0.152
Tumor size	1.06 (0.95–1.18)	0.280	1.07 (0.90–1.28)	0.459
Tumor thrombus level	1.58 (1.09–2.29)	0.016	2.12 (1.37–3.27)	0.001
Perinephric fat invasion	1.85 (0.97–3.52)	0.060	0.59 (0.21–1.66)	0.321
Adrenal gland invasion	4.91 (1.97–12.22)	0.001	8.04 (1.38–46.98)	0.021
N	2.60 (1.23–5.51)	0.013		
M	4.14 (2.17–7.93)	<0.001		
Histological subtype	0.94 (0.78–1.13)	0.491	0.85 (0.62–1.16)	0.305
Fuhrman grade	1.21 (0.66–2.21)	0.542	2.29 (0.88–5.98)	0.090

tumor thrombus extending into the IVC, when compared with RV involvement alone [7]. However, other researchers have not demonstrated that tumor thrombus level is a negative predictor [14, 19, 22]. In our study, for all patients, significant difference of CSS is only found between the early and advanced tumor thrombus groups ($p = 0.021$), when taking the hepatic vein as a cut-off line. Nevertheless, results of the N0M0 subgroup are different. Regardless of being between four different tumor thrombus levels, RV and IVC tumor thrombus, or early and advanced tumor thrombus, significant differences are found ($p = 0.011$, 0.036, and 0.004). Patients with higher tumor thrombus level tend to have worse prognosis. In the multivariate analysis, higher tumor thrombus level is also proved to be an independent prognostic risk factor whether for all patients or N0M0 patients.

The optimal stratification of venous tumor extension is still controversial. The classification of T3 subgroups has been changing over years. In the latest 2009 TNM system, diaphragm is taken as a division of patients with IVC tumor thrombus. In our study, we do not find significant survival difference between T3b and T3c patients. However, when compared to patients with tumor thrombus above the hepatic vein, significant benefit of prognosis is found in IVC level I patients. While considering the results mentioned above, it seems that the hepatic vein may be a more appropriate cut-off line instead of the diaphragm. So we propose the reclassification of T3 subgroups into T3b with IVC tumor thrombus below hepatic vein and T3c with IVC tumor thrombus above it.

We have found no significant predictive effect related to the status of perinephric fat invasion in all patients or in the N0M0 subgroup, which has been demonstrated in some previous studies [11, 20].

Within the last 3 years, we combined laparoscopic-assisted nephrectomy with open thrombectomy in carefully selected patients. Patients initially undergo laparoscopic transretroperitoneal nephrectomy in a lateral position and are then moved to a supine position for open thrombectomy. This surgical combination with laparoscopy allows for smaller incisions compared with a typical open surgical approach. Additionally, laparoscopy allows for clearer visualization and eases the manipulation of the renal pedicle and the abundant collateral vessels. In our experience, the combination of laparoscopic surgery decreases blood loss and shortens the operation time [23, 24]. The laparoscopic manipulation of lumbar veins is crucial. We should make sure that the circular dissection of IVC is completed and all the lumbar veins are ligated, in order to prevent unnecessary blood loss.

In our study, approximately 25% of patients had distant metastasis before surgery. For these patients, postoperative immunotherapy and targeted molecular therapy were suggested. The postoperative therapy information was also collected during follow-up (data not shown), but some patients were still undergoing clinical trials where the exact therapy strategies were not available. In recent years, the effectiveness of neoadjuvant and postoperative targeted molecular therapy for RCC patients with venous tumor thrombus has been reported [25–27]. With the combination of targeted therapy and surgery, patient may obtain an even better prognosis. However, evidences of high quality are urgently needed in this area.

Our study has some limitations. Because RCCs with venous extension are relatively uncommon, especially the lack of patients with advanced tumor thrombus (IVC level II and III IVC thrombus), we may require a consortium that includes more institutes to accumulate more patient data and attain more representative results. As our study is retrospective, we cannot collect patient information following a uniform approach, which may inevitably lead to some bias. Our study covered a long time period. There may be deviations for several variables, such as the pathological criterion of perinephric fat invasion. Nonetheless, to our knowledge, our study represents the largest group of Chinese RCC patients with venous tumor thrombus and fills a void in this area.

5. Conclusions

In our study, we confirmed the effectiveness of surgical resection for RCC patients with venous tumor thrombus, with a promising long-term survival rate, which was comparable to

previous studies. Higher tumor thrombus level was proved to be an important prognostic risk factor for these patients. Furthermore, the hepatic vein seems to be a more appropriate cut-off line for T3b and T3c tumor thrombus.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Qi Tang and Yi Song contributed equally.

References

[1] R. Siegel, E. Ward, O. Brawley, and A. Jemal, "Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths," *CA: A Cancer Journal for Clinicians*, vol. 61, no. 4, pp. 212–236, 2011.

[2] N. C. Center, *Chinese Cancer Registry Annual Report*, Military Medical Science Press, Beijing, China, 2012.

[3] V. Ficarra, R. Righetti, A. D'amico et al., "Renal vein and vena cava involvement does not affect prognosis in patients with renal cell carcinoma," *Oncology*, vol. 61, no. 1, pp. 10–15, 2001.

[4] J. Parra, S. J. Drouin, V. Hupertan, E. Comperat, M. O. Bitker, and M. Rouprêt, "Oncological outcomes in patients undergoing radical nephrectomy and vena cava thrombectomy for renal cell carcinoma with venous extension: a single-centre experience," *European Journal of Surgical Oncology*, vol. 37, no. 5, pp. 422–428, 2011.

[5] A. Haferkamp, P. J. Bastian, H. Jakobi et al., "Renal cell carcinoma with tumor thrombus extension into the vena cava: prospective long-term followup," *Journal of Urology*, vol. 177, no. 5, pp. 1703–1708, 2007.

[6] J. Rigaud, J.-F. Hetet, G. Braud et al., "Surgical care, morbidity, mortality and follow-up after nephrectomy for renal cancer with extension of tumor thrombus into the inferior vena cava: retrospective study since 1990s," *European Urology*, vol. 50, no. 2, pp. 302–310, 2006.

[7] M. L. Blute, B. C. Leibovich, C. M. Lohse, J. C. Cheville, and H. Zincke, "The Mayo-Clinic experience with surgical management complications and outcome for patients with renal cell carcinoma and venous tumour thrombus," *BJU International*, vol. 94, no. 1, pp. 33–41, 2004.

[8] I. M. Varkarakis, S. B. Bhayani, M. E. Allaf, T. Inagaki, M. L. Gonzalgo, and T. W. Jarrett, "Laparoscopic-assisted nephrectomy with inferior vena cava tumor thrombectomy: preliminary results," *Urology*, vol. 64, no. 5, pp. 925–929, 2004.

[9] A. N. Hoang, A. A. Vaporcyian, and S. F. Matin, "Laparoscopy-assisted radical nephrectomy with inferior vena caval thrombectomy for level II to III tumor thrombus: a single-institution experience and review of the literature," *Journal of Endourology*, vol. 24, no. 6, pp. 1005–1012, 2010.

[10] J. I. Martínez-Salamanca, W. C. Huang, I. Millán et al., "Prognostic impact of the 2009 UICC/AJCC TNM staging system for renal cell carcinoma with venous extension," *European Urology*, vol. 59, no. 1, pp. 120–127, 2011.

[11] B. Wagner, J.-J. Patard, A. Méjean et al., "Prognostic value of renal vein and inferior vena cava involvement in renal cell carcinoma," *European Urology*, vol. 55, no. 2, pp. 452–460, 2009.

[12] A. Moinzadeh and J. A. Libertino, "Prognostic significance of tumor thrombus level in patients with renal cell carcinoma and venous tumor thrombus extension. Is all T3b the same?" *Journal of Urology*, vol. 171, no. 2, pp. 598–601, 2004.

[13] C. Zhang, X. Li, H. Hao, W. Yu, Z. He, and L. Zhou, "The correlation between size of renal cell carcinoma and its histopathological characteristics: a single center study of 1867 renal cell carcinoma cases," *BJU International*, vol. 110, no. 11, pp. E481–E485, 2012.

[14] H. L. Kim, A. Zisman, K.-R. Han, R. A. Figlin, and A. S. Belldegrun, "Prognostic significance of venous thrombus in renal cell carcinoma. Are renal vein and inferior vena cava involvement different?" *Journal of Urology*, vol. 171, no. 2, pp. 588–591, 2004.

[15] V. Ficarra, A. Galfano, M. Mancini, G. Martignoni, and W. Artibani, "TNM staging system for renal-cell carcinoma: current status and future perspectives," *The Lancet Oncology*, vol. 8, no. 6, pp. 554–558, 2007.

[16] V. Ficarra, G. Novara, M. Iafrate et al., "Proposal for reclassification of the TNM staging system in patients with locally advanced (pT3-4) renal cell carcinoma according to the cancer-related outcome," *European Urology*, vol. 51, no. 3, pp. 722–731, 2007.

[17] R. H. Thompson, J. C. Cheville, C. M. Lohse et al., "Reclassification of patients with pT3 and pT4 renal cell carcinoma improves prognostic accuracy," *Cancer*, vol. 104, no. 1, pp. 53–60, 2005.

[18] R. J. Neves and H. Zincke, "Surgical treatment of renal cancer with vena cava extension," *British Journal of Urology*, vol. 59, no. 5, pp. 390–395, 1987.

[19] T. Klatte, A. J. Pantuck, S. B. Riggs et al., "Prognostic factors for renal cell carcinoma with tumor thrombus extension," *Journal of Urology*, vol. 178, no. 4, pp. 1189–1195, 2007.

[20] A. A. Glazer and A. C. Novick, "Long-term followup after surgical treatment for renal cell carcinoma extending into the right atrium," *Journal of Urology*, vol. 155, no. 2, pp. 448–450, 1996.

[21] D. G. Skinner, T. R. Pritchett, G. Lieskovsky, S. D. Boyd, and Q. R. Stiles, "Vena caval involvement by renal cell carcinoma: surgical resection provides meaningful long-term survival," *Annals of Surgery*, vol. 210, no. 3, pp. 387–394, 1989.

[22] N. K. Bissada, H. H. Yakout, A. Babanouri et al., "Long-term experience with management of renal cell carcinoma involving the inferior vena cava," *Urology*, vol. 61, no. 1, pp. 89–92, 2003.

[23] B. Xu, Q. Zhao, J. Jin, Z.-S. He, L.-Q. Zhou, and Q. Zhang, "Laparoscopic versus open surgery for renal masses with infrahepatic tumor thrombus: the largest series of retroperitoneal experience from China," *Journal of Endourology*, vol. 28, no. 2, pp. 201–207, 2014.

[24] Q. Tang, T. Wang, X. Li et al., "Renal cell carcinoma with infrahepatic vena caval tumor thrombus treated with a novel combined retroperitoneal and transperitoneal pure laparoscopic procedure," *Urology*, vol. 83, no. 5, pp. e9–e10, 2014.

[25] P. I. Karakiewicz, N. Suardi, C. Jeldres et al., "Neoadjuvant sutent induction therapy may effectively down-stage renal cell carcinoma atrial thrombi," *European Urology*, vol. 53, no. 4, pp. 845–848, 2008.

[26] N. G. Cost, S. E. Delacroix Jr., J. P. Sleeper et al., "The impact of targeted molecular therapies on the level of renal cell carcinoma vena caval tumor thrombus," *European Urology*, vol. 59, no. 6, pp. 912–918, 2011.

[27] X.-S. Li, X. Wu, P.-J. Zhao et al., "Efficacy and safety of sunitinib in the treatment of metastatic renal cell carcinoma," *Chinese Medical Journal*, vol. 124, no. 18, pp. 2920–2924, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 609549, 6 pages
http://dx.doi.org/10.1155/2015/609549

Clinical Study

Initial Experience of Sorafenib Neoadjuvant Therapy Combined with Retroperitoneoscopy in Treating T2 Large Renal Carcinoma

Chun-hua Lin, He-jia Yuan, Ke Wang, Ji-tao Wu, Qing-zuo Liu, Sheng-qiang Yu, Chang-ping Men, Zhen-li Gao, and Jiahui Wang

Department of Urology, Yantai Yuhuangding Hospital, Medical College of Qingdao University, 20 Yuhuangding East Road, Yantai, Shandong 264000, China

Correspondence should be addressed to Zhen-li Gao; gzlurology001@163.com and Jiahui Wang; jiahui.wang@outlook.com

Received 14 October 2014; Revised 30 January 2015; Accepted 26 February 2015

Academic Editor: Haifeng Yang

Objectives. To investigate the safety and feasibility of sorafenib neoadjuvant therapy combined with retroperitoneoscopic radical nephrectomy (RRN) in treating T2 large renal cell carcinoma (RCC). *Methods*. Retrospectively analyzed 5 cases (2 males and 3 females, aged 52–73 years) of T2 stage large RCC who receive preoperative sorafenib targeted treatment (400 mg bid for 1–3 months) and RRN between March, 2013, and July, 2014. Patient information, therapeutic regimen, drug adverse effect, tumor changes before and after surgery, and perioperative parameters were recorded. *Results*. During the sorafenib therapy adverse effects included 2 cases of hypertension (Grade I toxicity), 1 case of hand-foot syndrome (Grade I), and 1 case of diarrhea (Grade II), which were all tolerable for patients. CT scan and histopathological tests confirmed significant reduction in the longest dimension (LD) and medium density (MD) of the tumor after therapy as well as tumor hemorrhage, necrosis, and cystic degeneration. All 5 patients received RRN surgery successfully around 2 weeks after drug discontinuation with only 1 case of perioperative complication. *Conclusions*. Sorafenib neoadjuvant therapy could significantly reduce the size and aggressiveness of T2 large renal tumors, thus reducing the operative challenge and enabling patients who were previously disqualified for operation to receive surgical treatment.

1. Introduction

Renal tumor is the second most common carcinoma among urologic neoplasms with the highest fatality rate. It accounts for 2-3% of all malignancies and 80–90% of renal malignant tumors. The fatality rate is 30–40% comparing to the 20% of prostatic cancer or bladder cancer [1]. Radical nephrectomy is the main method for treating early stage renal tumors. However, it has limited use in treating metastatic renal cell carcinoma (RCC), for which molecular targeted drugs have huge advantages. According to multi-institutional studies worldwide, molecular targeted drug is effective against advanced renal tumor and increases the chance of advanced tumor patients receiving radical surgery or nephron-sparing surgery. Our hospital started using sorafenib as neoadjuvant targeted drug since 2013 in treating RCC patients with large tumors presumed not suitable for retroperitoneoscopic radical nephrectomy (RRN). Before 2013 RRN surgery on 3 patients with similar conditions had been performed but all failed in tumor removal. We hereby report our initial experience of successfully treating 5 cases of advanced RCC using preoperational sorafenib neoadjuvant therapy combined with RRN.

2. Materials and Methods

2.1. Patient Information. The clinical information of 5 renal tumor patients (2 males and 3 females; age 52–73, average of 66 years old), who voluntarily received sorafenib adjuvant therapy and RRN surgery between March, 2013, and July, 2014, was retrospectively analyzed. The inclusion criteria were patients diagnosed with RCC through needle biopsy, with tumor diameter > 7 cm, and CT scan showed large tumor with adhesion to surrounding tissues which was anticipated to be difficult for surgical removal or might cause large amount of intraoperative blood loss. The exclusion criteria were

RCC with clear boundary from surrounding tissues which could be surgically removed. Patients' cardiopulmonary and coagulation functions were assessed before operation and no apparent surgical contraindications were identified.

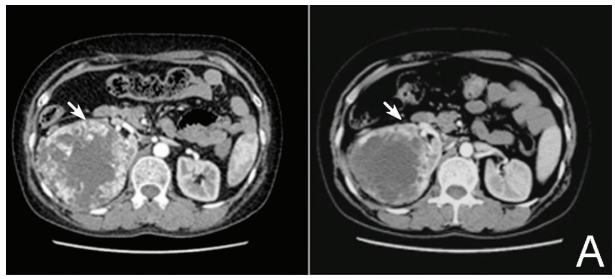
2.2. Treatment. All 5 patients were treated according to international recommendations [2]. Sorafenib was continuously delivered orally at 400 mg bid for 36–81 d, with average delivery time of 51.4 d. During the drug treatment, the cellular evaluation and biochemical analysis of blood and the coagulation indices (TT, FIB, PT, and APTT) of patients were regularly monitored and their cardiopulmonary functions were thoroughly evaluated to exclude surgical contraindications. Histopathological tests were performed before and after the sorafenib treatment. An average of 11.6 days after discontinuation of sorafenib treatment, patients received RRN under endotracheal general anesthesia. Surgical specimens were sent for pathological analysis.

2.3. Statistics. CT urography test was performed on all patients prior to medication and operation. Response evaluation criteria in solid tumor (RECIST) [3] were adopted to evaluate the effect of sorafenib on tumor. The longest dimension (LD) of primary tumors was measured from 3-dimensional reconstructed images from thin layer CT scan (1.5 mm) using Image Workstation (Terra Recon, San Mateo, CA). The medium density (MD) of tumor was measured using the Choi standard [4] according to their enhancement degree. Drug toxicity was graded according to the common terminology criteria for adverse events (CTCAE, version 4.0). The patients' general information, treatment protocol, and perioperative parameters were recorded. Statistical analysis was performed using SPSS 19.0 software. Significance was calculated using t-test; $P < 0.05$ indicated statistically significant difference.

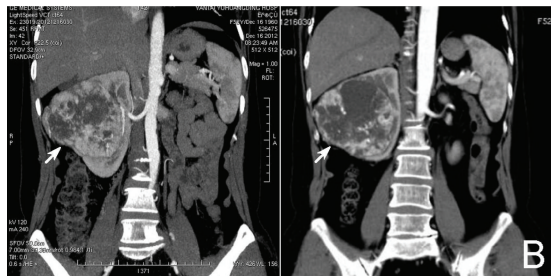
2.4. Statement of Human Rights. This experiment was conducted with the understanding and the consent of the human subject. All procedures were performed in agreement with the ethical standards of the Ethics Committee of Yantai Yuhuangding Hospital. Written informed consents were obtained from each patient for enrolling in this study.

3. Results

Patients' information, therapeutic outcome, and perioperative parameters were summarized in Table 1. During the course of drug treatment patient #1 had no particular discomfort (Grade 0). Patients #2, #3, and #4 experienced Grade I adverse event including mild hypertension (despite normal blood pressure before medication and no history of hypertension) and hand-foot syndrome with mild abnormal sensations one week after drug administration. None of them required medical intervention. Patient #5 had diarrhea 10 days after taking sorafenib. Taking the drug episodically with a 7-day gap relieved the symptom (Grade II). No other patient had dose reduction or gap of drug administration except patient #5. The adverse effects of sorafenib were tolerable.



(a)



(b)

Figure 1: Transverse (a) and coronal (b) CT scans of a patient before (left) and after (right) sorafenib treatment. Arrows indicated the renal tumor.

CTU test showed that in all 5 patients the tumor size reduced and enhancement receded in CT scan (Figure 1). The LD of primary tumors reduced significantly from 9.54 ± 1.85 cm to 8.72 ± 2.09 cm ($P = 0.034$) (Figure 2(a)) and the MD of tumors from 70.8 ± 8.26 HU to 63.6 ± 8.17 HU ($P = 0.019$) after sorafenib treatment (Figure 2(b)). Histopathologic test showed that after sorafenib treatment tumor hemorrhage, necrosis, and cystic degeneration were observed (Figure 3), indicating reduced aggressiveness of the tumor. Patients were successfully operated upon 7–16 days (average 11.6 days) after discontinuation of sorafenib. There were no severe perioperative complications except that patient #2 suffered a more severe adhesion of the tumor to the surrounding tissue than other patients. This resulted in prolonged operation, intraoperative hemorrhea, and damage in the peritoneum, which was immediately occluded with Hem-o-lok clip before the completion of the surgery. Surgical specimens were dissected, showing expanded lumen and apparent liquefaction (Figure 4). Pathological examination showed 4 cases were Grade II clear cell renal cell carcinoma (CCRCC) and one Grade I CCRCC. All patients were discharged within 2 weeks after the surgery.

4. Discussion

Despite the rising early stage diagnosis rate for renal tumors in recent years, there is still a large portion of patients who have missed the best time for operation upon diagnosis. Renal carcinoma tumors are highly resistant to radio- or chemotherapies for unknown reasons. Therefore palliative nephrectomy or immunotherapy is often used, such as IFN-α and IL-2. However, the response rate is as low as 15%. Currently,

Table 1: Patients' information and perioperative parameters of the sorafenib treatment group.

Patient	Gender	Age (yr)	TNM staging	Drug administration time (d)	Drug discontinuation time (d)	Drug side effects	Toxicity grading	LD before medication (cm)	LD after medication (cm)	MD before medication (HU)	MD after medication (HU)	Operation time (min)	Intraoperative blood loss (mL)	Perioperative complications	Fuhrman grading	Length of stay (d)
1	F	52	T2aN0M0	41	13	None	0	8.8	7.1	73	59	190	650	None	I	8
2	M	65	T2bN1M1	36	8	Hypertension	I	12.1	11.9	69	61	225	1980	Intraoperative hemorrhea and peritoneal damage	II	14
3	M	71	T2aN0M1	48	14	Hand-foot syndrome	I	9.1	8.2	82	77	210	530	None	II	11
4	F	69	T2aN1M1	51	16	Hypertension	I	10.5	9.6	59	56	102	810	None	II	13
5	F	73	T2aN0M0	81	7	Diarrhea	II	7.2	6.8	71	65	120	480	None	II	12

LD: longest dimension of the tumor; MD: medium density of the tumor.

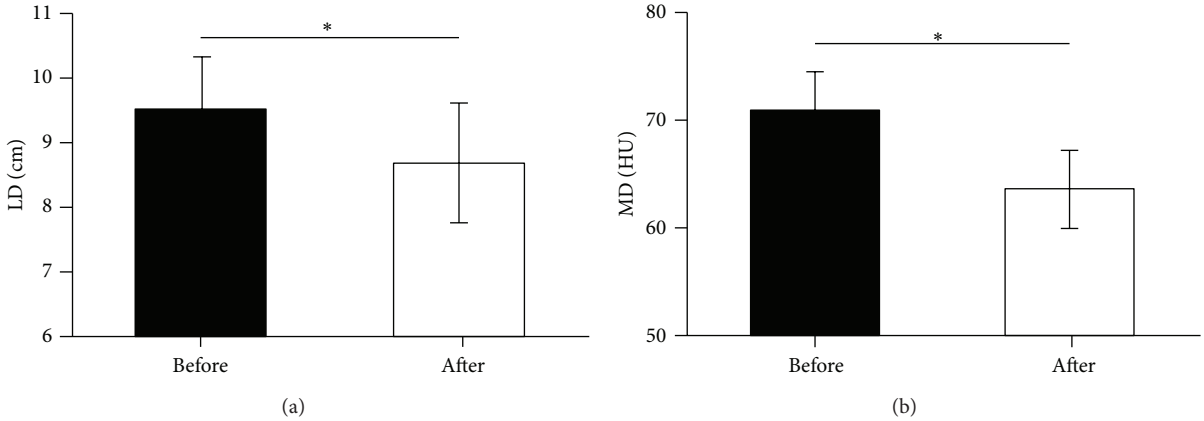


Figure 2: (a) Comparison of the longest dimension (LD) of tumors before and after sorafenib treatment. (b) Comparison of the medium density (MD) of tumors before and after sorafenib treatment. Error bars showed the standard error while ∗ represented significant difference ($P < 0.05$).

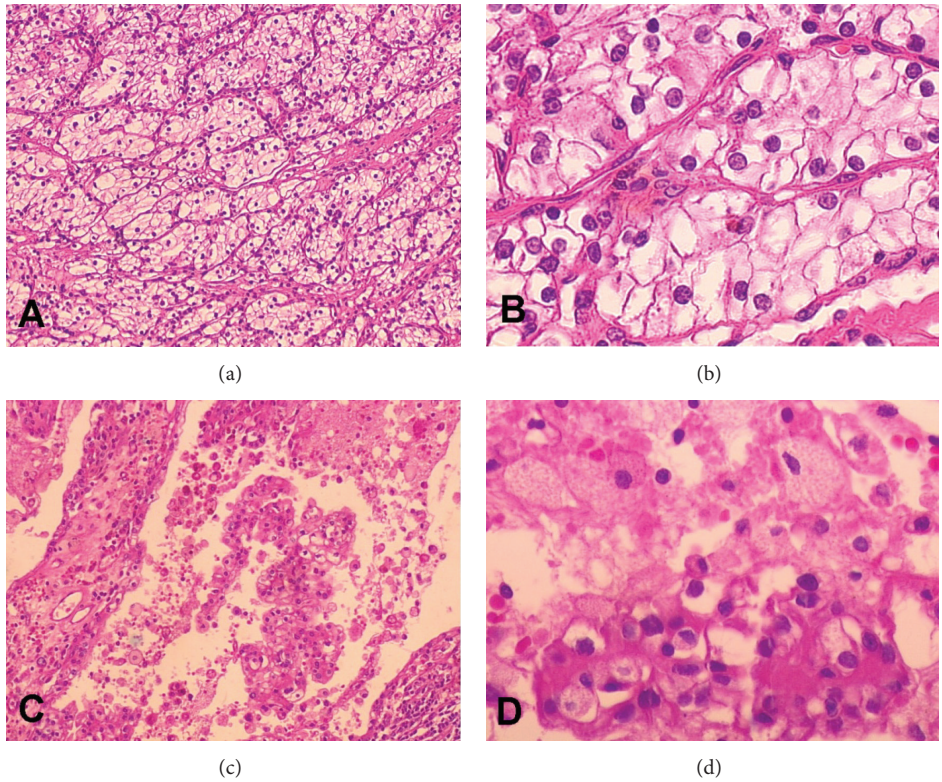
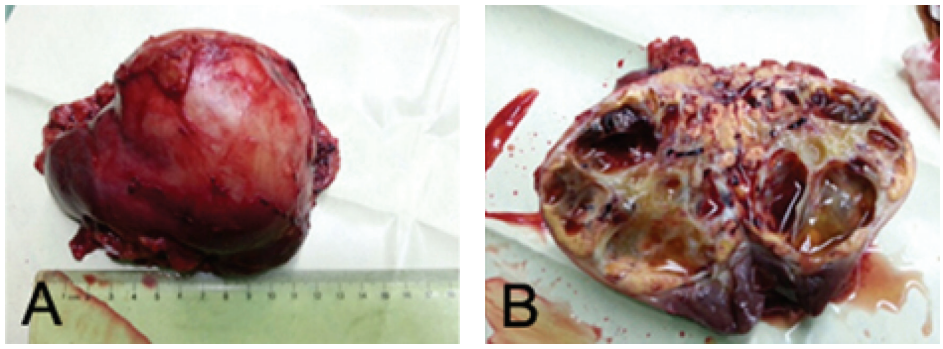


Figure 3: Histopathologic staining of RCC from the same patient before (a and b) and after (c and d) sorafenib treatment, where tumor hemorrhage, necrosis, and cystic degeneration were visible. (a) and (c) were at ×100 magnification. (b) and (d) were at ×400 magnification.

molecular sorafenib has been widely used in treating late-stage RCC. Escudier et al. carried out a phase III multicenter trial of sorafenib treatment in renal cancer which showed that patients treated with sorafenib had median PFS that was twice as long as the placebo group (5.5 versus 2.8 months) and their quality of life (QOF) was significantly higher [5]. Furthermore, Ye and Zhang reported that sorafenib was more effective in patients of Chinese ethnicity than in western patients and was well tolerated even at higher dosage and when used in combination with other anticancer agents [6].

There is controversy regarding the combination of molecular targeted therapy and retroperitoneoscopic surgery. It is currently understood that, for patients with localized advanced RCC, neoadjuvant therapy could be used first to reduce the tumor size, making the tumor easier for surgical resection or preserving renal functions as much as possible



(a) (b)

Figure 4: Image of a surgical extracted tumor (a) and its dissected view (b).

[7, 8]. For metastatic RCC, it is recommended to receive cytoreductive surgery before targeted treatment, the selection criteria of which are (A) ECOG score 0-1; (B) >75% of tumor load that could be resected; (C) no other obvious organ dysfunctions. On the other hand, for patients whose resection nidus comprises < 75% of the tumor load, neoadjuvant therapy is recommended [9]. In the application of RRN in resecting large tumors (>7 cm), the safety and effectiveness of the procedure, as well as the definition of the limit of tumor size, are still controversial. Albqami and Janetschek proposed that when tumor was confined within Gerota's fascia, RRN is applicable regardless of the size of the tumor. Many other researchers believed that, on the basis of good laparoscopic skills, RRN can be applied to all RCC confined in renal dispose capsule. Therefore the size of tumors is not the only standard to determine laparoscopy or open surgery.

Consensus has not been reached over the time window of preoperative sorafenib neoadjuvant treatment for RCC, the discontinuation time of the drug, and the time of performing the operation. There were large differences among various case reports. Most people believe that operation should only be considered after the primary or metastatic tumors had reduced by a certain extent and the tumor size should be continually monitored after discontinuation of sorafenib. If the tumor size increased again, it was not suitable for operation.

In this study, sorafenib treatment combined with retroperitoneoscopy was effective against RCC that was originally considered difficult to be surgically removed. In all 5 patients the renal tumors diminished to various extents. However, there is no unified criteria for evaluating the efficacy of sorafenib in treatment of primary RCC. We used the RECIST method to assess the changes of tumors after treatment. Histopathological changes could also reflect the necrosis of carcinoma, thus providing further evidence for using sorafenib in targeted therapy for RCC.

It has been reported that long-term use of sorafenib might severely delay the postoperative wound healing and increase the risk of postoperative bleeding and formation of thrombus. Kondo et al. reported it safe to stop sorafenib 7 days before operation [10] although many others suggested a 2-week gap between drug discontinuance and operation. In our study operations were performed 7–16 days after drug discontinuance. No obvious intraoperative or postoperative complications occurred except for #2 due to the heavy tumor adhesion. All 5 patients left hospital within 2 weeks after the operation.

At the moment, it is unknown whether sorafenib could reduce the recurrence risk of advanced RCC. According to the report by Cleveland Clinic Foundation, the positive surgical margin rates for laparoscopic removal of T1 stage (median LD 4.5 cm) and T2 stage (median LD 9.5 cm) RCC were close, with no difference in survival rates 2 years after operation [11].

This study is a retrospective study and relatively limited in the number of cases included. We did not assess the 5-year cancer-free survival rate or overall survival rate and, therefore, could not evaluate the long-term effect of this combined therapy. Larger sample size and longer follow-up period are required to further evaluate the clinical effect of sorafenib combined RPN in treating T2 large renal tumors.

In conclusion, neoadjuvant therapy based on multi-targeted drugs plays an important part in comprehensive treatment of late-stage RCC. Sorafenib neoadjuvant therapy could contribute to reducing the size and aggressiveness of tumors, thus reducing the operative challenge and increasing the radical resection rate of late-stage carcinoma, enabling patients to receive surgical treatment who were previously disqualified for operation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Chun-hua Lin and He-jia Yuan contributed equally to this paper.

References

[1] A. J. Pantuck, A. Zisman, and A. S. Belldegrun, "The changing natural history of renal cell carcinoma," *Journal of Urology*, vol. 166, no. 5, pp. 1611–1623, 2001.

[2] D. Strumberg, H. Richly, R. A. Hilger et al., "Phase I clinical and pharmacokinetic study of the novel raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors," *Journal of Clinical Oncology*, vol. 23, no. 5, pp. 965–972, 2005.

[3] P. Therasse, S. G. Arbuck, E. A. Eisenhauer et al., "New guidelines to evaluate the response to treatment in solid tumors," *Journal of the National Cancer Institute*, vol. 92, no. 3, pp. 205–216, 2000.

[4] H. Choi, C. Charnsangavej, S. C. Faria et al., "Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria," *Journal of Clinical Oncology*, vol. 25, no. 13, pp. 1753–1759, 2007.

[5] B. Escudier, T. Eisen, W. M. Stadler et al., "Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial," *Journal of Clinical Oncology*, vol. 27, no. 20, pp. 3312–3318, 2009.

[6] D. W. Ye and H. L. Zhang, "Critical appraisal of sorafenib in the treatment of Chinese patients with renal cell carcinoma," *OncoTargets and Therapy*, vol. 7, pp. 925–935, 2014.

[7] C. Amin, E. Wallen, R. S. Pruthi, B. F. Calvo, P. A. Godley, and W. K. Rathmell, "Preoperative tyrosine kinase inhibition as an adjunct to debulking nephrectomy," *Urology*, vol. 72, no. 4, pp. 864–868, 2008.

[8] F. di Silverio, A. Sciarra, U. Parente et al., "Neoadjuvant therapy with sorafenib in advanced renal cell carcinoma with vena cava extension submitted to radical nephrectomy," *Urologia Internationalis*, vol. 80, no. 4, pp. 451–453, 2008.

[9] Canadian Kidney Cancer Forum, "Management of kidney cancer: Canadian Kidney Cancer Forum consensus statement," *Canadian Urological Association Journal*, vol. 2, pp. 175–182, 2008.

[10] T. Kondo, Y. Hashimoto, H. Kobayashi et al., "Presurgical targeted therapy with tyrosine kinase inhibitors for advanced renal cell carcinoma: clinical results and histopathological therapeutic effects," *Japanese Journal of Clinical Oncology*, vol. 40, no. 12, pp. 1173–1179, 2010.

[11] A. P. Steinberg, A. Finelli, M. M. Desai et al., "Laparoscopic radical nephrectomy for large (greater than 7 cm, T2) renal tumors," *Journal of Urology*, vol. 172, no. 6, pp. 2172–2176, 2004.