

Clear Cell Sarcoma of the Kidney

—Immunohistochemical study and flow cytometric DNA analysis of 7 cases—

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Immunohistochemical study and flow cytometric DNA analysis were done on seven cases of clear cell sarcoma of the kidney (CCSK) to speculate its histogenesis and to access the diagnostic usefulness of these methods in the differential diagnosis of Wilms' tumor. Clinically, CCSK is a rare malignant renal tumor of children with a propensity to metastasize to bone. Arborizing vascular pattern surrounding the tumor cells which have clear cytoplasm is characteristic histologic finding. Immunohistochemically, only vimentin was diffusely demonstrated in the tumor cell membrane and cytoplasm. In flow cytometric DNA analysis, four cases showed diploidy and two cases near diploidy. CCSK is a separate disease entity with characteristic clinicopathologic, immunohistochemical and flow cytometric findings in distinction from Wilms' tumor. Considering the histologic and immunohistochemical findings, the possible histogenetic mechanism of CCSK seems to be in common with congenital mesoblastic nephroma (CMN), that is primitive mesenchymal cells which committed early stromagenic activity.

Key Words: Clear cell sarcoma of the kidney (CCSK), immunohistochemistry, flow cytometric DNA analysis, histogenesis

Clear cell sarcoma of the kidney (CCSK) is a rare malignant renal tumor of children with a characteristic histologic pattern and a marked propensity to metastasize to bone. As a clinicopathologic entity, CCSK was first recognized by Kidd in 1970. Since then, additional cases have been added in the literature under the names of bone metastasizing renal tumor of childhood (Marsden *et al.* 1978; Marsden and Lawler, 1980), undifferentiated sarcoma of the kidney (Morgan *et al.* 1978) or sarcomatous renal tumor of childhood (Novak *et al.* 1980). Although clinicopathologic characteristics of

CCSK have been well established and CCSK was known as a distinct entity from Wilms' tumor, there is still ongoing controversy to its histogenesis. A variety of histogenetic possibilities such as renomedullary interstitial cells (Beckwith 1982; Hass *et al.* 1984), transitional cells between mesenchymal and blastemal cells (Beckwith 1982, Hass *et al.* 1984) and blastemal cap cell (Marsden *et al.* 1978) have been proposed on the basis of ultrastructural and immunohistochemical studies.

In this study, immunohistochemistry and flow cytometric DNA analysis were performed on seven cases of CCSK to speculate the histogenesis of this tumor and to access the diagnostic usefulness of these methods in the differential diagnosis of Wilms' tumor.

MATERIALS AND METHODS

Six cases of CCSK diagnosed between 1980

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and 1990 from the files of Department of Pathology, Yonsei University College of Medicine, Seoul, Korea and one case provided by Kosin College of Medicine, Pusan, Korea, were analysed.

Review of medical records

Age, sex, age at diagnosis, primary site of tumor, clinical stage, treatment modality and follow-up data of each case were reviewed.

Gross and histopathologic examination

Gross description and gross photo of each case were reviewed and 5 μ m thick sections of formalin-fixed, paraffin-embedded tissue of each case were stained with hematoxylin-eosin, reticulin, periodic acid-Schiff (PAS) and PAS with diastase digestion (DPAS).

Immunohistochemical stain

4~5 μ m thick sections of formalin-fixed, paraffin-embedded tissue of each case were stained with a labeled streptavidin biotin (LSAB, DAKO Corp, Santa Barbara, U.S.A.) kit with diaminobenzidine (DAB) as a chromogen and counterstained with Mayer hematoxylin. Monoclonal antibodies used in this study include cytokeratin (cocktail, Biomed, U.S.A.), vimentin (Biomed), smooth muscle actin (Biomed) and desmin (Biomed).

Flow cytometric DNA analysis

Two or three 50 micron sections, cut from the tissue blocks were deparaffinized in Histo-Clear (National Diagnostics, Manville, U.S.A.) and rehydrated in a series of graded alcohols by the method of Hedley *et al.* (1985). After pepsin (2.5 ml of 0.5% pepsin at pH 1.5 at 37°C for 30 minutes, DIFCO, Michigan, U.S.A.) and ribonuclease (0.5ml of RNAse (2.50 mg/ml), Sigma Chemical Corp. St. Louis, U.S.A.) treatment, the samples were filtered through a 50 micron nylon mesh filter and stained with 0.025% propidium iodide (50 ul/ml, Sigma Chemical Corp. St. Louis, U.S.A.). Nuclei were analyzed in a FACScan (Becton-Dickinson, Sunnyvale, U.S.A.) with at least 20,000 nuclei read per sample. Normal kidney tissue was used as control. The first G₀/G₁ peak was assumed to be the diploid population and as-

signed a DNA index of 1.0. DNA aneuploidy was defined by the presence of a distinct, separate second peak to the right of the first G₀/G₁ peak accompanied by a low G₂M hump. The DNA index was calculated by the ratio of the channel number of the abnormal aneuploid peak to that of the normal diploid G₀/G₁ peak.

RESULT

Clinical findings

Clinical, flow cytometric characteristics and follow-up data of each patient are summarized in Table 1.

The age distribution of the patients was from 4 months to 3 years and one month. Male to female ratio was 5:2. All cases involved unilateral kidney (R:L=2:5). No associated congenital anomaly was noted in any of the cases. Clinical staging was done according to the National Wilms' Tumor Study (NWTs, D'Angio *et al.* 1976). At the time of diagnosis, tumor was confined to the kidney (stage I) in two patients, extended beyond the kidney (stage II) in three patients and spread to periaortic lymph nodes (Stage III) in two patients. All three cases with stage II eventually progressed to stage IV with metastatic lesion in bone and liver after operation.

Primary tumors were surgically excised and postoperative chemotherapy with cytoxan, vincristine and actinomycin D was performed in all the patients. In addition, radiotherapy was given to one patient (case No. 7).

Four patients (case No. 1, 2, 3 and 4) died of disease in 11, 11, 36 and 17 months after diagnosis, respectively. Three patients (case No. 5, 6 and 7) are alive with disease in 13, 18 and 14 months after finishing treatment.

Pathological findings

Gross appearance: The gross appearance of CCSK was distinguished from that of classic Wilms' tumor. The cut surfaces are usually homogeneous tan or gray-white with firm to rubbery consistency. The margin of the tumor revealed mostly infiltrative feature to the adjacent renal parenchyma. Hemorrhagic ne-

crisis was uncommon and cystic spaces with variable size were frequently found. All tumors weighed 450 gm or more; one of the tumors was exceptionally large, weighing 1080 gm (case No. 1).

Histologic findings: Microscopically, CCSK was typically composed of cells with poorly stained indistinct clear cytoplasm. On periodic acid-Schiff (PAS) and PAS with diastase digestion, cytoplasm were not stained. Nuclei

Table 1. Summary of cases and DNA index

No.	Age(year)	Sex	Weight(gm)	Stage	Metastasis	DNA Index	Follow up
2.		M	500	I		1.11	DOD
3.		M	800	III		1.13	DOD
4.		F	870	II→IV	liver (post op. 16 mo.)	1.0	DOD
5.	2 $\frac{5}{12}$	M	463	I	bone (post op. 12 mo.)	1.0	
6.	2	M	488	II→IV	bone (post op. 10 mo.)	1.0	
7.	2	F	710	II→IV	bone (post op. 10 mo.)	1.0	

DOD: died of disease
 AWD: alive with disease
 Post op.: post operation

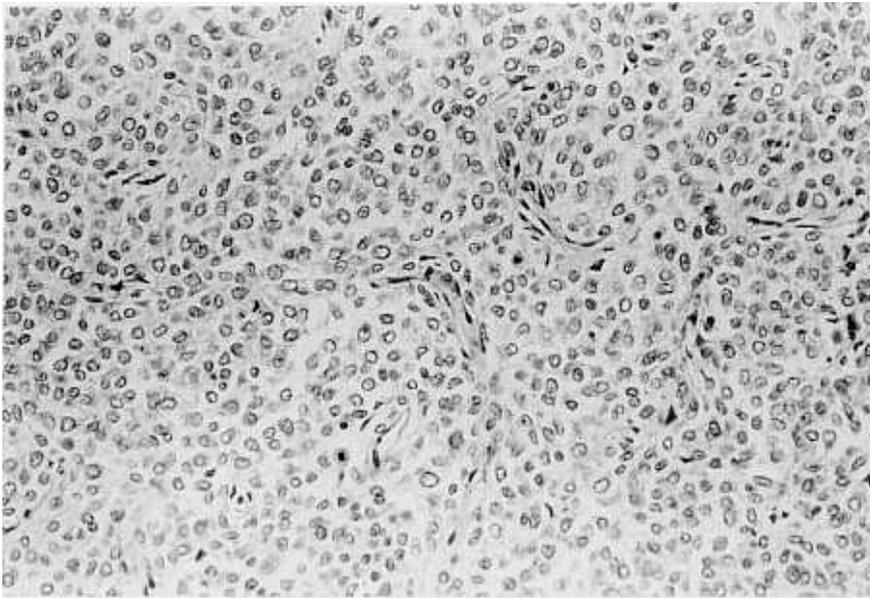


Fig. 1A. The tumor cells show round to ovoid vesicular nuclei and polygonal clear cytoplasm (H&E, ×100).

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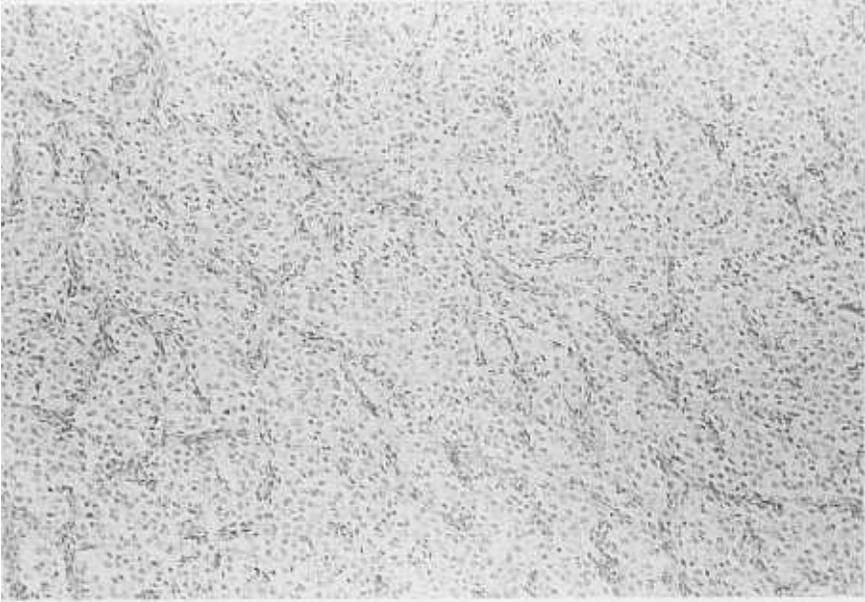


Fig. 1B. The tumor cells grow in the characteristic pattern of ill defined nests or sheets separated by arborizing fibrovascular stroma (H&E, $\times 40$).

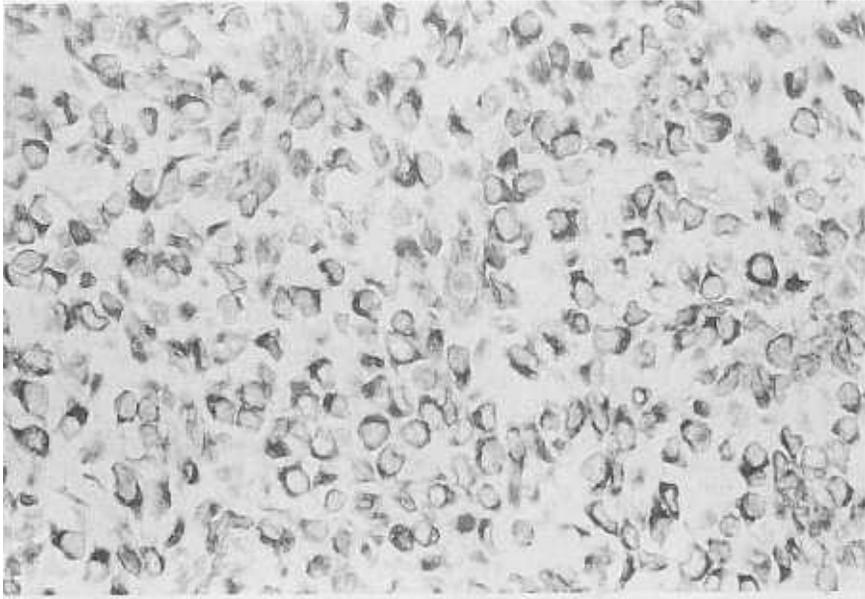


Fig. 2. The tumor cells show diffuse cytoplasmic positive reaction for vimentin (Immunoperoxidase, $\times 400$).

were oval to round, of uniform size with fine granular chromatin and showed a few mitotic figures from area to area (Fig. 1A). Tumor cells were divided into nests or cords by prominent, arborizing networks of small blood vessels supported by spindle cell stroma (Fig. 1B). Reticulin stain accentuated vascular pattern clearly, which was known to be an important clue to make a diagnosis (Beckwith, 1983). Some entrapped renal tubules, some of which forming cysts, were present in most cases. No blastemal tissue was found. Other histologic variants such as epithelioid trabecular, neurilemmoma-like nuclear palisading, fibrotic-hyalinized and angiectatic pattern were not present in our cases.

Immunohistochemical findings: Immunohistochemically, vimentin was diffusely demonstrated in the tumor cell membranes and cytoplasm (Fig. 2). Cytokeratin, actin and desmin were negative. In contrast, entrapped renal tubules which were located in peripheral portions of tumor revealed positive reaction for cytokeratin.

Flow cytometric DNA analysis: Four cases showed diploidy with DNA index of 1.0 and two cases had near-diploidy with DNA index of 1.11 and 1.13. One case (case No. 1) failed to be interpreted due to multiple broad peaks.

DISCUSSION

Clear cell sarcoma of the kidney is an uncommon renal malignancy estimated to constitute only 4% of childhood renal tumors (Beckwith, 1983) and is recognized as a distinct entity with an aggressive biologic behavior. There are considerable difficulty in differentiating this tumor from the more commonly occurring Wilms' tumor on the basis of clinical, radiological and histological features. Previously, CCSK diagnosed as Wilms' tumor showing sarcomatoid feature but having different histologic and clinical findings has been reported. CCSK was first recognized by Kidd (1970) as a sarcoma of the kidney with predisposition to metastasize to bone. The descriptive term 'clear cell' was originally proposed by Beckwith and Palmer (1978) to dis-

tinguish this tumor from the malignant rhabdoid tumor in terms of histologic characteristics. Sotelo-Avila *et al.* (1986) reviewed 21 cases of CCSK and reported additional 12 cases with single or multiple osseous metastasis to skull, spine, rib and femur, etc. In our small series, only two cases showed bone metastasis, one in tibia and one in vertebrae after surgery. The predilection for bone metastasis is well known but its pathogenesis is not clear. It probably appears to be determined partly by the anatomy of the venous return of kidney via Baston's plexus along the axial skeleton (Sotelo-Avila *et al.* 1986). In addition, renal vein and thoracic duct may play roles as disseminating routes if tumor cells are not filtered out by the capillaries of lung. Compared to Wilms' tumor (Table 2), CCSK reveals poorly demarcated tumor margin, firmer consistency and frequent peripheral cystic changes, grossly. Histologically, large vesicular nuclei with clear cytoplasm and characteristic arborizing fibrovascular network can help to establish the diagnosis. However, in some cases, distinct differential diagnosis is difficult especially in case of blastemal predominant Wilms' tumor in which blastemal cells often show clear cell change. In addition, entrapped normal tubules and glomeruli within the edge of the tumor can be misinterpreted as the neoplastic epithelial components of Wilms' tumor. Several immunohistochemical studies (Altmannsberger *et al.* 1984; Takagi *et al.* 1987) demonstrated only expression of vimentin in tumor cells of CCSK. And peanut agglutinin (PNA) lectin reacted only with luminal surfaces of the apparent tubular structures (Takagi *et al.* 1987). No tumor cells surrounding the tubular structures reacted with PNA lectin. Since the PNA lectin staining pattern of this structure in CCSK is the same as that of residual normal renal tubules, they are considered to be entrapped renal tubules. Some investigators also consider those structures to be nonneoplastic components because of their absence in the metastatic lesion (Beckwith, 1983). In our study, tumor cells only reacted with vimentin in contrast that the peripheral tubular structures reacted with cytokeratin. In Wilms' tumor, characteristic blastemal tissue reacts with both cytokeratin

Table 2. Comparison of clinical, pathological findings and DNA ploidy pattern between CCSK and Wilms' tumor

	CCSK	Wilms' tumor		
Clinical findings				
Bone metastasis	frequent (17~100%)	rare		
Recurrence	frequent	rare		
Pronosis	poor (mortality rate: >35%)	good (cure rate: 80~90%)		
Pathologic findings				
Gross				
Tumor margin	well or poorly demarcated	well demarcated		
Consistency	firm	soft&friable		
Cystic change	common (peripheral)	rare		
Microscopic				
Growth pattern	diffuse trabecular	triphasic (epithelial, blastemal, stromal)		
Cell shape	round to oval with clear cytoplasm	pleomorphic		
Arborizing vessel	+	-		
Entrapped normal tubule	+	-		
Immunohistochemical				
Vimentin	+	epi.	blastema	stroma
Cytokeratin	-	-	+/-	+
Actin	-	+	+/-	-
Desmin	-	-	-	+/-
DNA ploidy pattern	diploidy	favorable histology: diploidy unfavorable histology: aneuploidy		

epi: Epithelial component

and vimentin (Altmannsberger *et al.* 1984; Sibley *et al.* 1984; Takagi *et al.* 1987; Choi *et al.* 1993). Therefore, cytokeratin is greatly helpful in confirming the presence of blastemal component and useful in diagnosis of Wilms' tumor. In considering the combined histologic, immunohistochemical and electron microscopic features of CCSK, which was previously suggested by others (Altmannsberger *et al.* 1984; Hass *et al.* 1984; Takagi *et al.* 1987), a number of histogenetic possibilities deserve speculation. Recently, primitive mesenchymal cells which committed early stromagenic cells are strongly suggested as a origin of tumor cell of CCSK (Hass *et al.* 1984; Yun, 1993), which would explain the ability of CCSK to exhibit a wide range of histologic patterns. Congenital mesoblastic nephroma (CMN) is the most common renal neoplasm in the first 3 months of life (Bolande, 1973; Howell *et al.* 1982). It is

composed of interlacing bundles of plump to spindle shaped fibroblasts and myofibroblasts with eosinophilic cytoplasm and round to oval nuclei (Bolande, 1973; Pettinato *et al.* 1989). The term, atypical mesoblastic nephroma (AMN), have been proposed for a potentially aggressive CMN showing high cellularity and mitosis, necrosis and hemorrhage (Joshi *et al.* 1986). However, other have observed no correlation between these features and clinical behavior (Pettinato *et al.* 1989). The histogenesis of CMN remains controversial (Bolande, 1973) but the possibility of originating from secondary mesenchyme of mature mesoblastic derivatives is highly suggested (Wigger, 1975). In relation to CMN and AMN, early committed stromagenic cells can explain the histogenetic correlation with CCSK. Some authors proposed that CCSK may be originated by malignant transformation from CMN (Beckwith

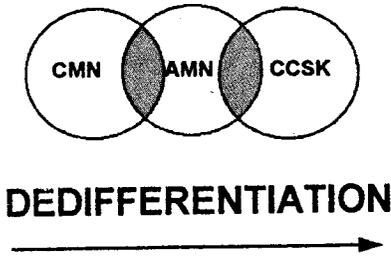


Fig. 3. A speculative histogenesis on the spectrum of congenital mesoblastic nephroma (CMN), atypical mesoblastic nephroma (AMN) and clear cell sarcoma of the kidney (CCSK).

and Yokomori, 1982; Hass *et al.* 1984) and CMN and CCSK represent both ends of the spectrum of mesenchymal renal tumors (Joshi *et al.* 1986). A speculative relationship between CMN and CCSK can be regarded then as an unique tumor with potential to differentiate into the stromal cell lineage showing varying degrees of differentiation in the spectrum of CMN, AMN and CCSK (Fig 3).

In flow cytometric DNA analysis, all cases represented diploidy or near-diploidy. These findings agreed with previous reports (Schmidt *et al.* 1986; Kumar *et al.* 1989) and thought to be different from those of Wilms' tumor with unfavorable histology. Most of Wilms' tumor reveals diploid DNA index but Wilms' tumor with unfavorable histology tends to be aneuploid DNA pattern with high DNA index (Schmidt *et al.* 1986; Kumar *et al.* 1989; Jung *et al.* 1991). Therefore, in differential diagnosis between CCSK and Wilms' tumor with predominantly monomorphic and unfavorable histology, DNA analysis appears to play a role in some extent.

In conclusion, CCSK is a separate entity with characteristic clinicopathologic, immunohistochemical and flow cytometric findings from Wilms' tumor which might reflect different histogenesis of these two tumors. But its histogenesis has not been proven conclusively. Further studies on histogenesis of CCSK in conjunction with CMN are required.

REFERENCE

- Altmansberger M, Osborn M, Schafer H, Schauer A, Weber K: Distinction of nephroblastomas from other childhood tumors using antibodies to intermediate filaments. *Virchows Arch (Cell Pathol)* 45: 113-124, 1984
- Beckwith JB, Palmer NF: Histopathology and prognosis of Wilms' tumor: Result from the First National Wilms' Tumor Study. *Cancer* 41: 1937-1948, 1978
- Beckwith JB: Histopathologic aspects of renal tumors in children. Renal Tumors. *Proceedings of the First international symposium on Kidney Tumors*. Ed by Kuss R, Murphy GP, Koury S. New York, Alan R Liss, 1982; 1-40
- Beckwith JB, Yokomori K: Clear cell sarcoma of kidney: Is it derived from mesoblastic nephroma (abstract). Presented at the *Fall Meeting of the Pediatric Pathology Club*, Vancouver, BC, Canada. October 6-8, 1982
- Beckwith JB: Wilms' tumor and other renal tumors of childhood: A selective review from the National Wilms' Tumor Study pathology center. *Hum Pathol* 14: 481-487, 1983
- Bolande RP: Congenital mesoblastic nephroma of infancy. *Perspect Pediatr Pathol* 1: 227-250, 1973
- Choi YJ, Jung WH, Shin DW, Park C, Lyu CJ: Histopathological and immunohistochemical features of Wilms' tumor. *Kor J Pathol* 27: 339-348, 1993
- D'Angio GJ, Evans AE, Breslow NE, Beckwith JB, Bishop HC, Fiegl P, Goodwin W, Leape LL, Sinks LF, Sutow W, Tefft M, Wolff J: The treatment of Wilms' tumor: Result of the National Wilms' Tumor Study. *Cancer* 38: 633-646, 1976
- Hass JE, Bonadio JF, Beckwith JB: Clear cell sarcoma of the kidney with emphasis on ultrastructural studies. *Cancer* 54: 2978-2987, 1984
- Hedley DW, Freidlander ML, Taylor IW: Application of DNA flow cytometry to paraffin embedded archival material for the study of aneuploidy and its clinical significance. *Cytometry* 6: 327-333, 1985
- Howell CG, Othersen HB, Kiviat NE, Norkool P, Beckwith JB, D'Angio GJ: Therapy and outcome of 51 children with mesoblastic nephroma: A report of the National Wilms' Tumor Study. *J Pediatr Surg* 17: 826-831, 1982
- Joshi VV, Kasznica J, Walters TR: Atypical meso-

- blastic nephroma. Pathologic characterization of a potentially aggressive variant of congenital mesoblastic nephroma. *Arch Pathol Lab Med* 110: 100-106, 1986
- Jung WH, Han JY, Park SY, Kim JE: Clear cell sarcoma of the kidney(Report of two cases). *Kor J Pathol* 25: 581-588, 1991
- Jung WH, Vawter GF, Kozakewich HP: Flow cytometric analysis(FCM) of DNA content in patients with Wilms' tumor(Abstract). *Lab Invest* 64(1): 6p, 1991
- Kidd JM: Exclusion of certain renal neoplasm from the category of Wilms' tumor. *Am J Pathol* 59: 16A, 1970
- Kumar S, Marsden HB, Cowan RA, Barnes JM: Prognostic relevance of DNA content in childhood renal tumors. *Br J Cancer* 59: 291-295, 1989
- Marsden HB, Lawler W, Kumar PM: Bone metastasizing renal tumor of kidney. *Cancer* 42: 1922-1928, 1978
- Marsden HB, Lawler W: Bone metastasizing renal tumor of childhood: Histopathological and clinical review of 38 cases. *Virchows Arch (A)* 387: 341-351, 1980
- Morgan E, Kidd JM: Undifferentiated sarcoma of kidney. *Cancer* 42: 1916-1921, 1978
- Novak RW, Cases JN, Johnson WW: Sarcomatous renal tumor of childhood: An electron microscopic study. *Am J Clin Pathol* 73: 622-625, 1980
- Oak SA, Hur B, Huh MH: Clear cell sarcoma of the kidney(A case report). *Kor J Pathol* 27: 81-84, 1993
- Pettinato G, Manivel JC, Wick HR, Dehner LP: Classical and cellular (atypical) congenital mesoblastic nephroma: A clinicopathologic, ultrastructural, immunohistochemical, and flow cytometric study. *Hum Pathol* 20: 682-690, 1989
- Schmidt D, Wiedemann B, Keil W, Sprenger E, Harms D: Flow cytometric analysis of nephroblastomas and related neoplasms. *Cancer* 58: 2494-2500, 1986
- Sibley RK, Wick MR, Cherwitz DL: An immunohistochemical study of Wilms' tumor and its variants(abstract). *Lab Invest* 52: 61A, 1985
- Sotelo-Avila C, Gonzalez-Crussi F, Sadowinski S, Gooch WM, Pena R: Clear cell sarcoma of the kidney: A clinicopathologic study of 21 patients with long-term follow-up evaluation. *Hum Pathol* 16: 1219-1230, 1986
- Takagi M, Takakuwa T, Ushigome S, Nakata K, Teruhiro F, Watanabe A: Sarcomatous variants of Wilms' Tumors. *Cancer* 59: 963-971, 1987
- Wigger HJ: Fetal mesenchymal hamartoma of kidney : A tumor of secondary mesenchyme. *Cancer* 36: 1002-1008, 1975
- Yun K: Clear cell sarcoma of the kidney expresses Insulinlike Growth Factor-II but not WT1 transcripts. *Am J Pathol* 142: 39-47, 1993