Identification of Anaplastic Lymphoma Kinase Fusions in Renal Cancer

Large-Scale Immunohistochemical Screening by the Intercalated Antibody-Enhanced Polymer Method

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BACKGROUND: Several promising molecular-targeted drugs are used for advanced renal cancers. However, complete remission is rarely achieved, because none of the drugs targets a key molecule that is specific to the cancer, or is associated with “oncogene addiction” (dependence on one or a few oncogenes for cell survival) of renal cancer. Recently, an anaplastic lymphoma kinase (ALK) fusion, vinculin-ALK, has been reported in pediatric renal cell carcinoma (RCC) cases who have a history of sickle cell trait. In this context, ALK inhibitor therapy would constitute a therapeutic advance, as has previously been demonstrated with lung cancer, inflammatory myofibroblastic tumors, and anaplastic large cell lymphomas. METHODS: Anti-ALK immunohistochemistry was used to screen 355 tumor tissues, using the intercalated antibody-enhanced polymer (iAEP) method. The cohort consisted of 255 clear cell RCCs, 32 papillary RCCs, 34 chromophobe RCCs, 6 collecting duct carcinomas, 10 unclassified RCCs, 6 sarcomatoid RCCs, and 12 other tumors. RESULTS: Two patients (36- and 53-year-old females) were positive for ALK as determined by iAEP immunohistochemistry. Using 5'-rapid amplification of complementary DNA ends, we detected TPM3-ALK and EML4-ALK in these tumors. The results of this study were confirmed by fluorescence in situ hybridization assays. The 2 ALK-positive RCCs were unclassified (mixed features of papillary, mucinous cribriform, and solid patterns with rhabdoid cells) and papillary subtype. They comprised 2.3% of non-clear cell RCCs (2 of 88) and 3.7% of non–clear cell and nonchromophobe RCCs (2 of 54). CONCLUSIONS: The results of this study indicate that ALK fusions also exist in adult RCC cases without uncommon backgrounds. These findings confirm the potential of ALK inhibitor therapy for selected cases of RCC.

KEYWORDS: anaplastic lymphoma kinase, molecular-targeted therapy, renal cell carcinoma, immunohistochemistry, intercalated antibody-enhanced polymer.

INTRODUCTION
Renal cancer is one of the major cancers. The incidence and mortality of cases are estimated at 273,518 and 116,368 in the world; 14,963 and 6957 in Japan; and 56,678 and 13,711 in the United States.1 The 5-year survival rate of patients with localized disease is relatively good: 65% to 93% and 47% to 77% for stages 1 and 2, respectively.2 For advanced renal cancers (34%-80% and 2%-20% 5-year survival rates in stages 3 and 4, respectively),2 several molecular-targeted drugs have been recently approved by the US Food and Drug Administration. These drugs, which include sunitinib, sorafenib, temsirolimus, everolimus, bevacizumab, pazopanib, and axitinib, are promising. However, none of them targets a key molecule that is specific to the cancer, or is associated with “oncogene addiction” of renal cancer, namely, the dependence on one or a few oncogenes for maintenance of the malignant phenotype and cell survival.

Anaplastic lymphoma kinase (ALK) fusion is a potential vulnerability, an “Achilles’ heel”, of many types of human cancer, including lymphoma, sarcoma, and carcinoma.6,7 Experimentally, lung adenocarcinomas developed in EML4-ALK (fusion of ALK with echinoderm microtubule–associated protein like 4) transgenic mice were successfully treated with an ALK inhibitor.8 The ALK inhibitor crizotinib has recently been used in patients with lung cancer, inflammatory myofibroblastic tumors (IMTs), or anaplastic large cell lymphomas (ALCLs), which harbor various ALK fusions. The compound showed an 81% response rate in ALK-positive lung cancers defined by at least 2 diagnostic methods,9,10 and a
strong response in IMT for several months. Two patients with ALCL who were receiving crizotinib achieved complete remission. These findings indicate that ALK fusion addiction is one of the most promising targets in cancer therapy.

To ensure that such molecular-targeted therapy is effective and less toxic, accurate screening methods to detect ALK fusions are crucial. However, although immunohistochemistry has been a gold standard for the detection of ALK fusions in ALCL and IMT, conventional anti-ALK immunohistochemistry is not sensitive enough to detect EML4-ALK, which was first described in lung cancer in 2007. To overcome this, we developed a sensitive intercalated antibody-enhanced polymer (iAEP) method (Fig. 1). Combined with a conventional anti-ALK mouse monoclonal antibody 5A4, the iAEP method efficiently and consistently detected EML4-ALK in paraffin-embedded sections. In various studies on ALK-positive lung cancer, anti-ALK immunohistochemistry by iAEP or essentially equivalent methods was used to examine surgically resected specimens, transbronchial lung biopsy specimens, and endobronchial ultrasound-guided transbronchial needle aspiration specimens. More importantly, some of the patients screened by anti-ALK iAEP immunohistochemical analysis received crizotinib therapy and showed a good response. Novel ALK fusions, including v6 and v7 of EML4-ALK, kinesin family member 5B (KIF5B)-ALK, sequestosome 1 (SQSTM1)-ALK, and PTPRF interacting protein, binding protein 1 (PPFIBP1)-ALK have been identified using anti-ALK iAEP immunohistochemical analysis. Thus, anti-ALK iAEP immunohistochemistry constitutes a powerful tool for clinical and also research purposes.

The development of anti-ALK antibodies has facilitated the investigation of many types and cases of cancer, including lung cancer. Since 1994, ALK-positive tumors have been identified exclusively in lymphoma...
(ALCL and ALK-positive large B-cell lymphoma\(^{28}\)) and sarcoma (IMT,\(^{5}\) rhabdomyosarcoma,\(^{26}\) and neuroblastoma\(^{25}\)). It was not until 2007 that the presence of an ALK fusion was described in lung cancer.\(^{6}\) This seems to be mainly because EML4-ALK is barely detectable by conventional anti-ALK immunohistochemistry. Considering in reverse, in cases of a tumor that is positive by anti-ALK iAEP immunohistochemistry, but negative by conventional anti-ALK immunohistochemistry, the tumor may have a novel ALK fusion partner, or express wild-type ALK at a modest level. Indeed, in “ALK-negative” IMT cases defined by conventional ALK immunohistochemistry, PPFIBP1-ALK was identified through reassessment for ALK fusions, using anti-ALK iAEP immunohistochemistry.\(^{24}\) This prompted us to reevaluate other types of solid cancers for ALK fusions. Here, we describe the identification of TPM3-ALK (fusion of tropomyosin 3 and ALK) and EML4-ALK in renal cancer, by anti-ALK iAEP immunohistochemistry.

MATERIALS AND METHODS

Materials

We examined 355 renal tumor tissues from patients who had received surgery in the Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, between 1994 and 2010. Renal tumors included 255 clear cell renal cell carcinomas (RCCs), 32 papillary RCCs, 34 chromophobe RCCs, 6 collecting duct carcinomas, 10 unclassified RCCs, 6 sarcomatoid RCCs, and 12 other tumors (4 oncocytomas, 3 angiomylolipomas, 1 solitary fibrous tumor, 2 spindle cell sarcomas, 1 desmoplastic sarcoma, and 1 anaplastic carcinoma). Surgically removed tumor specimens were routinely fixed in 20% neutralized formalin and embedded in paraffin for conventional histopathological examination. Immunohistochemical screenings were performed using tissue microarrays. For the 2 cases positive for anti-ALK immunohistochemistry, total RNA was extracted from the corresponding snap-frozen specimen, and purified with the use of an RNaseasy Mini kit (Qiagen, Tokyo, Japan). Informed consent was obtained from the patients. The study was approved by the institutional review board of the Japanese Foundation for Cancer Research.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was sliced at a thickness of 4 \(\mu\)m, and the sections were placed on silane-coated slides. For antigen retrieval, the slides were heated for 45 minutes at 102°C in antigen retrieval solution (Nichirei Bioscience, Tokyo). For conventional immuno-staining, the slides were incubated at room temperature with primary antibodies: ALK (5A4), vimentin, epithelial membrane antigen (EMA), cytokeratin 7, AE1/AE3, CAM5.2, 34βE12, \(\alpha\)-methylacyl-coenzymeA racemase (AMACR), clusters of differentiation 10 (CD10), transcription termination factor 1 (TTF1), renal cell carcinoma marker (RCC Ma), paired box 2 (PAX2), and paired box 8 (PAX8) for 30 minutes. The immune complexes were then detected with polymer reagent (Histofine Simple Stain MAX PO; Nichirei Bioscience, Tokyo, Japan). For the sensitive detection of ALK fusion proteins, the ALK Detection Kit (Nichirei Bioscience), which is based on the iAEP method, was used.

Isolation of ALK Fusions

To obtain complementary DNA (cDNA) fragments corresponding to a novel ALK fusion gene, we used a 5’ rapid amplification of cDNA ends (5’-RACE) method with the SMARTer RACE cDNA Amplification Kit (Clontech, Takara Bio Inc., Shiga, Japan). We followed the manufacturer’s instructions, with a minor modification: the ALK2458R primer (5’-GTATGTTGGGTATGTCGTCATGATGGT-3’) was used as the gene-specific reverse primer. From the deoxythymidine oligomer–primed cDNA obtained from RNA from case 1, a 385–base pair (bp) cDNA fragment containing the fusion point was specifically amplified with the primers TPM3-705F (5’-AGAGACCCGTGTGCAGTTTGTCTG-3’) and ALK3078RR (5’-ATCCAGTTCTGCTGTGTTCAAGC-3’). From case 2, a 454-bp cDNA fragment containing the fusion point was specifically amplified with the primers EML4-72F (5’-GTCAAGCTCTGGATGCAGTGGTCTGTCCAAGC-3’) and ALK3078RR. Polymerase chain reaction (PCR) analysis of genomic DNA for TPM3-ALK in case 1 was carried out with a pair of primers flanking the putative fusion point: TPM3-705F (5’-AGAGACCCGTGTGCAGTTTGTCTG-3’) and TPM3-705R (5’-TCTTGCCAGCAAAGCAGTAGTTGG-3’). For genomic PCR analysis of EML4-ALK in case 2, we used primers EML4-107F (5’-ATGAAATCACTGTGCTAA-3’), EML4-423R (5’-ATCCAGTTCTGCTGTGTTCAAGC-3’), and Fusion-RT-AS (5’-TCTTGCCACAGAAGCAGTAGTGGG-3’). For genomic PCR analysis of EML4-ALK in case 2, we used primers EML4-107F (5’-ATGAAATCACTGTGCTAAAGGCGGCT-3’), Fusion-RT-AS (5’-TCTTGCCACAGAAGCAGTAGTGGGG-3’), and Fusion-RT-AS (5’-TCTTGCCACAGAAGCAGTAGTGGG-3’).

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) analysis of gene fusion was carried out with DNA probes for ALK, TPM3, EML4, and transcription factor E3 (TFE3). Unstained sections (4 \(\mu\)m thick) were subjected to hybridization with an ALK-split probe set (Dako, Tokyo, Japan), TFE3-split probe set (Kreatech, Amsterdam, The Netherlands), or bacterial artificial chromosome (BAC) clone-derived...
probes for ALK (RP11-984I21, RP11-62B19, RP11-701P18), TPM3 (RP11-809B24), and EML4 (RP11-996L7). Hybridized slides were then stained with 4',6-diamidino-2-phenylindole and examined using a fluorescence microscope BX51 (Olympus, Tokyo, Japan).

**Mutation Analyses for MET**

A 1007-bp cDNA fragment containing the MET kinase domain was amplified using the primers MET-3186F (5'-GTCCATTACTGCAAAATACTGTCC-3') and MET-4193R (5'-CACCTCATCATCAGCGTATCC-3'). The PCR product was sequenced after subcloning.

**RESULTS**

**Identification of ALK Fusions in RCC Samples**

Sections of tissue microarray were immunostained for ALK by the iAEP method, resulting in the detection of 2 positive cases (case 1, Fig. 2A-C; case 2, Fig. 2D-F). The positive results were also confirmed using corresponding whole histopathological sections, in which all of the tumor cells stained for ALK as other ALK-positive cancers usually do. We carried out 5'-RACE assays to determine whether these cases expressed ALK fusion or full-length ALK (mutated or unmutated). We isolated a cDNA fragment containing the exon 8 of TPM3 fused in-frame to the exon 20 of ALK (Fig. 3A) in case 1, and the exon 2 of EML4 fused to the exon 20 of ALK in case 2 (Fig. 3B). This EML4-ALK is called variant 5 (E2;A20) in lung cancer.30 Reverse transcription PCR (RT-PCR) assays designed for the TPM3-ALK or E2;A20 successfully amplified cDNAs containing the fusion points (Fig. 3C,D). To confirm the genomic rearrangement, we performed FISH assays (Fig. 4) and genomic PCR (data not shown) for each fusion. All our results were consistent with the presence of t(1;2)(p21;p23)/TPM3-ALK in case 1, or inv(2)(p21p23)/E2;A20 in case 2. No other cases were positive for ALK by iAEP immunohistochemistry. All 355 cases were further examined by ALK-split FISH assay. In 12 of the cases, FISH was unsuccessful and not evaluable. In the other cases, the results were identical to those obtained by anti-ALK iAEP immunohistochemistry.

**Case Presentation**

**Case 1**

The patient was a 36-year-old woman who had a complaint suggestive of pyelonephritis. Magnetic resonance imaging and computed tomography showed a mass (4.0 cm × 4.0 cm × 3.5 cm) in the left kidney. No metastatic lesions or lymph node enlargements were identified. The patient had no past medical history of malignancy.
She underwent a translumbar left-radical nephrectomy and is currently alive and well without evidence of disease at 2 years of follow-up.

**Case 2**

A 53-year-old woman was found incidentally to have microscopic hematuria by medical check-up. Ultrasonography and magnetic resonance imaging showed a change in the left kidney, but the diagnosis was indefinite at that time. One year later, adenocarcinoma cells were detected by urinary cytology, and computed tomography revealed an isodense left renal mass (2.5 cm × 2.5 cm × 2.3 cm). The patient underwent a translumbar left-radical nephrectomy. She is currently alive and well at 7 years after surgery.

The patients had no episodes or family history indicative of sickle cell trait. To the best of our knowledge, there is no reported case of (genetically) Japanese individuals with sickle cell trait/disease.

**Histopathological Examinations**

The 2 ALK-positive renal cancers were papillary subtype and unclassified (with mixed features of papillary, mucinous cribriform, and solid patterns with rhabdoid cells). They comprised 2.3% of non–clear cell RCCs (2 of 88) and 3.7% of non–clear cell and nonchromophobe RCCs (2 of 54).

**Case 1**

Histologically, tumor cells were composed of papillary, tubular, or cribriform growth of cuboidal cells with...
eosinophilic cytoplasm. The cribriform morphology consisted of tubular structures with flattened epithelial cells, compressed by mucinous pool and inter- or intracytoplasmic vacuoles. Solid sheets of tumor cells with occasional deeply eosinophilic intracytoplasmic inclusions and eccentric nuclei, resulting in rhabdoid features, were focally identified. Nuclei were round to ovoid, and the nuclear size was basically uniform. Irregular nuclear membranes and nuclear grooves were occasionally observed. Mitotic figures were scant. The background stroma in the tumor area possessed abundant mucin. Frequent deposition of psammoma bodies and infiltration of numerous foamy macrophages were also seen. A large amount of mucinous matrix was highlighted with Alcian blue stain. These histological features resembled the mucinous cribriform pattern frequently observed in ALK-positive lung adenocarcinoma, and also a representative case of unclassified RCC by Lopez-Beltran et al., favoring a diagnosis of unclassified RCC. Immunohistochemically, neoplastic cells showed a diffuse and strong positivity for ALK (iAEP), vimentin, EMA, cytokeratin 7, AE1/AE3, cytokeratin CAM5.2, and cytokeratin 34BE12, and focally staining for PAX2, PAX8, AMACR, and CD10. TTF1 and RCC Ma were completely negative. Intracytoplasmic inclusions corresponded to aggregates of intermediate filaments of vimentin. The ALK-staining pattern appeared to be accentuated around the cell membrane of rhabdoid cells. The MIB1 (mindbomb homolog 1) labeling index was less than 1%.

Case 2

Histologically, the tumor consisted of papillary configuration of cuboidal or low columnar cells, with eosinophilic cytoplasm and small uniform round to oval nuclei. A clear cell change was focally seen. Nuclei showed a round to oval shape, and nuclear grooves were frequently observed. The size variation of nuclei was minimal, and the irregularity of the nuclear membrane was evident. Nuclear pseudoinclusions were seldom seen. Small nucleoli were occasionally identified, but mitoses were absent. The fibrovascular cores of papillary architecture contained numerous psammoma bodies and foamy macrophages. In addition, glandular lumens of tumor cells focally contained myxoid materials. These findings morphologically corresponded to papillary RCC, but did not fit to types 1 and 2 by the classification of Delahunt and Eble. In contrast, the features resembled papillary RCC, type 2A, described by Yang et al. Alcian blue stain highlighted a small amount of stromal-type mucin. Upon immunohistochemical analysis, neoplastic cells were diffusely and
strongly positive for ALK (iAEP), vimentin, EMA, cytokeratin 7, AE1/AE3, cytokeratin CAM5.2, cytokeratin 34BE12, and AMACR, and focally positive for PAX2 and PAX8, but negative for TTF1, CD10, and RCC Ma.

**DISCUSSION**

Recently, 2 independent groups have reported vinculin-ALK (VCL-ALK) in renal cancer (Table 1).35,36 These findings broaden the spectrum of ALK fusion–positive tumors. Interestingly, the 2 patients described in the reports share several uncommon backgrounds for renal cancer: very early onset (6- and 16-year-old boys), a history of sickle cell trait, and uncommon histopathological subtypes (medulillary subtype and indeterminate subtype with mixed features of medulillary, chromophobe, and transitional cell subtypes). In this study, we screened 355 renal tumors, including 343 RCCs, and identified ALK fusions in 2 RCCs. Significantly, we identified ALK fusions in adult patients (36- and 53-year-old females) without sickle cell trait. This finding will provide a key to ALK inhibitor therapy for more common renal cancers.

RCC associated with TFE3 gene fusions is already a distinctive entity in the World Health Organization classification,37,38 and MET mutation has been described in 13% of sporadic papillary RCCs.39 In the present study, we identified neither MET nor TFE3 aberrations in our ALK-positive renal cancer cases. ALK rearrangements are recognized as almost mutually exclusive to other mutations such as EGFR (epidermal growth factor receptor) and KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene) in lung cancer.40-42 All of the tumor cells in the 2 ALK-positive renal cancers observed by immunohistochemistry expressed ALK fusion protein, suggesting that all tumor cells harbor one or more ALK fusion genes. Therefore, as well as other ALK-positive tumors, ALK rearrangement in renal cancer probably occurs at a very early phase of carcinogenesis, and is likely to be a driver mutation and mutually exclusive to other driver mutations. As in the case of ALK-positive ALCCL, ALK-positive renal cancer will be a distinct molecular pathological entity.

**Examinations of Other Gene Aberrations**

For MET, a cDNA fragment with the predicted size was obtained by RT-PCR in case 1. In case 2, no products were identified, indicating that the tumor of the patient did not express MET. No mutations were identified in case 1 by sequencing. TFE3 split signals were not observed in either of the 2 cases by FISH.

TPM3-ALK was first identified in ALCL in 1999,41 and subsequently found in IMT in 2000.42 Therefore, RCC is the third type of cancer that may harbor TPM3-ALK. The organ distribution of EML4-ALK is somewhat controversial. Since its discovery, EML4-ALK has been reported to be identified in lung, breast, and colon cancers. Many research groups have reported the presence of EML4-ALK in a small subset of lung adenocarcinomas (2%-10%). Interestingly, a group in the United States reported the presence of EML4-ALK in breast (5 of 209) and colorectal (2 of 83) cancers, identified by RT-PCR optimized for variants 1, 2, and 3, without showing histopathological evidence.43 In contrast, 2 Japanese groups examined these cancers (90 breast and 96 colon cancers by RT-PCR for EML4-ALK variants 1 and 2, and 48 breast and 50 colon cancers by multiplex RT-PCR for all possible fusions), but detected no positive cases.30,43 One possible reason for this discrepancy may be differences in ethnicity. In the present study, we showed histopathological features of the 2 ALK-positive renal cancers. In addition to morphology, the positivity of PAX2 and PAX8 and the negativity of TTF1 strongly indicated that the ALK-positive cancers of the present cases were primary RCCs, and not metastatic lesions of ALK-positive lung cancer.

The oncogenic activities of TPM3-ALK and EML4-ALK have previously been documented,30,44 and therefore we did not demonstrate them in the present study. As in the case of other ALK-positive tumors, ALK-positive renal cancer is a promising candidate disease for ALK inhibitor therapy. In the present study, we screened surgically removable cases; the prognoses for the 2 ALK-positive patients were good, without recurrence. To realize the full potential of ALK inhibitors in renal cancers, it is important to identify the detailed clinicopathological features of ALK-positive cases, especially those of advanced or recurrent cases, by large-scale screening. For this purpose, anti-ALK immunohistochemistry can most readily be carried out as a primary screening tool. However, caution is needed; the screening immunohistochemical assay should be appropriately sensitive, because our present findings indicate that renal cancer involves EML4-ALK, which is barely detectable by conventional immunohistochemistry methods.13,45

Is morphology a clue to the presence of ALK fusion in renal cancers? Almost all ALK-positive lung cancers are adenocarcinomas, and more frequently show mucinous cribriform patterns and signet-ring cells than do ALK-negative adenocarcinomas.18,31,46 ALK fusion is probably very rare in clear cell RCC, which is the most common
subtype of renal cancer; 2 previously reported cases with VCL-ALK were not clear cell RCC, and we identified no ALK-positive cases in 255 clear cell RCCs in this study. Interestingly, case 1 showed a mucinous cribriform pattern. This may be a characteristic feature of ALK-positive carcinomas, universally applicable to carcinomas of various organs. Further study with a larger number of cases is warranted.

Molecular-targeted therapy of advanced renal cancers is starting to realize its full potential. However, complete remission is rarely achieved, because no agent targets a key molecule associated with “oncogene addiction” of renal cancer. In this context, ALK fusion constitutes a promising advance in renal cancers, as has previously been demonstrated with various other types of cancer. In the present study, we identified 2 adult cases of ALK-positive renal cancer in patients without uncommon backgrounds. Our findings confirm the potential of ALK inhibitor therapy for RCC. More detailed clinicopathological features of ALK-positive renal cancers, especially at higher clinical stages, are desirable. Hunting the “ALKoma” in various types of carcinomas, as well as in lung and kidney cancer, will provide an answer to these pathological and clinical questions.

### Table 1. ALK-Positive Renal Cancers: Present Cases and Review of Literature

| Characteristic                  | VCL-ALK (Debelenko et al) | VCL-ALK (Marino-Enriquez et al) | TPM3-ALK (Case 1) | EML4-ALK (Case 2) |
|--------------------------------|----------------------------|----------------------------------|-------------------|------------------|
| Age, y                         | 16                         | 6                                | 36                | 53               |
| Sex                            | Male                       | Male                             | Female            | Female           |
| Ethnicity                      | African American           | African American                 | Japanese          | Japanese         |
| Past history                   | Sickel cell trait          | Sickel cell trait                | Tuberculosis (22 y old) | Pleomorphic adenoma (50 y old) |
| Karyotype                      | Abnormal complex karyotype | 46,XY(2;10)(p23;q22), add(14)(p11) | Not examined      | Not examined     |
| Symptom                        | Right flank pain, gross hematuria | Intermittent periumbilical pain, hematuria | Pyelonephritis | Microscopic hematuria |
| Stage                          | Stage III                  | Stage I                          | Stage I           | Stage I          |
| Follow-up                      | 9 mo, alive. No evidence of disease | 21 mo, alive. No evidence of disease | 2 y, alive. No evidence of disease | 3 y, alive. No evidence of disease |
| Gross findings                 | 6.5-cm irregularly shaped solid tumor mass with infiltrative borders centered in the right renal medulla | 4.5-cm irregularly spherical mass with lobulated, fleshly light tan appearance centered in the medulla | Papillary, tubular, or cribriform growth of cuboidal cells with eosinophilic cytoplasm. Nuclei round to ovoid; nuclear size basically uniform | Double cancer. A: 2.5 cm x 2.5 cm x 2.3 cm solid yellow tumor in the cortex of the left intermediate pole. B: 0.6-cm yellow mass in the cortex of the left inferior pole |
| Microscopic findings           | Diffuse sheet-like pattern; round, oval, and polygonal tumor cells; eosinophilic cytoplasm; moderately polymorphic and vesicular nuclei | Solid growth pattern; spindle-shaped cells with large vesicular nuclei; clear coarse chromatin and abundant eosinophilic cytoplasm | Papillary, tubular, or cribriform growth of cuboidal cells with eosinophilic cytoplasm. Nuclei round to ovoid; nuclear size basically uniform | A: Papillary structure of cuboidal or low columnar cells with eosinophilic cytoplasm and small uniform round to oval nuclei. B: Clear cell |
| Immunohistochemistry           | Positive: AE1/AE3, CAM5.2, CK7, EMA, INI1, TFE3. Negative: CD10, S100, HMB45, WT1 | Positive: AE1/AE3, CAM5.2, EMA | Positive: ALK, vimentin, EMA, cytokeratin 7, AE1/AE3, CAM5.2, 34E12, AMACR (focal), CD10 (focal), PAX2 (focal), PAX8 (focal). Negative: TTF1, RCC Ma | A: Positive: ALK, vimentin, EMA, cytokeratin 7, AE1/AE3, CAM5.2, 34E12, AMACR, PAX2 (focal), PAX8 (focal). Negative: CD10, TTF1, RCC Ma |
| Diagnosis                      | Renal cell carcinoma, indeterminate subtype (medullary, chromophobe, transitional cell carcinoma mixed) | Renal medullary carcinoma | Renal cell carcinoma, unclassified | A: Papillary renal cell carcinoma, type 2A. B: Clear cell renal cell carcinoma |

ALK indicates anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein like 4; TPMS, tropomyosin 3; VCL, vinculin.
ALK-Positive Renal Cancer/Sugawara et al

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CONFLICT OF INTEREST DISCLOSURE
Dr. Takeuchi is a scientific advisor for the anti-ALK iAEP immunohistochemistry kit (ALK Detection Kit, Nichirei Bioscience, Tokyo, Japan). All remaining authors have made no disclosures.

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