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Renal cell carcinoma with synchronous ipsilateral urothelial carcinoma of the renal pelvis

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Abstract. The simultaneous occurrence of renal cell carcinoma (RCC) and urothelial carcinoma (UC) in the same kidney is extraordinarily rare, and is also known as multiple primary malignant tumors. The present study reports the case of a 76-year-old female with synchronous ipsilateral RCC and UC of the renal pelvis, who underwent operation, chemotherapy and reoperation when recurrence of RCC or UC was identified. Cluster of differentiation 44 (CD44) is one of the promising markers for identifying cancer stem cells in various solid tumors, along with aldehyde dehydrogenase 1 A1 (ALDH1A1). Detection of CD44 and ALDH1A1 prior to and subsequent to chemotherapy could provide useful prognostic information. New treatments against the cancer stem cells fraction should be used in combination with chemotherapy to improve the outcome for patients with overexpression of CSC markers.

Introduction

Combined renal cell carcinoma (RCC) and urothelial carcinoma (UC) of the renal pelvis is a rare type of multiple primary malignant tumor, which is characterized by the coexistence of two histologically distinct malignant tumors in the same organ with a shorter median time to relapse and mortality compared with a solitary tumor (1). There are only a few such cases in the world (2,3). Research into prognostic markers of multiple primary malignant tumors is important to establish adequate therapeutic strategies. Cancer stem-like cells (CSCs) are a small population of cancer cells that have the properties of tumor-initiating ability, self-renewal and differentiation (4). CSCs are more resistant to chemotherapy and radiotherapy than non-CSC populations via various mechanisms (5). Several studies have indicated that detection of CSC markers such as cluster of differentiation 44 (CD44) and aldehyde dehydrogenase 1 A1 (ALDH1A1) in urologic neoplasms can provide useful prognostic information (6-8). Furthermore, CD44 and ALDH1A1 have demonstrated high levels of activity in several types of solid cancer (9).

The present study reports a case of synchronous RCC and UC of the left kidney with poor prognosis. Abnormal expression of CD44 and ALDH1A1 CSC markers investigated prior to and subsequent to chemotherapy may indicate poor prognosis.

Materials and methods

Patient. A 76-year-old female with a 5-month history of left flank pain presented to the Department of Urology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School (Nanjing, China) without a history of fever, fatigue, weight loss or gross hematuria in March 2014. Her medical history included an 8-year history of hypertension, and there was no family history of urologic malignancies. Physical examination revealed that her vital signs were stable, and no palpable abdominal mass could be detected. Results from routine examinations, including electrocardiogram, chest radiography, pulmonary function test and laboratory tests of blood and urine, were all within the normal limits, with the exception of 8.4 red blood cells (RBC)/μl in urine (normal range, 0-5 RBC/μl). Ultrasonography suggested a left renal mass. A computed tomography (CT) scan revealed a 7.5-cm solid mass on the posterior aspect of the lower pole of the left kidney (Fig. 1A). In addition, a solid mass protruding into the upper collecting system was suspicious for RCC with invasion into the collecting system or for UC of the renal pelvis (Fig. 1B). The contralateral kidney was normal. The glomerular filtration rates of the left and right kidneys were 21.4 and 38.2 ml/min, respectively (normal range, >36.5 ml/min).

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Abbreviations; RCC, renal cell carcinoma; UC, urothelial carcinoma; CSCs, cancer stem-like cells; ALDH1A1, aldehyde dehydrogenase 1 A1; CD44, cluster of differentiation 44; CT, computed tomography

Key words: synchronous, renal cell carcinoma, urothelial carcinoma, CD44, ALDH1A1

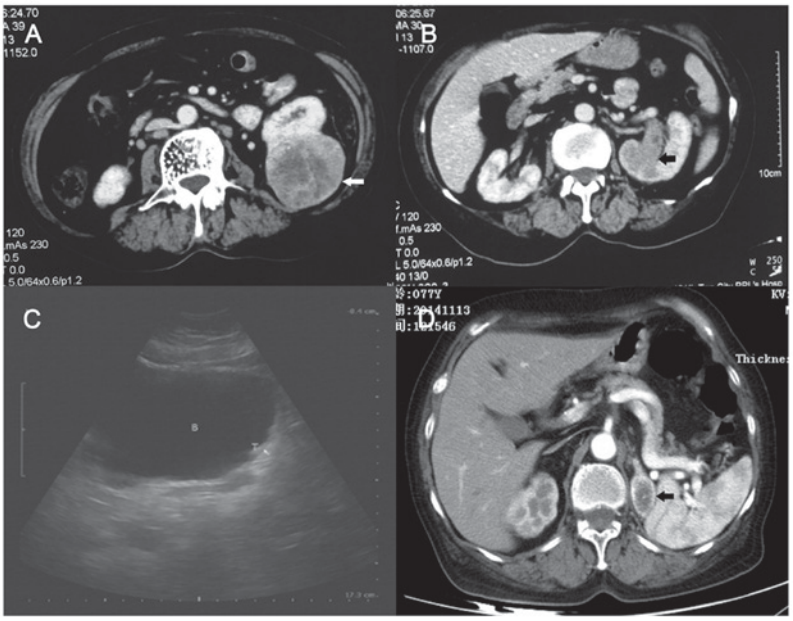

Figure 1. (A) CT scan demonstrates a 7.5-cm solid mass on the posterior aspect of the lower pole of the left kidney. (B) CT scan demonstrates a solid mass protruding into the upper collecting system. (C) Ultrasound demonstrates recurrence of bladder cancer at the follow-up of 5 months. (D) Restaging CT scans demonstrate metastasis of the left adrenal tumor at the follow-up of 15 months (black arrow). CT, computed tomography.

The patient underwent transperitoneal laparoscopic left radical nephrectomy. Upon dissecting the kidney, it was obvious that there were two morphologically distinct masses in the kidney. Intraoperative frozen section of the suspicious mass confirmed a UC of the renal pelvis; thus, transperitoneal laparoscopic left ureterectomy was performed. The cut surface of the gross specimen displayed two masses: A 7.5x5.0x4.5-cm yellowish, sharply marginated solid tumor in the lower pole of the kidney; and a 4.0x3.0x2.5-cm mass in the superior aspect of the renal pelvis (Fig. 2A). Tissue blocks were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Histologically, the larger tumor exhibited the characteristics of stage T2a clear cell carcinoma, with Fuhrman's nuclear grade 3 of 4 and without invasion of the renal capsule or pelvis (Fig. 2B). The following key was used to assess immunohistochemical staining: -, negative; ±, weak positive; +, moderate positive; and ++, strong positive Immunohistochemical staining (2 h at room temperature) of RCC demonstrated 30% Ki-67^{+} (1:400; cat. no., 19972-1-AP; ProteinTech Group, Inc., Chicago, IL, USA), P53$^{\pm}$ (1:1,000; cat. no., 10442-1-AP; ProteinTech Group, Inc.), cyclooxygenase 2^{++} (1:200; cat. no., 38024; Signalway Antibody LLC, College Park, MD, USA), vascular endothelial growth factor$^{\pm}$ (1;200; cat. no., 19003-1-AP; ProteinTech Group, Inc.), epidermal growth factor receptor^{-} (1:100; cat. no., 42520; Signalway Antibody LLC) and O6-methylguanine DNA methyltransferase^{+} (1:100; cat. no., 17195-1-AP; ProteinTech Group, Inc.). The second tumor was a stage T3, high-grade papillary UC with invasion of the renal parenchyma (Fig. 2C). The surgical margins were negative, and no metastasis of lymph nodes was detected. The patient received gemcitabine (1000 mg/m^2; day 1 and day 8) and cisplatin (70 mg/m^2; day 2) chemotherapy following surgery, every 3 weeks. Bladder recurrence of UC occurred at the follow-up of 5 months (Fig. 1C), and left adrenal metastasis of RCC occurred at the follow-up of 15 months (Fig. 1D). The patient accordingly underwent transurethral resection of a bladder tumor and laparoscopic left adrenalectomy.

At present, the patient remains under follow-up. The patient gave informed consent for their data to be published as part of the present study.

Immunoblotting. Tumor samples were lysed in radioimmunoprecipitation assay buffer containing cOmplete™, Mini Protease Inhibitor Cocktail (Roche Diagnostics, Basel, Switzerland) following surgery. The proteins in the lysates (20 μg) were separated by 30% SDS-PAGE and transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Upon blocking with 5% non-fat milk in PBS containing Tween 20 (PBST), primary antibodies against CD44 (Cat# 3570S; Cell Signaling Technology, Inc., Danvers, MA, USA), ALDH1A1 (Cat# 22109-1-AP; ProteinTech Group, Inc., Chicago, IL, USA) and β-actin (Cat# 05-0079; AbMax, Beijing, China) were used. Dilutions for all antibodies were 1:1,000, and membranes were incubated for 16 h at 4˚C. The membranes were then washed with PBST three times (5 min, room temperature) and incubated (1 h at room temperature) with a horseradish peroxidase-conjugated secondary antibody (1;1,000; cat. no., L3052-2; Signalway Antibody LLC). The western blots were visualized using enhanced chemiluminescence reagents (Cat# WBKLS0100; EMD Millipore).

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNAs were extracted using TRIzol® (Cat# 15596018; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. RT was conducted using random primers provided in Takara system

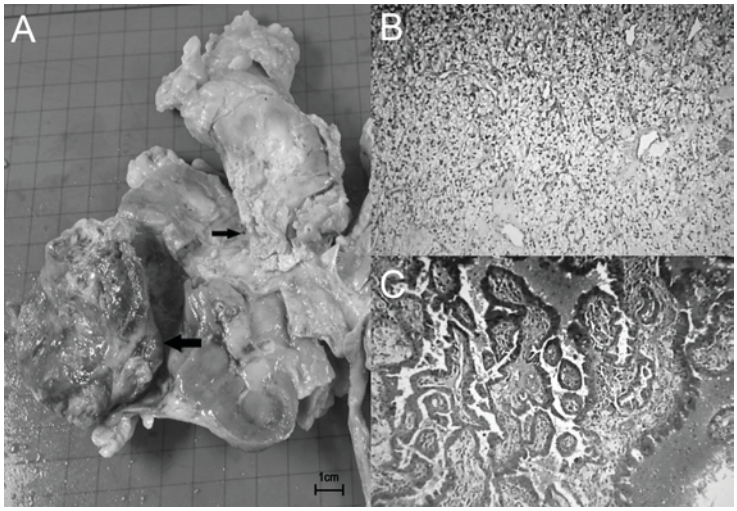

Figure 2. (A) Gross specimen demonstrating synchronous ipsilateral RCC (wide arrow) and UC of the renal pelvis (narrow arrow). (B) RCC of clear cell type with Fuhrman's nuclear grade 3 of 4 (H&E staining; magnification, x100). (C) High-grade papillary UC of the renal pelvis (H&E staining; magnification, x100). RCC, renal cell carcinoma; UC, urothelial carcinoma; H&E, hematoxylin and eosin.

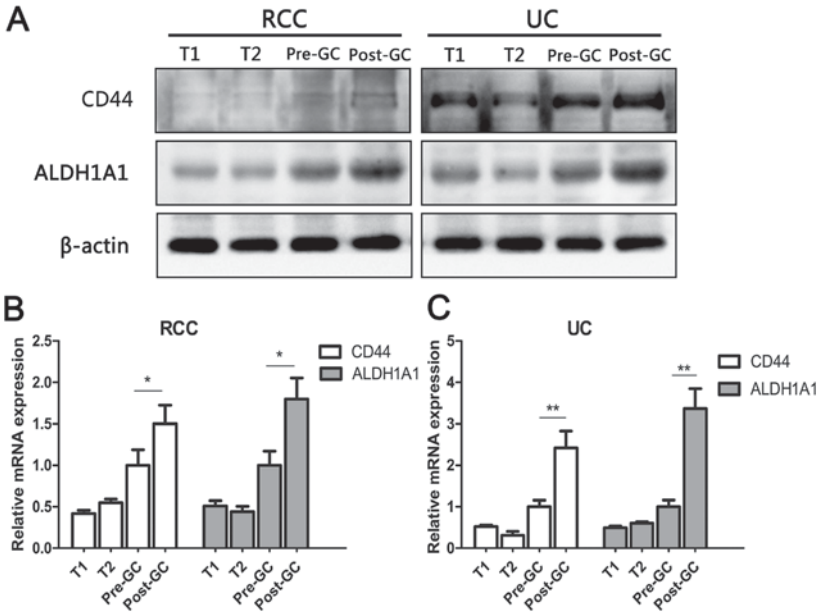

Figure 3. The expression levels of CD44 and ALDH1A1 were detected by (A) western blotting and (B and C) reverse transcription-quantitative polymerase chain reaction prior to chemotherapy (Pre-GC) and subsequent to chemotherapy (Post-GC). T1 and T2 represent single type of tumor of RCC or UC. Pre-GC and Post-GC represent primary and recurrent lesions, respectively, of the present case. *P<0.05; **P<0.01. CD44, cluster of differentiation 44; ALDH1A1, aldehyde dehydrogenase 1 A1; RCC, renal cell carcinoma; UC, urothelial carcinoma; mRNA, messenger RNA; GC, gemcitabine and cisplatin.

(PrimeScript RT Reagent kit with gDNA Eraser; Takara Biotechnology Co., Ltd., Dalian, China). The expression of relative genes was measured by RT-qPCR using SYBR Green (Takara Biotechnology Co., Ltd.) in an ABI 7500 StepOnePlus Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers used were as follows: CD44 forward, 5'-ATCGCTCTCCTGCTAACA GTC-3' and reverse, 5'-CTCGTACTGGATGGGTGA ACT-3'; ALDH1A1 forward, 5'-CACCACGTACAAGGG TCAGGTGC-3' and reverse, 5'-CAGCCTCCCACGCTG GGGTAT-3'; and β-actin forward, 5'-CATGTACGTTGC TATCCAGGC-3' and reverse, 5'-CTCCTTAATGTCACG CACGA-3'. The thermocycling conditions were as follows: Pre-denaturation at 95˚C for 10 sec, followed by denaturation at 95˚C for 5 sec, and annealing and extension at 60˚C for 31 sec. The expression of target genes was calculated based on the quantification cycle (Cq) values compared with a reference gene β-actin, using the formula $2^{-\Delta\Delta Cq}$ (?). RT-qPCR was performed in triplicate for each sample in a 10-μl reaction mixture, which consisted of template complementary DNA (0.2 μl), primers (0.4 μl, 1.0 M), ROX Reference Dye II (0.2 μl; SYBR® Premix Ex Taq kit; Takara Biotechnology Co., Ltd.), distilled H_2O (4.2 μl) and SYBR Premix Ex Taq (5 μl; SYBR® Premix Ex Taq kit; Takara Biotechnology Co., Ltd.). All reactions were performed in triplicate.

Results

Western blotting revealed that the protein levels of CD44 and ALDH1A1 were higher when RCC and UC of the renal pelvis occurred simultaneously. In addition, the expression level of cancer stem cell markers in metastatic lesions was higher than that in primary lesions following chemotherapy in the present case (Fig. 3A), although RCC was not sensitive to chemotherapy according to the guidelines (10).

RT-qPCR analysis revealed that gene expression of CD44 and ALDH1A1 in UC of the present case increased more significantly than that of RCC following chemotherapy (Fig. 3B and C).

Discussion

Multiple primary malignant neoplasms are characterized by the coexistence of two adjacent but histologically distinct malignant tumors (1). The incidence of this kind of tumor in the kidney is lower than that in other organs (11). RCC is the most common lesion of the kidney, and accounts for ~70% of renal malignancies (12). Primary UC of the renal pelvis is a relatively rare disease, which accounts for 5-7% of urinary tract tumors (13). The combination of these two types of tumor has rarely been reported previously in the literature. The earliest case was reported by Graves and Templeton (12) in 1921, and the most recent one was reported by Atilgan *et al* (2) in 2013. According to Pubmed search results, ~40 cases of synchronous ipsilateral RCC and renal pelvic UC have been reported in the literature to date (2,3,14,15). The average age of the reviewed patients was 65±11 years; the male/female ratio was 1.6; and the left-to-right-side ratio was 1.9. In total, 73% of the cases presented with hematuria, 37% with flank pain and 10% without obvious symptoms, and no identifiable past medical history could be observed (2,3,14-16).

Accurate preoperative diagnosis of RCC with synchronous ipsilateral UC of the renal pelvis is important to guide the selection of a surgical operation method. The rareness of such disease causes a high misdiagnosis rate (3). Preoperative examination should be comprehensive to obtain as much information as possible and to ensure the identification of suspicious masses. Intraoperative frozen section of the suspicious solid mass may confirm the diagnosis during operation, so that ureterectomy can be performed (3).

The standard surgical procedure of RCC is radical nephrectomy or partial nephrectomy, according to the characteristics of the tumor (17). For UC of the renal pelvis, the recurrence rate is 30-70%, and nephroureterectomy represents the main line of treatment (18). The 5-year survival rate of high-grade pT3 UC of the upper tract, such as the one described in the present case, is only 25% (19). In summary, radical nephroureterectomy should be performed in cases with synchronous ipsilateral RCC and UC, and transperitoneal laparoscopic nephroureterectomy is a less invasive method for suspicious UC of the renal pelvis.

Multiple primary malignancies tend to exhibit poor prognosis. In total, 24% of such cases had tumor metastases at initial examination, and 34% of the patients had bladder neoplasms (15). In the present report, routine follow-up demonstrated recurrence of RCC and UC, despite the fact that the patient received chemotherapy and the lesion was resected completely according to the pathological results. To the best of our knowledge, the current study discusses the first reported patient who has suffered recurrence of both RCC and UC during follow-up.

CSCs are considered to possess resistance to chemotherapy, and there is a direct link between the expression of CSC markers and patient survival (5,20). The abnormal detection of CSC markers in primary or recurrent lesions prior and subsequent to chemotherapy may partly explain the high rate of metastatic recurrences and short survival, which is clearly reflected in this case. However, the roles of CD44 and ALDH1A1 in UC require further investigation.

In conclusion, adjuvant therapy should be administered according to the staging and pathological grading of RCC with synchronous ipsilateral UC of the renal pelvis, and new treatments against the cancer stem cells fraction should be used in combination with chemotherapy to improve the outcome of such patients.

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