Assessment of Cell Cycle-related Elements p53, p21WAF1/Cip1, Cyclin D1 and PCNA in a Mixed Transitional Cell Carcinoma and Adenocarcinoma of the Renal Pelvis: a Case Report

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A case featuring a well differentiated adenocarcinoma mixed with a transitional cell carcinoma (TCC) arising in the renal pelvis of a 63-year-old woman is presented. Daughter tumors, located in the ureter and the uretero-vesical junction, were entirely TCC in character. Immunohistochemical assessment of cell cycle-related proteins revealed overexpression of cyclin D1 but reduced p21WAF1/Cip1 or PCNA expression in the adenocarcinomatous regions. Conversely, expression of p21WAF1/Cip1 and PCNA was high in the TCC components. Immunohistochemical staining for p53 was negative and PCR–SSCP analyses confirmed the absence of any mutation. Therefore, assessments on the altered expression of cell cycle-related elements may contribute to our understanding of tumor biology in adenocarcinomas and TCCs of the renal pelvis and to identifying the similarities and differences between the two different cell types.

Key words: adenocarcinoma – renal pelvis – p21WAF1/Cip1 – p53 – cyclin D1

INTRODUCTION

Tumors of the renal pelvis are almost exclusively transitional cell carcinomas (TCCs) (1,2) and adenocarcinomas are very rare (3), accounting for less than 1% of all primary neoplasms in this region (4). We report here a case of adenocarcinoma of the renal pelvis coexisting with TCC components with special emphasis on the expression and topological distribution of cell cycle-related proteins. In addition, the mutational status of p53 was determined.

CASE REPORT

A 63-year-old woman was referred to our urology department with a suspected renal pelvic tumor. Prior to admission, the patient had noted gross hematuria for approximately 1 year but she was otherwise asymptomatic and did not pay much attention to it. Abdominal sonography was performed when she sought medical treatment for a peptic ulcer and hydronephrosis of the right kidney was detected. Intravenous pyelography (IVP) revealed a non-functioning right kidney and a filling defect of the right uretero-vesical junction. Magnetic resonance imaging (MRI) and cystoscopy confirmed the existence of right renal pelvic, ureter and uretero-pelvic junction tumors. A right nephro-ureterectomy with bladder cuff resection was performed and the patient has now been followed-up for 18 months without recurrence. The patient did not have a history of chronic phenacetin abuse.

PATHOLOGIC FINDINGS

The right kidney was divided sagitally in half and the ureter was opened to reveal its contents. The gross specimen consisted of papillary tumors of the right renal pelvis, pelvic-uretero junction, upper ureter and uretero-vesical junction. Moderate hydronephrotic change was noted in the resected kidney. The topological arrangement of the tumors and the histologic appearances of the various lesions are illustrated in Fig. 1. The two renal pelvic tumors measured ~3 cm (Fig. 1A) and ~6 cm (Fig. 1D, E, F) in the largest dimension. Those at the right pelvic-uretero junction (Fig. 1B), the upper ureter (Fig. 1C) and the uretero-vesical junction (Fig. 1G) measured ~2.5, ~1.5 and ~2.5 cm in diameter, respectively. Histopathological diagnoses were as follows: for the smaller renal pelvic tumor (Fig. 1A), TCC, G2, pTa, pT1, pVO; for the larger renal pelvic tumor (Fig. 1D, E, F), TCC, G2, pT2, pL1, pV0; for the pelvic-uretero junction tumor (Fig. 1B), TCC, G2, pTa, pL1, pV0; for the ureter tumor (Fig. 1C), TCC, G2, pTa, pL0, pV0; and for the uretero-vesical junction tumor (Fig. 1G), TCC, G2 (G2 > G3),...
Figure 1. Topological arrangement of the tumors and the histology of the various lesions.

pT1b, pT0, pV0, INF0. High-magnification microphotographs of the adenocarcinoma component are presented in Fig. 2. A well differentiated adenocarcinoma was surrounded by grade II TCC components (Fig. 2A). A higher magnification of the adenocarcinoma component showed numerous glandular formations intermixed with small amounts of stromal cells (Fig. 2B). Similar histopathology of well differentiated adenocarcinoma surrounded by TCC components was observed elsewhere within the renal pelvic tumor (Fig. 2C).

MATERIALS AND METHODS

TUMOR MATERIAL

The surgical specimens were fixed in 10% neutral-buffered formalin (pH 7.4) for 2 days at 4°C. Detailed sketches were made and the sizes and locations of the grossly visible tumors were recorded. The surgical specimens were routinely processed for embedding in paraffin. Histological diagnosis was made on the hematoxylin/eosin-stained specimens.

IMMUNOHISTOCHEMICAL ANALYSIS

Serial sections of 3 μm thickness were spread on poly-L-lysine (PLS)-coated slides, immersed in three changes of xylene and hydrated using a graded series of alcohol. Antigen retrieval was performed by immersion of sections in distilled water inside a plastic container and by microwave heating for 20 min at low power. For p53 and proliferating cell nuclear antigen (PCNA) immunohistochemistry, sections were blocked with goat serum at 37°C for 30 min and then incubated with, respectively, mouse monoclonal anti-p53 antibody (DO-7, IgG2b; Dako, Glostrup, Denmark) at 1:100 dilution and mouse monoclonal anti-PCNA antibody (PC-10, IgG2a; Dako) at 1:500 dilution overnight at 4°C. For cyclin D1 and p21WAF1/Cip1 immunohistochemistry, sections were blocked with horse serum and then incubated with, respectively, rabbit polyclonal antihuman cyclin D1 antibody...
DNA EXTRACTION

DNAs were extracted from formalin-fixed, paraffin-embedded specimens. Sections of 10 μm thickness were made, spread on glass slides and deparaffinized as described above. Microdissections were performed under a dissection microscope. Dissected specimens were placed in 1.5 ml siliconized microfuge tubes and digested in 100 μl aliquots of buffer consisting of 20 mM Tris, pH 8.0, 1 mM EDTA, 0.5% SDS and 200 mg/ml proteinase K for up to 72 h at 42°C. DNAs were precipitated with 2-propanol and then further purified by phenol-chloroform extraction and ethanol precipitation. DNA pellets were resuspended in Instagene (Bio-Rad) at a final concentration of 50 ng/μl (6).

DETERMINATION OF MUTATIONS

Mutations of p53 were evaluated by polymerase chain reaction (PCR)–single-strand conformation polymorphism (SSCP) analysis. Histologically normal regions of kidney cortex served as controls for PCR amplification. The primers for the PCR–SSCP analysis were as follows:

| Exon 5:       | Upstream  5-TCT CIT CCA GTA CTC TCC TC-3 |
|              | Downstream 5-AGG CGG TGT TGA GGG CTT AC-3 |
| Exon 6:       | Upstream  5-GGC TTC TGA CTT ATT CTT GC-3 |
|              | Downstream 5-CAA CTG TCT CTA AGA CGC AC-3 |
| Exon 7:       | Upstream  5-TCA CCT GCA TCC TGT GTC TT-3 |
|              | Downstream 5-CAG GCT AAC CTA ACC TAC CA-3 |
| Exon 8:       | Upstream  5- ACT GCC TTG TGCTGGTCCTI-3 Exon |
|              | Downstream 5-TGA AGC TCA ACA GCC TCC TC-3 |

Sample DNA (50 ng) was mixed in a total of 5 μl of reaction solution containing 50 nM [32P]dCTP and amplified for 35 cycles (94°C, 30 s; 55°C, 30 s; 72°C, 30 s). PCR products were mixed with 20 μl of SSCP stop solution containing 95% formamide–20 mM EDTA–0.05% bromphenol blue–0.05% xylene cyanol, heated at 90°C for 5 min and then applied to modified acrylamide (MDE) gels (AT Biochem, Malvern, PA). Electrophoresis was carried out at 6 W for 16 h and the acrylamide gels were then dried and autoradiographed between intensifying screens at −80°C.

RESULTS

IMMUNOHISTOCHEMICAL ANALYSIS

Serial sections were made for all tumors and immunohistochemical assessment of p53, p21WAF1/Cip1, cyclin D1 and PCNA expression was performed. An adenocarcinoma of the renal pelvis is shown surrounded by TCC elements. It is presented at low magnification in Fig. 3A–D to illustrate the topographic distributions of the various cell cycle-related elements and at a higher magnification in Fig. 3E–H to show the detailed features of histology; these illustrations correspond to the lesion in Fig. 1A. In Fig. 4A–D, islands of well differentiated adenocarcinoma are intermixed with TCC; Fig. 4E–H illustrate the numerous glandular formations within the adenocarcinoma; they are representative of the lesions illustrated in Fig. 1E–F. By immunohistochemistry, p53-positive cells represented less than

![Figure 2. Hematoxylin/eosin staining of tumor specimens. (A) Well differentiated adenocarcinoma of the renal pelvis surrounded by a grade II TCC. (B) Higher magnification of the adenocarcinoma. (C) Island of well differentiated adenocarcinoma cells surrounded by grade II TCC components. (Magnifications: A, x25; B, x50; C, x100.)](https://academic.oup.com/jjco/article-abstract/28/3/227/916684)
5% of tumor cell population in both the TCC or the adenocarcinoma (Figs 3A, 3E, 4A and 4E). p21WAF1/Cip1 staining was diffusely positive within the TCC elements but absent from the adenocarcinoma components (Figs 3B, 3F, 4B and 4F). Cyclin D1 was positively stained in both the TCC elements and the adenocarcinoma but the intensity and the extent of staining were regionally variable; they were appreciably stronger within the adenocarcinoma (Figs 3C, 3G, 4C and 4G). PCNA was positively stained in both the TCC and the adenocarcinoma; however, the intensity of staining was strong and diffuse within the TCC elements but weak and scattered within the adenocarcinoma (Figs 3D, 3H, 4D and 4H).

Figure 3. Immunohistochemical assessment of the adenocarcinoma. (A, E) p53-positive nuclei were rare in the TCC and were absent from the adenocarcinoma. (B, F) Nuclear staining of p21WAF1/Cip1 was weak in the TCC and was absent from the adenocarcinoma. (C, G) Cyclin D1 overexpression was present in both the adenocarcinoma and TCC components with a higher intensity of staining in the adenocarcinoma. (D, H) PCNA staining was predominantly localized to the TCC and was negligible in the adenocarcinoma. (Magnifications: A-D, ×25; E-H, ×50.)
Figure 4. Expression of cell cycle-related proteins in adenocarcinoma and TCC components of a renal pelvic tumor. (A, E) Only a few nuclei within the TCC were positively stained with p53 while the adenocarcinoma was devoid of p53 staining. (B, F) Nuclei in the TCC, but not the adenocarcinoma, were weakly stained with p21WAF1/CIP1. (C, G) A large number of cells within the adenocarcinoma showed nuclear staining of cyclin D1. (D, H) PCNA staining was intense within the TCC components and was negligible within the adenocarcinoma components. (Magnifications: ×50.)

GENETIC ANALYSIS

We evaluated the p53 mutational status of the tumors by PCR–SSCP analysis of the microdissected specimens. Mutations were not detected within exons 5, 6, 7 and 8 of the p53 gene.

DISCUSSION

We recently described a close association between expression of cyclin D1 in TCCs of the urinary bladder and tumor differentiation (5). In the present case, we observed intense cyclin D1
staining within the adenocarcinoma elements, which demonstrated less proliferation than the surrounding TCC as assessed by PCNA staining. Therefore, the overexpression of cyclin D1 in the adenocarcinoma elements may have been linked to cellular transformation of the transitional epithelium of the renal pelvis rather than a direct consequence of increased cell proliferation.

Nuclear accumulation of the p53 protein generally correlates with mutations in the p53 gene in invasive TCCs of the human urinary bladder (7). In the present case, immunohistochemistry revealed p53-positive nuclei in less than 5% of the tumor cell population in both the adenocarcinoma elements and the TCC elements; therefore, we hypothesized that p53 mutations was probably absent in both the TCC and the adenocarcinoma components. PCR-SSCP analysis of exons 5, 6, 7 and 8 of the p53 gene confirmed the absence of mutations. These results suggest that functional inactivation of the p53 gene may not be involved in the carcinogenesis of the present case.

Assessment of nuclear expression of the CDK inhibitor p21WAF1/Cip1 demonstrated staining of the TCC but not the adenocarcinomatous components. Similarly, PCNA staining was strongly positive in the TCC components but very weakly positive in the adenocarcinoma. Recent reports suggest that p21WAF1/Cip1 can inhibit DNA replication by binding to PCNA (8,9) and we noted here that the patterns of staining for these two proteins corresponded in most of the TCC components. The differences in staining intensity of PCNA between the TCC components and the adenocarcinoma components may be explained by the difference in the rate of cell proliferation.

Transitional epithelium of the renal pelvis may undergo glandular metaplasia (10). In the present case, the well differentiated adenocarcinoma was admixed with TCC components; therefore, it may have originated by transformation of transitional epithelium of the renal pelvis. p53 and p21WAF1/Cip1 are negative regulators of the cell cycle and they are subject to precise control; conversely, cyclin D1 is among the positive regulators of cell cycle and its overexpression is commonly observed in malignancy; dysregulated expression of these proteins may be a critical factor in the generation of malignancy. The present case suggests that aberrant expression of cell cycle-related proteins may be responsible for differences in the behavior of adenocarcinomas and TCCs of the renal pelvis. Furthermore, assessments on the altered expression of cell cycle-related elements may contribute to our understanding of tumor biology in adenocarcinomas and TCCs of the renal pelvis and to identifying the similarities and differences between the two different cell types.

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