Carcinosarcoma of the Urinary Bladder Following Cyclophosphamide Therapy

Evidence for Monoclonal Origin and Chromosome 9p Allelic Loss

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• We report a case involving a 45-year-old man with a 12-year history of Wegener granulomatosis, who developed a carcinosarcoma of the urinary bladder after long-term cyclophosphamide therapy. Cyclophosphamide is well recognized as an etiologic agent for urothelial carcinoma of the urinary bladder. However, only 5 cases of carcinosarcoma of the urinary bladder following cyclophosphamide therapy have been reported. We used loss of heterozygosity studies and microsatellite markers to define the molecular basis of this rare neoplasm. These studies revealed evidence supporting a monoclonal origin for the 2 components of this tumor. We also demonstrated allelic loss of chromosome 9p. This loss associated with carcinosarcoma of the urinary bladder is in agreement with previous studies, suggesting a possible role for the tumor suppressor gene *p16* in the pathogenesis of this tumor.

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Cyclophosphamide therapy has greatly improved the survival of patients with Wegener granulomatosis. However, cyclophosphamide is also a known carcinogen, well documented to cause urothelial carcinoma of the urinary bladder.[1] Although cyclophosphamide is clearly linked to the development of carcinoma of the urinary bladder, its role in the development of other malignant bladder tumors is less clear. A recent report documented the interesting association between cyclophosphamide therapy and bladder leiomyosarcoma.[2] The authors of the article reported 2 cases and found a total of 7 additional cases of postcyclophosphamide leiomyosarcoma in the literature. Carcinosarcoma of the urinary bladder is an even rarer sequel to cyclophosphamide therapy; only 5 cases have been reported.[3,4]

Here, we report a case of carcinosarcoma of the urinary bladder in a patient with Wegener granulomatosis who was given cyclophosphamide therapy. Loss of heterozygosity (LOH) analyses using microsatellite-flanking markers suggested a monoclonal origin for the 2 components of the tumor and provided evidence for allelic loss of chromosome 9p.

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REPORT OF A CASE

A 45-year-old man presented with hematuria and groin pain. He had been diagnosed 12 years earlier with Wegener granulomatosis and was treated with steroids, methotrexate, and cyclophosphamide. During an 8-year period, he received a cumulative dose of approximately 293 g of cyclophosphamide. At the current admission, cyclophosphamide-induced hemorrhagic cystitis was considered the cause of the hematuria, and cyclophosphamide was therefore discontinued. However, the hematuria persisted, and computerized tomography of the abdomen revealed a bladder mass, which was biopsied transurethrally. Results of computerized tomography and bone scans were negative for metastasis. A cystoprostatectomy with ileal conduit formation was performed. The patient was offered adjuvant radiotherapy but declined. In the 12 months since diagnosis, no evidence of recurrence or metastasis of the tumor has been detected.

MATERIALS AND METHODS

The LOH analyses were performed using protocols published previously.[5] All primer sequences are available from the Web (http://www.ncbi.nlm.nih.gov/genome/guide/human/) except the informative primer pair for the short arm of chromosome 17. This primer pair was designed to flank a (CA)n repeat from BAC clone AC109333 located at 17p13.2 close to the GP1Bα gene. The primers (sequences) for 17p are GT6250F (AGAACTAGGCACAG AGGACACAGTG) and GT6250R (GCCCACGGCATGTTAAAG TTT), which amplify an amplicon of 163 base pairs with 17 (CA)n repeats. Amplification conditions for all primers used involved 35 cycles at 57°C for annealing and 1.5mM magnesium chloride. Microsatellite-flanking markers were selected from all autosomal arms, except the short arms of the acrocentric chromosomes (Table 1). Amplification was performed on DNA extracted from nontumorous prostate (control) and the carcinomatous and sarcomatous components from formalin-fixed paraffin-embedded tissue. Two blocks composed exclusively of sarcomatous and carcinomatous elements were used for DNA extraction, obviating the need for microdissection. If the nontumor tissue was not heterozygous, a second and rarely a third primer pair were used so as that an informative (ie, heterozygous) microsatellite marker for all chromosome arms was obtained.

RESULTS

Gross, Histologic, and Immunohistochemical Findings

Histopathologic examination of the transurethral resection revealed a high-grade, poorly differentiated malig-

Table 1. Microsatellite Marker Analysis to Detect Chromosomal Deletions and Microsatellite Instability

	Short Arm (p)			Long Arm (q)		
Chromosome	Locus	Region	Aberrations*	Locus	Region	Aberrations
1	D1S1612	p36.23	None	D1S659	q41	None
2	D2S1360	p24.1	None	D2S362	q	MSI (CA)
3	D3S2432	p24.1	None	D3S1262	q	None
4	D4S2639	p15.31	MSI (CA, SA)	D4S1607	q	None
5	D5S1457	p13.1	None	D5S816	q	None
6	D6S271	p21.1	None	GATA184 A08	q	None
7	D7S2514	p21.3	None	D7S1824	q	None
8	D8S264	p23.2	None	D8S1132	q	None
9	D9S286	p24.1	LOH (CA, SA)	D9S1776	q	None
10	D10S211	p12.31	None	D10S1432	q	None
11	D11S922	p15.5	None	D11S1986	q	None
12	D12S1685	p13.32	None	D12S1064	q	None
13	NA†	NA	NA	D13S325	q	None
14	NA	NA	NA	D14S67	q	None
15	NA	NA	NA	D15S659	q	None
16	D16S2616	p13.32	None	D16S3253	q	None
17	GP1Bα (GT6250)	p13.3	LOH (CA, SA)	D17S515	q	None
18	D18S542	p11.21	None	D18S55	q	None
19	D19S591	p13.3	None	D19S433	q	None
20	D20S470	p12.1	None	D20S481	q	None
21	NA	NA	NA	D21S1899	q	None
22	NA	NA	NA	D22S685	q	LOH (CA)
22	NA	NA	NA	D22S420	q	LOH (CA)

* MSI indicates microsatellite instability; CA, carcinoma; SA, sarcoma; and LOH, loss of heterzygosity.
† NA indicates no analysis performed.

nant neoplasm invading the bladder musculature with essentially only vimentin reactivity in the tumor cells. During subsequent radical cystoprostatectomy, a 9.0-cm polypoid tumor was found that occupied 80% of the bladder lumen, grossly invading the muscle wall and extending into the surrounding adipose tissue. Examination of sections revealed a biphasic tumor composed of well-differentiated squamous cell carcinoma, interfacing with poorly to well-differentiated malignant mesenchymal elements (Figure 1, A).

The carcinoma was cytokeratin positive and vimentin negative (Figure 1, B and C). Urothelial carcinoma was not detected in multiple sections obtained from the tumor and the adjacent mucosa, although areas of squamous metaplasia were identified. The mesenchymal component was vimentin positive and cytokeratin negative (Figure 1, B and C). Extensive sampling of this component revealed foci resembling a well-differentiated liposarcoma and areas consistent with pleomorphic liposarcoma and dedifferentiated liposarcoma. The well-differentiated liposarcomatous component was composed of relatively mature adipocytes, with lipoblasts and atypical stromal cells present (Figure 2, A). The pleomorphic component contained lipoblasts and pleomorphic spindle and giant cells (Figure 2, B). The dedifferentiated component consisted of a high-grade sarcoma, which appeared nonlipogenic and cellular, with spindle cells and a fascicular pattern of growth (Figure 2, C). Desmin-positive pleomorphic rhabdomyosarcomatous differentiation was also focally detected (Figure 3, A through C). A diagnosis of carcinosarcoma of the urinary bladder was made; the sarcomatous elements had features of a mixed-type liposarcoma with focal rhabdomyosarcomatous differentiation.

Molecular Genetic Analysis

Both the sarcoma and carcinoma samples shared LOH for microsatellite markers on the short arms of chromosomes 9 and 17. They also showed microsatellite instability; they were identical for the same gain of a repeat at the D4S2639 marker on 4p. In addition, the carcinoma but not the sarcoma showed LOH for the long arm of chromosome 22 and the gain of an allele for the D2S362 microsatellite marker on the long arm of chromosome 2. No evidence of microsatellite instability or LOH was evident from the other markers.

COMMENT

A substantial percentage of patients treated with long-term cyclophosphamide develop urothelial carcinoma of the urinary bladder (5% at 10 years and 16% at 15 years).[1] Cyclophosphamide's urotoxicity is thought to be mediated by its metabolite acrolein,[1,6] which is excreted in the urine, making it especially toxic to the urinary bladder. In contrast to the relatively common occurrence of postcyclophosphamide urothelial carcinomas, there are only 5 previously reported cases of carcinosarcoma of the urinary bladder subsequent to cyclophosphamide therapy (Table 2).[3,4] In those cases, however, the patients were being treated for malignancies (lymphoma or recurrent urothelial cancer). To the best of our knowledge, this is the first reported case in which a patient being treated with cyclophosphamide for a nonneoplastic disease (Wegener granulomatosis) has developed a carcinosarcoma of the bladder.

Review of the data in Table 2 reveals that cyclophosphamide-related carcinosarcomas tend to occur in the elderly and to present at an advanced stage and are treated surgically. The prognosis is variably poor and may depend on the extent of resection. Carcinosarcoma of the urinary bladder, even outside the setting of cyclophosphamide therapy, is a rare tumor. A review of the literature revealed only approximately 90 reported cases to date. Liposarcomatous differentiation of the sarcomatous compo-

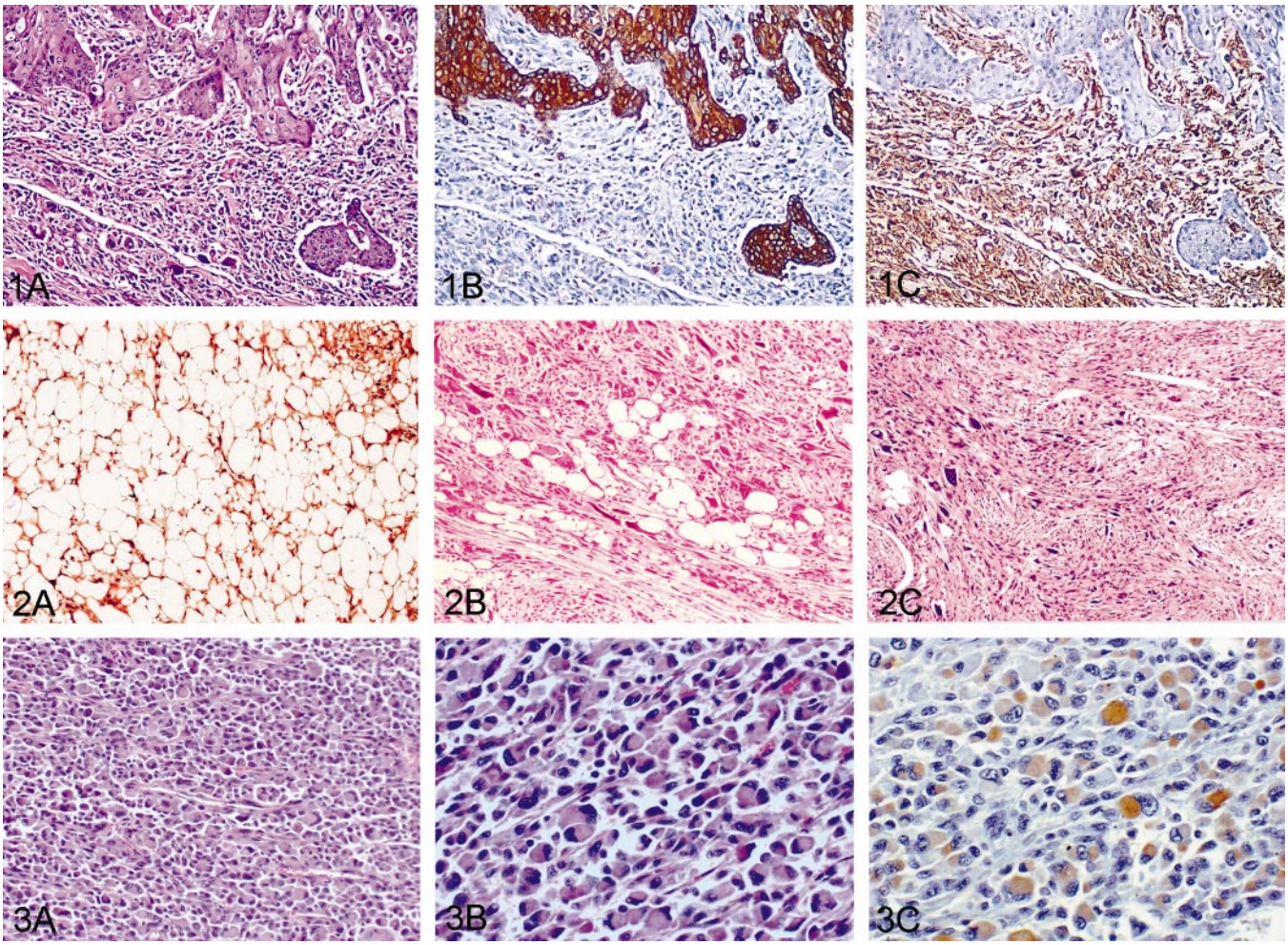

Figure 1. *Junction of carcinoma and sarcoma (hematoxylin-eosin [A], cytokeratin [B], vimentin [C]; original magnification ×60).*

Figure 2. *Liposarcomatous differentiation. A, Well-differentiated liposarcoma (hematoxylin-eosin, original magnification ×24). B, Pleomorphic liposarcoma (hematoxylin-eosin, original magnification ×40). C, Dedifferentiated liposarcoma (hematoxylin-eosin, original magnification ×40).*

Figure 3. *Rhabdomyosarcomatous differentiation (hematoxylin-eosin, original magnification ×40 [A]; hematoxylin-eosin, original magnification ×256 [B]; desmin, original magnification ×256 [C]).*

Table 2. Cyclophosphamide-Induced Bladder Carcinosarcoma: Cases Reported to Date

Case No.	Age, y	Sex	Indication for Cyclophosphamide	Histology	Treatment; Follow-up
1[3]	50	F	Non-Hodgkin lymphoma	Urothelial carcinoma, leiomyosarcoma	Preoperative radiation and radical cystectomy; alive with metastasis at 4 y
2[4]	53	F	Lymphoma	Urothelial carcinoma, osteosarcoma, leiomyosarcoma	Cystectomy and irradiation; dead of disease in 11 mo
3[4]	82	M	Lymphoma	Small cell carcinoma, osteosarcoma, leiomyosarcoma	Transurethral resection; dead of disease in 2 mo
4[4]	52	M	Recurrent urothelial cancer	Small cell carcinoma, malignant fibrous histiocytoma	Transurethral resection; not available
5[4]	66	M	Recurrent urothelial cancer	Urothelial carcinoma, leiomyosarcoma	Cystectomy; dead of intercurrent disease
Current case	45	M	Wegener granulomatosis	Squamous cell carcinoma, mixed-type liposarcoma, rhabdomyosarcoma	Cystoprostatectomy; no evidence of disease at 12 mo

nent, as seen in our case, is very rare. Only 2 such cases have been reported.[7,8]

The histogenesis of carcinosarcoma is controversial. The divergence theory of a monoclonal origin for both components[9] implies that the tumors diverge from a single multipotent common precursor. The convergence theory of multiclonal origin implies 2 stem cells, one epithelial and the other mesenchymal. This theory was originally

proposed by Virchow and involved a ''collision'' between 2 independently developing tumors. Of these conflicting concepts, the monoclonal theory seems to be gaining ground in the published literature.[9,10] Our results are consistent with a monoclonal origin for carcinosarcoma of the urinary bladder. The sarcoma and carcinoma in our case show a number of common genetic abnormalities, including LOH on 9p and 17p and microsatellite instability at the D4S2639 marker on 4p. These shared abnormalities suggest a monoclonal origin for these 2 components. The carcinoma shows additional abnormalities (divergence) in the form of LOH at 22q and microsatellite instability at D2S362 on 2q, presumably acquired during tumor progression.

Previous studies on carcinosarcoma of the urinary bladder have used comparative genomic hybridization and LOH to delineate the genetic events occurring in this tumor.[10,11] Halachmi et al[11] (6 cases) showed loss of 9p, 9q, 8p, and 8q in both components. Gronau et al[10] (1 case) showed losses of 9p and 11q in both components. The short arm of chromosome 9 was also lost in our case. Thus, loss of 9p seems to be the most consistent chromosomal aberration in carcinosarcoma of the urinary bladder in the literature to date and may be the key genetic event in the pathogenesis of these tumors. As pointed out by Gronau et al,[10] 9p is the location of the tumor-suppressor gene *p16*. The genetic locus for *p16* (9p21) is close to the location of the microsatellite we used for chromosome 9 in this study (9p24.1). Losses of 9p, including deletion of *p16*, have been previously reported in both components in carcinosarcomas of various sites and are an early finding in superficial urothelial carcinomas.[12] Another notable feature in the present case is LOH at 17p (in both the carcinoma and the sarcoma), the location of the tumor suppressor gene *p53*. The *p53* gene mutation may have played a role in the development of the tumor; however, loss of 17p was not noted in previous studies on the molecular genetics of carcinosarcoma of the urinary bladder.[10,11] Therefore, in contrast to allelic loss of chromosome 9p, loss of chromosome 17p appears to be a finding peculiar to the present tumor rather than a consistent genetic event in urinary bladder carcinosarcomas.

Here, we present a case of carcinosarcoma of the urinary bladder developing in a patient with Wegener granulomatosis that had been given cyclophosphamide therapy. This case adds to the growing evidence that cyclophosphamide may be associated with bladder neoplasms other than urothelial carcinoma. We also present evidence for the monoclonal origin of carcinosarcoma using microsatellite-flanking markers and LOH analyses. The demonstrated allelic loss of chromosome 9p in carcinosarcoma of the urinary bladder suggests direction for further research on the role of the tumor suppressor gene *p16* in the development of this tumor.

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