Inflammatory myofibroblastic tumor of the urinary bladder with FN1-ALK gene fusion: A case report

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Abstract. Inflammatory myofibroblastic tumors (IMTs), which are rare tumors, exhibit myofibroblastic differentiation, often with anaplastic lymphoma kinase (ALK) gene rearrangements. A subset of IMTs identified in the urinary tract have been shown to harbor a fibronectin 1 (FN1)-ALK gene fusion. In this case report, a case of an IMT with FN1-ALK fusion in the urinary bladder was presented, and its clinicopathological characteristics were reviewed. A 45-year-old female was referred to Chungbuk National University Hospital with gross hematuria. Cystoscopy revealed a solid mass in the bladder. The patient subsequently underwent transurethral resection of the lesion. The mass comprised stellate and spindled myofibroblastic cells that were arranged in loose fascicles, with a myxoid background and a mixed inflammatory infiltrate. Immunohistochemical analysis revealed that the tumor cells were positive for vimentin, cytokeratin AE1/AE3 and ALK, and focal-positive for desmin. Targeted next-generation sequencing was subsequently employed to identify the FN1-ALK fusion. To date, the patient has undergone outpatient follow-up for 18 months, with no signs of tumor recurrence. To conclude, in total, FN1 has been identified as an ALK fusion partner almost exclusively in cases of genitourinary IMTs [13 bladder IMTs (including the present case) and two uterine IMTs]. In the present case, the FN1-ALK fusion was found to involve ALK exon 19 and FN1 exon 23. By contrast, the majority of the other IMTs with an ALK fusion have involved ALK exon 20, whereas ALK fusion involving ALK exon 18 or 19 has been reported only in genitourinary IMTs. Therefore, the FN1-ALK fusion involving ALK exon 18 or 19 may be specific to a subset of IMTs arising in the urinary bladder.

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Introduction

Inflammatory myofibroblastic tumors (IMTs) are rare mesenchymal neoplasms with myofibroblastic differentiation. IMTs can occur at any age, although the disease has a predilection for children, adolescents and young adults (1). IMTs typically arise in the abdominal cavity, retroperitoneum, pelvis, lung, and head and neck; they occur less commonly in the gastrointestinal tract, pancreas, bladder and uterus (2). The preferred option of treatment for IMTs of the urinary bladder is surgical resection; however, treatment options are limited for patients with advanced disease and/or unresectable cases (3).

~50% of IMTs harbor anaplastic lymphoma kinase (ALK) gene rearrangements, resulting in constitutive gene activation and cytoplasmic ALK protein expression (4). A targeted therapeutic approach against ALK fusion has been proposed as a novel and promising option for therapy. A patient with a RANBP2-ALK-positive IMT was found to have a partial response to the ALK tyrosine kinase inhibitor (TKI), crizotinib (5). In addition, a neoadjuvant therapy for a patient with FN1-ALK fusion treated with the ALK TKI lorlatinib exhibited a reduction in tumor size.

The majority of urinary tract IMTs with an ALK gene rearrangement harbor a fibronectin 1 (FN1)-ALK gene fusion (6). In the present case report, another case of an IMT of the urinary bladder with FN1-ALK fusion was presented. In the current case, the FN1-ALK fusion involved ALK exon 19 and FN1 exon 23. By contrast, the majority of IMTs with ALK fusion at other organs involve ALK exon 20, whereas ALK fusions involving exon 18 or 19 have been reported only in genitourinary IMTs with a 5'-gene fusion partner FN1 (3,6-9). Together with a review of the clinicopathological characteristics of such cases, it is suggested that the presence of FN1-ALK fusions with ALK exon 18 or 19 rearrangements may be specific to a subset of IMTs arising in the urinary bladder.

Case report

A 45-year-old woman was referred to the Chungbuk National University Hospital institute with a 1-week history of gross

hematuria. Computed tomography (CT) revealed the presence of a 2.2x2 cm, heterogeneously enhancing round mass located in the anterior/superior wall of the urinary bladder (Fig. 1). Cystoscopy identified it as a solid erythematous mass (Fig. 2). The patient subsequently underwent transurethral resection of the lesion in May 2021. Microscopically, the mass was found to comprise stellate and spindled myofibroblastic cells in loose fascicles, with a myxoid background and a mixed inflammatory infiltrate comprising neutrophils, eosinophils and lymphocytes (Fig. 3A). The tumor cells had a pale eosinophilic cytoplasm, with long tapering processes. The nuclei were found to be elongated, ovoid or round, with a bland appearance, and contained fine chromatin and small-to-inconspicuous nucleoli (Fig. 3B). Mitotic figures were present (3-4 per 10 high-power fields), along with focal necrosis. The formalin-fixed, paraffin-embedded tumor specimens were sectioned (4-μm thickness) and then stained. Fully automated immunostaining was performed using a BenchMark XT autostainer (Ventana Medical Systems, Inc.) Immunohistochemical analysis revealed that the tumor cells were positive for vimentin (cat. no. 790-2917, ready-to-use; Ventana Medical Systems, Inc.), cytokeratin AE1/AE3 (cat. no. PA0909; ready-to-use; Leica Biosystems) and ALK (cat. no. CMC20421040; 1:50; Cell Marque; MilliporeSigma), with cytoplasmic staining and focal-positive staining for desmin (cat. no. CMC24321040; 1:50; Cell Marque; MilliporeSigma) (Fig. 4A-C), but negative for EMA (cat. no. 790-4463; ready-to-use; Ventana Medical Systems, Inc.), S-100 (cat. no. CMC33021060; 1:200; Cell Marque; MilliporeSigma) and α-SMA (cat. no. PA0943; 1:150; Leica Biosystems Nussloch GmbH), leading to a suspicion of IMT. Therefore, targeted next-generation sequencing was subsequently performed. RNA was extracted using the RecoverAll™ Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Inc.). RNA was reverse transcribed using an Ion Torrent NGS Reverse Transcription Kit (Thermo Fisher Scientific, Inc.). Automated library preparation was performed using Oncomine™ Comprehensive Assay Plus, RNA, Chef-Ready panel (Thermo Fisher Scientific, Inc.) and an Ion AmpliSeq™ Kit for Chef DL8 (Thermo Fisher Scientific, Inc.). Templating and sequencing were performed using an Ion 550™ Chip Kit (cat. no. A34537; Thermo Fisher Scientific, Inc.), using IonS5 XL (Thermo Fisher Scientific, Inc.) and Ion Chef instrument systems (Thermo Fisher Scientific, Inc.). All procedures were performed per the manufacturer's protocols. Next-generation sequencing succeeded in identifying FN1-ALK fusion between exon 23 of FN1 and exon 19 of ALK. To date, the patient has undergone outpatient follow-up for 18 months, with no signs of tumor recurrence.

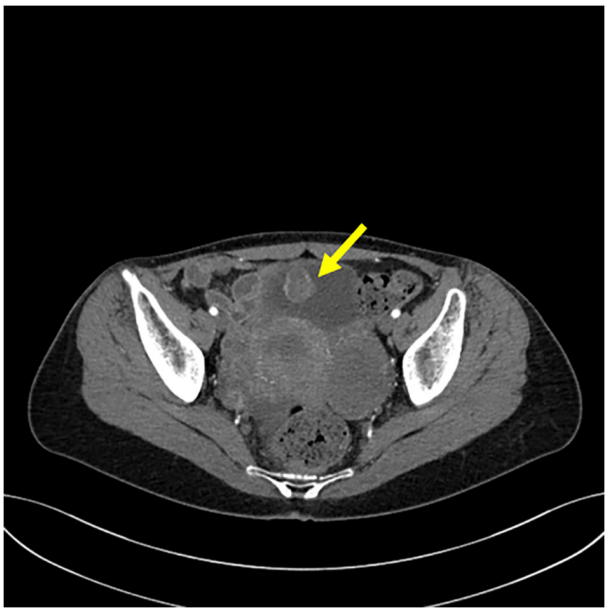

Figure 1. Enhanced abdominopelvic computed tomography image showing an enhanced 2.2x2 cm bladder mass (yellow arrow).

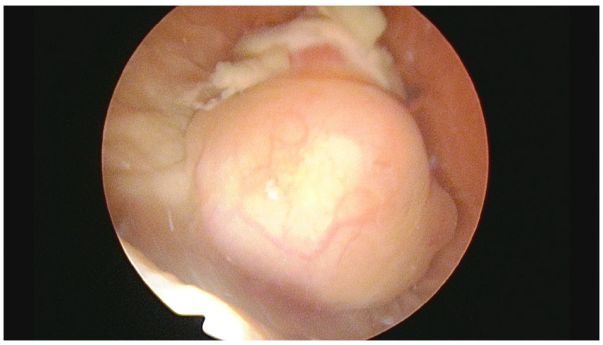

Figure 2. Cystoscopy, revealing a single solid erythematous tumor in the anterior/superior wall of the urinary bladder.

Discussion

IMT of the urinary bladder is a rare tumor that follows an indolent clinical course. IMTs are often associated with ALK gene rearrangement, and the FN1 gene is the most common fusion partner in the urinary bladder (6,10). Including the present case, 13 cases of urinary bladder IMTs with FN1-ALK fusion have been reported (3,6,7,9,11). It should be noted that seven cases reported that were by Acosta *et al* (6) were designated by these authors as pseudo-sarcomatous myofibroblastic proliferation (PMP), a separate entity from IMTs; however, these tumors are currently classified as IMTs by the WHO Classification of Tumors of the Urinary System and Male Genital Organs (5th edition) (12), which details that IMTs and PMP share histological features and a myofibroblastic immunohistochemical phenotype, even though no definite gene fusion is detected in the case of PMP. The clinicopathological characteristics of this subtype were investigated [with the exception of the seven cases reported by Acosta *et al* (6) that featured no detailed clinical data] (Table I). Of the remaining six cases, the neoplasm was detected in two children and four young-to-middle aged adults; the majority of the patients were female. The most common clinical symptom identified was hematuria. Patients with large tumors underwent neoadjuvant treatment and partial cystectomy, whereas the others underwent transurethral resection. All three patients with available data concerning prognosis lived uneventfully. ALK immunohistochemistry was positive in all cases. Notably, immunostaining for α-SMA was positive in most bladder IMTs (13), but negative in the present case.

Overall, the recurrence rate for IMTs is ~20%, although it is markedly higher for abdominal and pelvic IMTs (up

Table I. Clinical features of previously reported cases of a primary retroperitoneal mucinous cystic neoplasm with borderline malignancy.

First uthor, year	Age, years	Sex	Size, cm	Clinical symptoms	Treatment	Outcome	Fusion	ALK IHC	(Refs.)
Lovly *et al*, 2014	8	F	3	ND	ND	ND	Exon 23 of FN1 Intron 18 of ALK		(3)
Lovly *et al*, 2014	26	F	3	ND	ND	ND	Exon 23 of FN1 Intron 18 of ALK	Positive	(3)
Ouchi *et al*, 2015	12	M	ND	Gross hematuria	Neoadjuvant treatment (meloxicam) + partial cystectomy	NED, 12 months	Exon 20 of FN1 Exon 19 of ALK	Positive	(7)
Bertz *et al*, 2020	64	F	ND	ND	TUR-B	ND	ND	Positive	(11)
Reinhart *et al*, 2020	43	F	7	Dysuria and macrohematuria	Neoadjuvant therapy (crizotinib → lorlatinib) + partial cystectomy	NED, 12 months	Exon 36 of FN1 Exon 19 of ALK	Positive	(9)
Present case	45	F	2.2x2	Gross hematuria	TUR-B	NED, 18 months	Exon 23 of FN1 Exon 19 of ALK	Positive	

F, female; IHC, immunohistochemistry; M, male; ND, not described; NED, no evidence of disease; TUR-B, transurethral resection of the bladder; ALK, anaplastic lymphoma kinase; FN1, fibronectin.

to 85%), and lower for IMTs of the lung (<2%) and bladder (<4%) (2,14-16). Metastasis occurred in <5% of the IMT cases (2,16). The clinical behavior of IMT, particularly that of the urinary bladder, was found to be indolent; therefore, the treatment of choice is surgical resection, and adjuvant chemotherapy is not usually necessary. However, neoadjuvant therapy may be required to reduce the size of the tumor prior to surgery.

The current RNA sequencing results revealed the presence of FN1-ALK fusion. In cases of IMT of the urinary bladder, the most common fusion partner is FN1 (6). IMTs of other organs harbor ALK fusions with various fusion partners, most commonly TPM3 and CLTC (3). Other partners of ALK include HNRNPA1 (17), TPM4 (18) and ATIC (19). Gene fusions involving other kinases, including ROS1 and PDGFRB, have been reported in small subsets of IMT (10). To date, FN1 has been identified as a fusion partner for ALK almost exclusively in genitourinary IMTs [i.e., the reported 13 bladder IMTs (including our case) and two uterine IMTs] (3,6-9,11). All 13 bladder IMTs were found to be immunopositive for ALK (3,6,7,9,11).

FN1 is located at chromosome 2q35, and encodes fibronectin, a glycoprotein widely distributed in plasma and the extracellular matrix. *In vitro,* fibronectin is required for transforming growth factor-β-induced myofibroblastic differentiation (20). As in most other ALK fusion genes, the oncogenic activity of the fusion gene is probably due to the strong promoter function of FN1, which facilitates activation of ALK via homodimerization of the fusion protein (21).

In the present case study, the FN1-ALK fusion involved ALK exon 19 and FN1 exon 23. The majority of previously reported ALK fusions have featured a common breakpoint between exons 19 and 20 (22). Thus, the ALK fusions have always been found to include exons 20-29, which code for the cytoplasmic ALK tyrosine kinase domain (23), whereas in genitourinary IMTs, all the reported cases with the FN1-ALK fusion have involved ALK exon 18 or 19, with the exception of the cases that lacked data (3,6-9). Therefore, the transmembrane domain of the ALK protein encoded by exons 19-20 was retained in genitourinary IMTs with FN1-ALK fusion, resulting in membrane/cytoplasmic localization of ALK (22,24).

Tumors with FN1-ALK fusion have shown different treatment responses, depending on the type of ALK inhibitors used to treat the patient. Reinhart *et al* (9) reported that, in IMTs of the urinary bladder with FN1-ALK fusion, neoadjuvant treatment with the first-generation ALK inhibitor crizotinib showed no tumor response, whereas, by contrast, treatment with the next-generation ALK inhibitor lorlatinib led to a rapid and deep response. In uterine leiomyosarcoma with the FN1-ALK fusion, the tumor continued to progress when the patient was administered therapies including crizotinib, but a remarkable patient

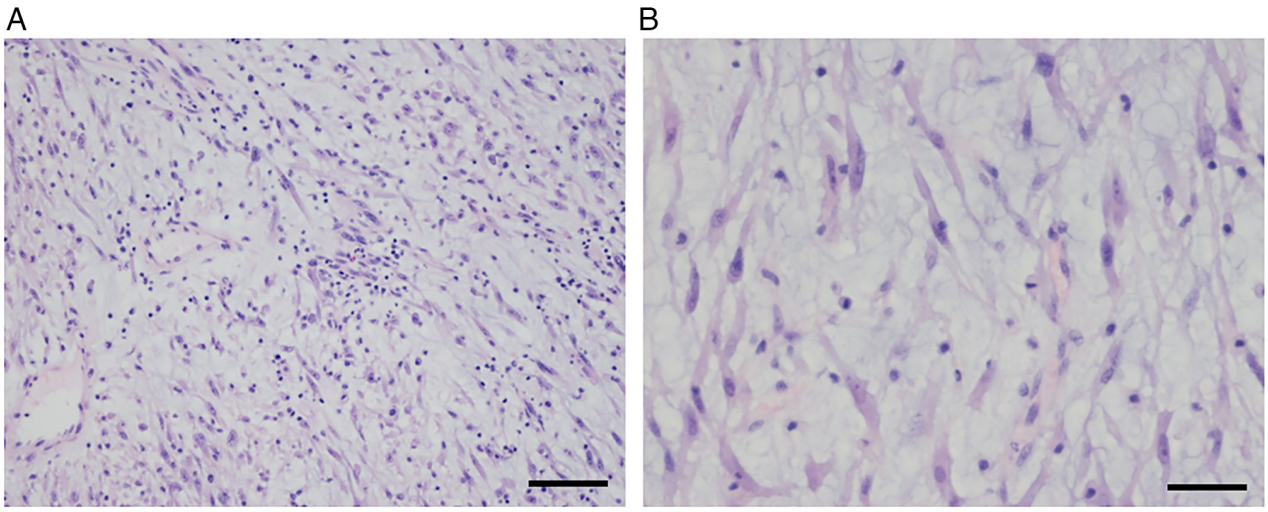

Figure 3. (A) The mass comprised stellate and spindled myofibroblastic cells arranged in a 'tissue culture-like' pattern, with a myxoid background and a mixed inflammatory infiltrate comprising neutrophils, eosinophils and lymphocytes (original magnification, x200; scale bar, 100 μm). (B) The tumor cells had a pale eosinophilic cytoplasm, with long tapering processes. The nuclei were elongated, ovoid or round, with a bland appearance and containing delicate chromatin and small-to-inconspicuous nucleoli (original magnification, x400; scale bar, 50 μm).

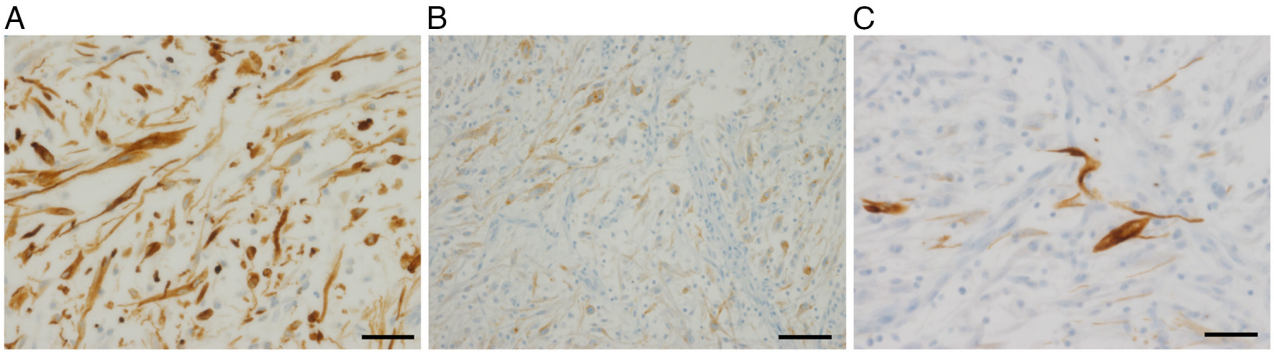

Figure 4. The tumor cells showed strong immunoreactivity for (A) cytokeratin AE1/AE3 and (B) ALK protein. The immunoreactivity for (C) desmin was focal (original magnification, x400; scale bar, 50 μm). ALK, anaplastic lymphoma kinase.

response resulted from administering the second-generation ALK inhibitors, alectinib and lorlatinib (25). In neither case were resistance mutations against the first generation TKI, crizotinib, identified. In an *in vitro* study by Childress *et al* (24), tumor cells expressing FN1-ALK were found to have a significantly higher sensitivity to lorlatinib compared with crizotinib.

It is unclear why the FN1-ALK fusion has different sensitivities, depending on the type of ALK inhibitor used for the therapy. Childress *et al* (24) showed that FN1-ALK forms significantly more foci and more colonies in soft agar compared with the positive control (note that full-length ALK receptor retains the ALK transmembrane domain harboring the active ALK mutation). Therefore, in addition to the unique properties of retaining the transmembrane domain in the FN1-ALK fusion, the 5'-fusion partner FN1 itself may affect the biochemical and cellular properties of the ALK fusion protein, including its kinase activity, protein stability, transformative potential and response to ALK TKIs.

In conclusion, a rare case of an IMT with FN1-ALK fusion in the urinary bladder was reported in the present case study. The presence of FN1-ALK fusions with ALK with exon 18 or 19 rearrangement may be specific to a subset of IMTs, wherein ALK is inhibited upon treatment with second-generation ALK inhibitors, but not with crizotinib. This could be of importance both in the neoadjuvant and in the palliative settings, particularly in terms of negating the need for radical surgery. Therefore, the detection of a distinct FN1-ALK fusion not only assists the diagnosis of IMTs, but also helps to determine the most appropriate course of treatment. However, since only one case was reported, the current conclusions are limited and may not represent all patients. Considering the rarity of this condition, gathering data from all cases of urinary bladder IMTs with FN1-ALK fusion will help to confirm the molecular characteristics and the efficacy of ALK TKIs in the treatment for IMTs.

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Availability of data and materials

The data analyzed during the current study is available at https://www.ncbi.nlm.nih.gov/sra/PRJNA935024.

Authors' contributions

SMS and HCL made substantial contributions to the conception and design of the work, and drafted and revised the manuscript. CGW and OJL interpreted the pathological data. YJK analyzed the patient data. SMS and CGW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study adhered to the guidelines established by the Declaration of Helsinki and was approved (approval no. 2022-11-018) by the Institutional Review Board of Chungbuk National University Hospital (Cheongju, Korea).

Patient consent for publication

Written informed consent for the publication of anonymous case information was provided by the patient.

Competing interests

The authors declare that they have no competing interests.

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