SHORT REPORT

Primary primitive neuroectodermal tumour of the urinary bladder: a clinico-pathological study emphasising immunohistochemical, ultrastructural and molecular analyses

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Primary primitive neuroectodermal tumours (PNETs) of the bladder are extremely rare and aggressive neoplasms, and only six examples have been reported in the literature. The case of a 21-year-old woman, who remains disease free 3 years after tumour resection, is reported here. Morphological features were found to correspond to a small round blue cell tumour without rosette formation and with extensive areas of necrosis. Strong expression of CD99, vimentin and CD117 (c-kit), and focal reactivity to cytokeratin and S-100 protein was observed in tumour cells. Ultrastructurally, sparse neurosecretory granules were observed. Diagnosis of PNET was supported by molecular genetic analysis, showing the EWS–FLI-1 fusion transcript type 2 by RT-PCR and EWS gene rearrangement by fluorescence in situ hybridisation. A normal genetically balanced genotype was shown by comparative genomic hybridisation, which, together with the expression of c-kit, a known therapeutic target for imatinib, may have prognostic and therapeutic implications.

Primitive neuroectodermal tumours are malignant small round blue cell tumours, which exhibit a variable degree of neural differentiation and arise outside the brain, spinal cord and sympathetic nervous system.¹ These tumours are closely related to osseous or extraosseous Ewing's sarcomas, with which they share the same chromosomal abnormality: t(11; 22) (q12; q24),² and, in fact, both are considered to be members of the Ewing family of tumours. Ewing's sarcoma is therefore considered to be a less differentiated form of primary PNET.¹ PNETs arising in the urinary bladder are extremely rare, and to our knowledge only six cases have been reported so far in the English literature.^{3–8} An additional case of PNET in the bladder is documented, emphasising the clinicopathological, immunohistochemical, ultrastructural and molecular genetic features of value in the diagnostic and prognostic evaluation of these uncommon tumours.

MATERIAL AND METHODS

Case report

A 21-year-old woman was referred to the urology clinic because she had developed frequency, dysuria and gross haematuria. Ultrasound examination revealed a large filling defect in the bladder. On cystourethroscopy, a tumour occupying the posterior and right and left sides of the bladder wall was found to be the cause for this. Biopsies were taken from two different areas of the tumour for histological

analysis. Chest x ray, bone scan and computed tomography scan did not show extravescicle extension, and the patient subsequently underwent radical cystectomy and total hysterectomy with bilateral salpingo-oophorectomy, followed by ileal loop diversion. The urethra was not removed. After pathological evaluation, it was thought to be diagnostic of PNET. The patient received 1 year of adjuvant systemic chemotherapy, followed by maintenance with imatinib. After 3 years of follow-up, she is well, with no signs of recurrence or metastasis.

RESULTS

Pathological findings

The first biopsy specimen included two irregular fragments of tissue, 0.3 and 0.2 mm in diameter. Once open, the cystectomy specimen showed a 9×8×6 cm grey-white focally necrotic polypoid fleshy tumour (table 1) that appeared to arise from the bladder wall, infiltrated the full thickness of the bladder and focally extended through perivesicle fat (fig 1A). A histological analysis of available specimens showed a highly cellular, focally necrotic malignant tumour composed of sheets of uniform small round cells containing hyperchromatic nuclei with evenly distributed fine chromatin and inconspicuous nucleoli (fig 1B). The cytoplasm had glycogen, as shown by the periodic acid–Schiff stain (positivity was abolished by diastase digestion), and indistinct borders (fig 1C).

Apoptotic cells were present and mitotic activity was moderate (16 per 10 high-power fields). The tumour cell nests were surrounded by fibrovascular stroma. Flexner–Wintersteiner or Homer–Wright rosettes and neurofibrillar material were not noticed in multiple evaluated sections. The tumour included the adjacent vesicle muscularis propria, and focally perivesicle fat and uterus. The bladder mucosa away from the tumour, the endometrium, bilateral adnexa and pelvic nodes were unremarkable. Therefore, no coexisting urothelial carcinoma or carcinoma in situ was present. This was thought to be consistent with the diagnosis of PNET.

Immunohistochemistry

The tumour cells were strongly immunoreactive, with antibodies to the MIC2 gene product (CD99, clone O13) and to vimentin and CD117 (c-kit), which had a membranous pattern. Focal staining of cytokeratin (AE1 or AE3) and S-100 protein (fig 1D–G) was also observed. The stains for LCA, Myo-D1, α -inhibin, actin, desmin, neurofilaments, NSE, chromogranin and synaptophysin were negative.

Abbreviations: CGH, comparative genomic hybridisation; PNET, primitive neuroectodermal tumour

Figure 1 Primary peripheral primitive neuroectodermal tumour of the urinary bladder. (A) Cystectomy specimen showing a large tumour infiltrating the bladder wall. (B,C) Histological picture showing a small round blue cell tumour with no rosette formation (haematoxylin and eosin, original magnification $\times 250$). (D–F) Immunoreactive diagnostic features of CD99, vimentin and cytokeratin, respectively, as compared with normal overlying urothelium (streptavidin–biotin–peroxidase, original magnification $\times 250$). (G) Strong membranous immunostaining of CD117 (c-kit) in tumour areas (streptavidin–biotin–peroxidase, original magnification $\times 250$). (H) Sparse organelles, cell processes and rare neurosecretory granules at electron microscopy (uranyl acetate–lead citrate, original magnification $\times 55\,000$). (I) Reverse transcription–polymerase chain reaction with primers EWS 22.8 and FLI-1 11.3. Lane 1, patient sample showing a 394-bp product (splicing variant type 2); lane 2, negative control; lane 3, positive control showing a 355-bp product (splicing variant type 1); lane 4, white sample. (J) Fluorescent in situ hybridisation analysis of paraffin-wax-embedded sections of the tumour with a commercial EWS probe. Although results of hybridisation on the normal interphase nucleus of paraffin-wax-embedded tissue show two fusion signals, several tumour interphase nuclei (white arrows) show a pattern including one fusion signal, one red signal and one green signal, indicative of a rearrangement of one copy of the EWS gene.

Electron microscopy

Tumour cells were poorly differentiated with sparse organelles, which included mitochondria, a small Golgi apparatus, some rough endoplasmic reticulum and polyribosomes. Cells with elongated interdigitating cytoplasmic processes and a few dense-core neurosecretory granules (fig 1H) were present, together with moderate intracytoplasmic glycogen, scattered microtubules and bundles of intermediate filaments. No desmosomes, tonofilaments or myofilaments were noticed.

EWS/FLI-1 gene fusion, comparative genomic hybridisation (CGH) analysis and fluorescence in situ hybridisation analysis

Molecular analysis with RT-PCR with primers EWS 22.3 and FLI-1 11.3, which included appropriate negative and positive controls, showed a 394-bp product band (corresponding to a type 2 EWS/FLI1 fusion), which supported the diagnosis of PNET (fig 1I). In CGH analysis, DNA extracted from the tumour was labelled with SpectrumGreen dUTP by nick translation. Reference DNA from peripheral lymphocytes was labelled with SpectrumRed dUTP. The sizes of the probes were optimised to a range from 500 bp to 2 kbp. CGH results were analysed by using an epifluorescence microscope equipped with a 100-W mercury lamp and a cooled charge-coupled device camera controlled by a cytovision image analysis system. The results showed a genetically balanced tumour by CGH, with no secondary chromosome changes. Fluorescence in situ hybridisation analysis was carried out in paraffin-wax-embedded sections by using a commercially available EWS probe (Sarcopath TM, Master-Diagnostica, Granada, Spain). Although results of hybridisation on the normal interphase nucleus of paraffin-wax-embedded tissue showed two fusion signals, most tumour interphase nuclei (white arrows) showed a pattern that included one fusion signal, one red signal and one green signal, which was indicative of a rearrangement of one copy of the EWS gene (fig 1J).

DISCUSSION

To our knowledge, six cases of primary intravesicle PNET have been reported so far, and we report an additional case occurring in a 21-year-old woman with no immunosuppressive status.³⁻⁸ The ages of patients with bladder PNET, including ours, ranged from 15 to 81 years (mean age

43 years). Two patients had a history of kidney transplant or Hodgkin's disease and they were treated with various combinations of surgery, chemotherapy and radiation therapy.⁹⁻¹⁰ Two of the four patients remained free of disease at follow-up 18 months and 3 years after surgery. One died of disease and the other died of a massive pulmonary embolism, but an autopsy showed PNET of the bladder. The diagnosis of PNET was established in our case by combining histological, immunohistochemical, ultrastructural and molecular analyses. Light microscopy showed a glycogen-rich, small round cell tumour without rosettes or intercellular fibrillar material, which virtually excluded neuroblastoma. Ultrastructural study was useful in our case, showing occasional dense-core granules with complex cytoplasmic processes, which are common in PNETs.⁴⁻⁷ Tumour cells expressed CD99, vimentin, cytokeratin and S-100 protein, a profile highly suggestive of PNET. Although not specific for PNET or Ewing's sarcoma, CD99 is almost always present in these tumours.¹⁻¹¹ It must be used with antibodies to vimentin, seen in most cases of PNET, or cytokeratin stain, as reported in our case, in up to 57% of soft tissue samples.¹ Neurone-specific enolase, S-100 protein, neurofilaments and other neural markers showed a variable degree of expression in reported cases. Moreover, the lack of staining of muscle, lymphoid or neuroendocrine markers virtually excludes rhabdomyosarcoma, haematolymphoid neoplasm or neuroendocrine carcinoma (particularly rare in this age group; table 2).¹²

PNETs occasionally stain with chromogranin and synaptophysin, and small-cell carcinomas are often cytokeratin positive.¹² In this situation, ultrastructural or genetic studies may be helpful. The molecular genotype of PNET is characterised by fusion of the EWS gene (22q12) with a member of the external transcribed spacer family of transcription factors (usually FLI-1 gene in 11q24) in about 95% of samples.⁸⁻¹¹ Ours is the first reported case showing the type 2 EWS/FLI-1 fusion transcript, which has been associated with a worse prognosis.¹¹ By contrast, it is also the first bladder PNET showing, by CGH analysis, a genetically balanced tumour with no secondary chromosome changes, a finding that can be associated with a better prognosis.² Interestingly, in our case, tumour cells expressed c-kit, a finding that seems to be reported for the first time in a bladder PNET,⁹ which was used in this particular case as a therapeutic target with imatinib. Imatinib is known to enhance the cytotoxic effect of some chemotherapeutic drugs

Table 1 Reported series of primary peripheral primitive neuroectodermal tumours of the urinary bladder

Histochemical and immunohistochemical findings						Molecular genetic findings				Clinicopathological findings				
Ref	PAS	CD99	VIM	NSE	CK	S-100	c-kit	EWS/FLI-1 fusion	CGH	Age/sex	Bladder location/tumour size/perivesicle extension	HRR	DCG	Follow-up
5	+	+++	NA	+Focal	NA	Neg	ND	Yes*	ND	15/F	NA/3×2×2/No	No	Yes	NA
7	+	+++	ND	+Focal	Neg	Neg	ND	ND	ND	21/M†	RLW/8×6×4 cm/Yes	No	Yes	NED/18 months
6	+	+++	+++	+++	Neg	Neg	ND	ND	ND	38/F‡	Anterior, right and left lateral walls/12×7×3.5 cm/Yes	No	ND	NA
4	+	+++	+Focal	+Focal	Neg	+Focal	ND	ND	ND	62/M	TURB (Huge tumour)/NA/Yes	Yes	NA	DOC/3 weeks
8	+	+++	+++	NA	Neg	Neg	ND	Type-1	ND	61/F	Mass in bladder wall (CT scan)/No	NA/Yes	ND	NA
3	+	+++	Neg	+++	Neg	Neg	ND	ND	Gains: 3p,6,8; q,12,1; 7q,21q	81/M	Entire cavity (x ray)/NA/Yes	No	Yes	DOD/2 weeks
Our case	+	+++	+++	Neg	+Focal	+Focal	++	Type-2	Balanced genotype	21/F	Posterior, right and left lateral walls/9×8×6 cm/Yes	No	Yes	NED/3 years

CGH, comparative genomic hybridisation; CK, cytokeratin; DCG, dense-core granules at electron microscopy; DOC, dead of other causes (pulmonary embolism); DOD, dead of disease; M, male; F, female; HRR, Homer-Wright rosettes; NA, not available; ND, not done; NED, no evidence of disease; Neg, negative; NSE, neurone-specific enolase; PAS, periodic acid-Schiff; Ref, reference number; TURB, transurethral resection of bladder tumour; VIM, vimentin.

*Translocation between the long arms of chromosomes 11 and 22 by cytogenetic chromosome analysis (G-banding).

†Renal transplant recipient under immunosuppressive treatment.

‡Patient had Hodgkin's disease, which was treated with chemotherapy.

Table 2 Main immunohistochemical and genetic features in differential diagnosis of small round blue cell tumours of the bladder

Small round blue cell tumours	LCA	CD99	MDM	NEM	CD117	CKAE1/AE3	EWS/FLI fusion
PNET	—	+	Variable +	Variable +	+	Variable +	+
SCC	—	—	—	+	+	Variable +	—
Rhabdomyosarcoma	—	—	+	—	—	—	—
Lymphoma	+	Variable +*	—	—	—	—	—

LCA, leucocyte common antigen; MDM, muscle differentiation markers, such as actin, desmin or myo-S100, chromogranin, synaptophysin, NSE or TTF-1; PNET, primitive neuroectodermal tumour; SCC, small-cell undifferentiated carcinoma.

*Some T cell lymphomas.

Take-home messages

- Primary primitive neuroectodermal tumours (PNETs) of the bladder are extremely rare and aggressive.
- PNETs are malignant small round blue cell tumours closely related to Ewing's sarcomas, with which they share the same chromosomal abnormality. Both are considered to be members of the Ewing family of tumours.
- Tumour cells show strong expression of CD99, vimentin and CD117 (c-kit).
- Molecular genetic analysis supported the diagnosis of PNET by showing the EWS-FLI-1 fusion transcript type 2 by RT-PCR and EWS gene rearrangement by FISH.
- The expression of c-kit, a known therapeutic target for imatinib, may have prognostic and therapeutic implications for the patients.

on PNET cells and, in fact, tumour relapse was not observed after 3 years of follow-up, an interval exceeding the median relapse time of PNET.¹⁰

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