Virchows Arch (1997) 431:365–369

CASE REPORT

Masanori Hisaoka · Hiroshi Hashimoto · Tetsu Murao

Peripheral primitive neuroectodermal tumour with ganglioneuroma-like areas arising in the cauda equina

Received: 30 April 1997/Accepted: 16 June 1997

Abstract Peripheral primitive neuroectodermal tumour (pPNET or peripheral neuroepithelioma) is one of the malignant small round cell tumours of peripheral nerves, soft tissues and bones, but rarely originates in the spinal canal. We report an example of pPNET arising in the cauda equina of a 14-year-old Japanese boy. At surgery, a well-demarcated tumour measuring 2×4 cm in diameter and involving one of the nerve roots of the cauda equina was located within the intradural space with no evidence of extradural extension. Microscopically the tumour was made up of sheets of closely packed small round cells, associated with ganglioneuroma-like islands. Immunohistochemically, the small round tumour cells were intensely positive for neuron-specific enolase (NSE), an *MIC2* gene product (O13) and β2-microglobulin, whereas the foci with ganglion cell-like cells reacted positively to NSE, synaptophysin and β2-microglobulin but were negative for O13. A chimeric transcript of the *EWS/FLI-1* fusion gene detected by a nested reverse transcriptase–polymerase chain reaction using formalin-fixed paraffin-embedded tissue justified the diagnosis of pPNET. Only 6 cases of PNET in the cauda equina have been described in the literature, and this is the first case of a pPNET with ganglioneuroma-like areas. This finding suggests that the primitive tumour cells of pPNET may respond to unknown inductive effects and express a ganglion cell-like morphology.

Key words Primitive neuroectodermal tumour · Ganglion cell · Cauda equina · *EWS/FLI-1* gene · *MIC2* gene

M. Hisaoka · H. Hasimoto (✉)
Department of Pathology and Oncology,
University of Occupational and Environmental Health,
1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807, Japan
Tel.: (81)93-691-7239, Fax: (81)93-692-0189

T. Muorao
Department of Orthopedics, Kyushu Rosai Hospital, Japan

Introduction

Peripheral primitive neuroectodermal tumours (pPNET), also refered to as peripheral neuroepitheliomas, are a group of malignant small round cell tumours presenting mostly in children or young adults and arising outside the central and sympathetic nervous systems. Despite their histological resemblance to neuroblastoma, rhabdomyosarcoma and malignant lymphoma, pPNET have distinct clinicopathological features with limited neuroectodermal development, as suggested their immunohistochemical and ultrastructural profiles. Both pPNET and Ewing's sarcoma strongly express the glycoprotein p30/32 (CD99), which is encoded by the *MIC2* gene [4, 5, 20, 22]. Moreover, recent cytogenitical and molecular analyses have demonstrated that pPNET is characterized by a highly consistent chromosomal translocation t(11; 22)(24; q12), resulting in the formation of a unique chimeric gene EWS/FLI-1, which is also shared by Ewing's sarcomas of bone and in extraskeletal sites [1, 4, 5, 20, 27]. Thus, the concept tht pPNET and Ewing's sarcoma are part of a disease spectrum, forming a PNET/Ewing family of tumours, has been considered [1, 4–6, 16, 19].

Although pPNET usually occurs in peripheral nerves, soft tissues of extremities and trunk, and bones, tumours arising from the nerve roots in the spinal canal are rare. We describe here a case of pPNET in the cauda equina of an adolescent boy together with immunohistochemical and molecular analyses of the tumour.

Clinical history

A 14-year-old Japanese boy was admitted to Kyushu Rosai Hospital with 3-month history of lumbago after exertion and a recent onset of intensified low backache and pain in the left lower extremity, which led to difficulties in continued standing and walking. Routine laboratory tests were within normal limits. Radiological examination revealed an intraspinal mass in the cauda equina (Fig. 1), which was removed surgically. At surgery, the lesion was found to be confined to the intradural space of the lumbar spinal canal, and one of the nerve roots of the cauda equina was involved

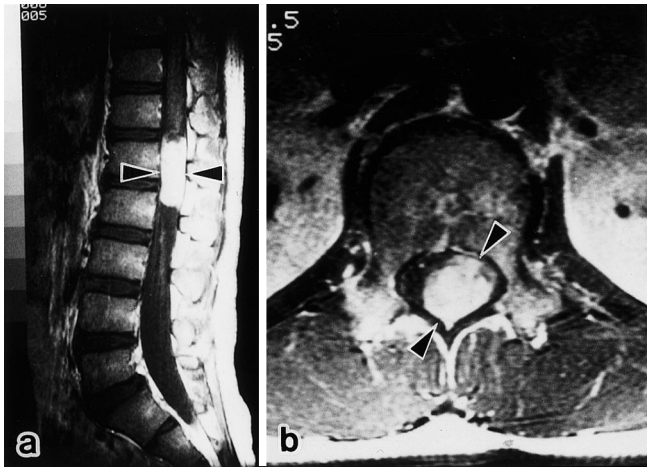


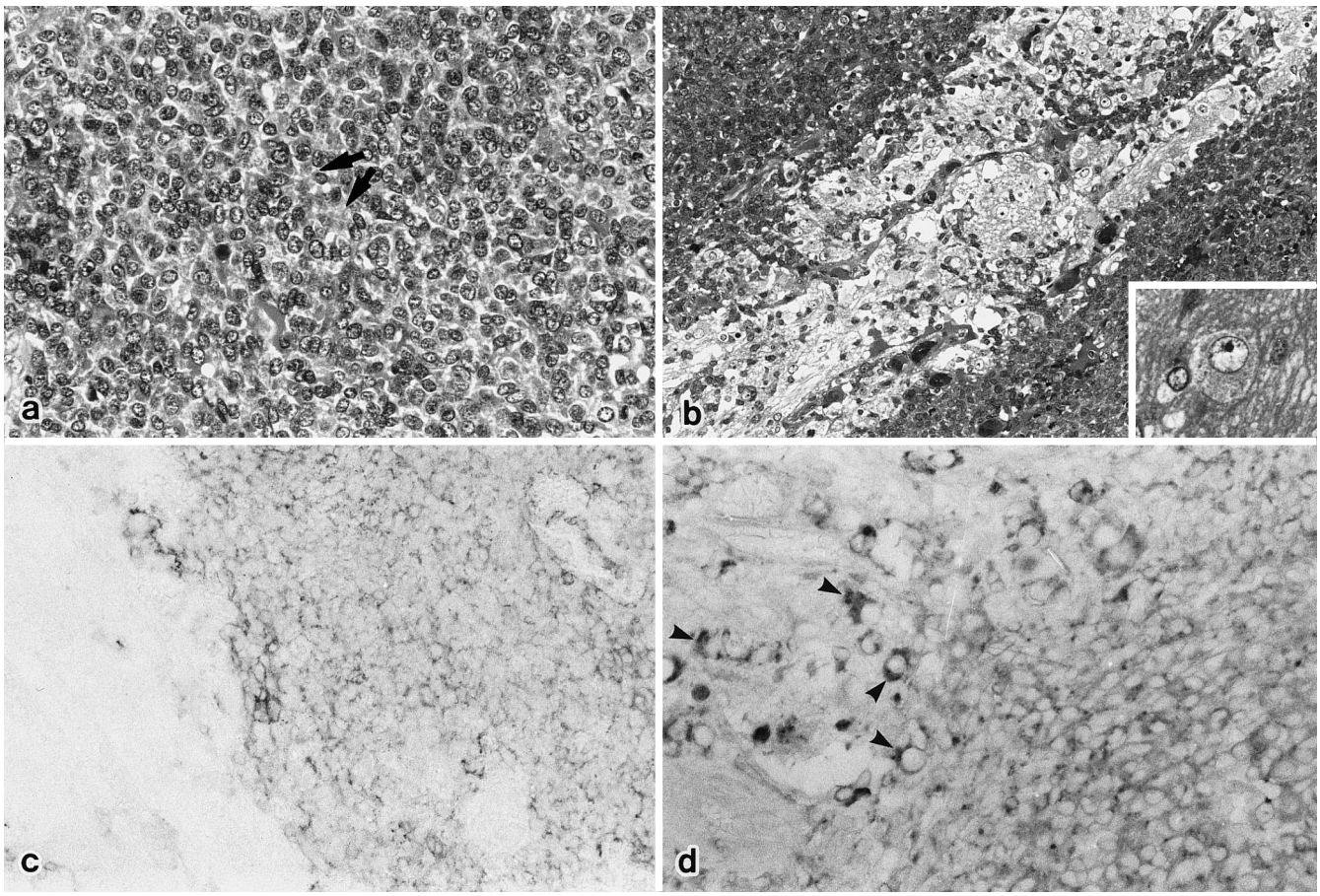
Fig. 1 **a** Sagittal and **b** axial views of magnetic resonance imaging after enhancement with contrast medium, showing a solid mass with an enhanced signal intensity in the lumbar spinal canal (*arrowheads*)

Fig. 2a–d Photomicrographs of the intraspinal tumour. **a** The tumour is made up of a monotonous proliferation of small round cells, partially forming abortive rosettes (*arrows*) H&E, ×150. **b** Islands of large ganglion cell-like cells are seen between sheets of the small round cells, H&E, ×100. *Inset* displays an enlarged view of one of the ganglion cell-like cells. **c** The small round cells are diffusely positive for a *MIC2* gene product (O13), while the ganglioneuroma-like areas are negative for this marker (*left*). Immunostaining, ×150. **d** Both small round cells and ganglion cell-like cells (*arrowheads*) are intensely stained for β2-microglobulin. Immunostaining, ×200

by the tumour with no extradural extension. The patient was well, without evidence of disease at 3 months after the surgery.

Materials and methods

The surgical specimen obtained was routinely fixed in 10% formalin and embedded in paraffin. In addition to haematoxylin-eosin staining, periodic acid–Schiff preparations (PAS) with and without prior diastase treatment were done. For immunohistochemistry, deparaffinized sections were treated with 3% hydrogen peroxide in absolute methanol to block endogenous peroxidase activity, and incubated with the following primary antibodies: neuron-specific enolase (NSE; Dako Japan, Kyoto, Japan), synaptophysin (Dako Japan), S-100 (Dako Japan), glial fibrillar acid protein (GFAP; Dako Japan), PGP9.5 (Ultraclone, Cambridge, UK), cytokeratin (AE1/AE3; Boehringer Mannheim, Indianapolis, Ind.), cytokeratin (CAM5.2; Becton Dickinson, Mountain View, Calif.), desmin (Dako Japan), muscle-specific actin (Enzo Diagnostics, New York, N.Y.) α-smooth muscle actin (Sigma, St. Louis, Mo.), O13 (Signet, Dedham, Mass.), leucocyte common antigen (LCA; Dako Japan) and β2-microglobulin (Dako Japan). A labelled streptavidin-biotin peroxidase method using an LSAB kit (Dako Japan) was employed. For molecular analysis of the tumour, total RNA was extracted from the formalin-fixed paraffin-embedded tissue specimen as previously described [1, 22]. Briefly, five 4-µm-thick sections were deparaffinized in two exchanges of xylene and two washes of ethanol. Then 1.5 ml of Trizol reagent (Gibco BRL, Gaithersburg, Md.) was added, and the tissue sections were homogenized. Extraction of RNA was performed in accordance with the manufacturer's instructions. A nested reverse transcriptase–polymerase chain reaction (RT-PCR) for the EWS/FLI-1 fusion transcript was carried out on the extracted RNA according to the method described by Adams et al. [1]. To determine the type



of fusion transcript, the PCR product was cloned into a pCR2.1 vector (Invitrogen, San Diego, Calif.) by TA ligation and sequenced using an automated sequencing system, ALFexpress DNA sequencer (Pharmacia Biotech, Uppsala, Sweden). As positive and negative controls of the nested RT-PCR reaction in this study, another case of pPNET occuring in the skeletal muscle of the thigh with t(11; 22)(q24; q12) resulting in the junction of genes between EWS exon 7 and FLI-1 exon 5 and an example of ependymoma in the spinal cord were used, respectively.

Pathological findings

Grossly, the tumour was elongated, ovoid, and well-demarcated, and measured about 2×4 cm at the greatest diameter. The cut surface was grey to tan, and there were haemorrhagic foci in the peripheral portion.

Microscopically, the lesion was made up of a lobular or sheet-like arrangement of closely packed uniform small round cells with scant cytoplasm and hyperchromatic nuclei having marginated or dispersed chromatin and small nucleoli (Fig. 2a). No distinct true or pseudorosettes were formed, though abortive forms of Homer Wright rosettes were occasionally seen. Mitotic figures were frequently seen. Many tumour cells possessed intracytoplasmic PAS-positive granules, and the PAS positivity disappeared after a diastase treatment, indicating glycogen granules. There were areas of variable size and shape with scattered large ganglion cell-like cells with oval vesicular nuclei containing distinct nucleoli and faintly eosinophilic or amphorphilic cytoplasm embedded in an eosinophilic fibrillar background reminiscent of ganglioneuroma (Fig. 2b). No glial elements or satellite cells, as seen in the spinal ganglia, were identified in these areas.

Immunohistochemically, most of the small round tumour cells were diffusely and intensely positive for NSE, β2-microglobulin and O13 (Fig. 2c, d), and the small cells that were positively reactive to S-100 or synaptophysin or PGP9.5 were frequently intermingled in the tumour. The areas of large ganglion cell-like cells were positive for NSE, synaptophysin, PGP9.5 and β2-microglobulin, but were negative for O13 (Fig. 2c, d).

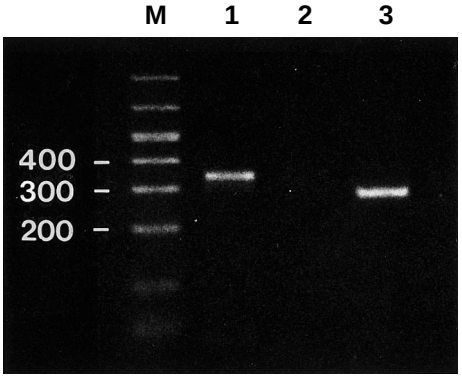


Fig. 3 Nested RT-PCR for the *EWS/FLI-1* fusion gene showing a 277-bp positive band in this case (*lane 3*). *M* 100-bp DNA ladder, *lane 1* PNET of soft tissue with t(11; 22)(q24; q12) (positive control), *lane 2* ependymoma in the spinal cord (negative control)

Desmin, actins, GFAP, cytokeratins and LCA were consistently negative in both elements.

A 277-bp product was obtained by a nested RT-PCR for chimeric transcripts of the *EWS/FLI-1* gene using extracted RNA from the archival tissue specimen (Fig. 3). A subsequent sequence analysis of the PCR product revealed the fusion gene containing the junction between EWS exon 7 and FLI-1 exon 6 [27].

Discussion

A widely used term, "PNET" generally outlines a group of primitive or poorly differentiated small round cell neoplasms both in the central and sympathetic nervous systems and in peripheral systems such as bone and soft tissues. Stout first described a primitive neuroepithelial neoplasm arising in the ulnar nerve in 1918 [26], and early reports of PNET in the peripheral sites were composed of those originating from the peripheral nerves. Recent studies of the tumours, however, have focused mainly on the lesions in soft tissues or bones without close relation to peripheral nerves. Recently, Akeyson et al. reviewed 36 cases of the tumours involving the peripheral nerves in the literature [2]. PNET arising in the cauda equina is rare, and only 6 cases have been reported previously [12, 14, 15, 25]. These tumours in the cauda equina affected exclusively young or middle-aged adults with a mean age of 36 years and a male predominance.

With regard to an intraspinal location, a number of differential diagnostic considerations emerged in this case. However, the distinction of the present tumour from the commonest intraspinal tumours, such as meningioma, schwannoma, neurofibroma and ependymoma, was straightforward microscopically. Other small round cell tumours, including malignant lymphoma, rhabdomyosarcoma and small cell carcinoma, were eliminated by the negative immunoreactivities to LCA, desmin, muscle actin and cytokeratin.

The morphological aspect of the small cell areas of the present tumour is comparable to that of less highly differentiated pPNET lacking in distinct rosettes and resembling classic Ewing's sarcoma. Peripheral PNET is characterized by primitive small round cells with distinct neural differentiation, and histological evidence of its neural differentiation is commonly indicated by rosette formation [4–6, 14]. However, some pPNET may have few distinct rosettes or only abortive forms of Homer Wright-type rosettes as seen in our case. Some investigators diagnose any tumour with evidence of neuroectodermal differentiation as pPNET [4], and others have suggested the presence of two or more neural markers or ultrastructural evidence of neural differentiation to distinguish pPNET from Ewing's sarcoma [6]. According to these notions of pPNET, the current case is considered an example of pPNET, because a neural or neuroectodermal derivation of the tumour is substantiated by the cellular immunoreactivies to NSE, PGP9.5 and synaptophysin along with abortive rosette formation.

The diffuse cellular expressions of an *MIC2* gene product recognized by an antibody O13, β2-microglobulin, and the presence of a chimeric transcript of the *EWS/FLI-1* fusion gene are considered firm supports for the idea that the present tumour is a member of the PNET/Ewing family. Recent immunohistochemical analyses have suggested that immunoreactivities to the surface glycoprotein, p30/32^{MIC2} or CD99, encoded by the *MIC2* gene, and β2-microglobulin, an HLA-1 class antigen-associated glycoprotein, are useful markers to distinguish the PNET/Ewing family from other tumours such as neuroblastoma [20, 22]. The aberrant transcript generated by a unique chromosomal translocation t(11; 22)(q24; q12) or a fusion of the segments of the *FLI-1* gene on chromosome 11 and the *EWS* gene on chromosome 22 have been almost consistently identified in the tumours belonging to the Ewing family [1, 19, 22, 27]. The variant translocations, such as t(21; 22)(q22; q12) involving the *ERG* gene on 21q22, t(7; 22)(p22; q12) involving the *ETV1* gene on 7p22 and more recently t(17; 22)(q12; q12) involving the *E1AF* gene on 17q21, have been described in a minority of this family [10, 11, 27]. Our nested RT-PCR analysis results could reinforce the utility and feasibility of the detection of t(11; 22)(q24; q12) translocation breakpoint in paraffin-embedded tissue of the Ewing family tumours that has been recently demonstrated by Adams et al. [1].

It is notable that the present tumour had ganglioneuroma-like areas with well-differentiated large ganglion cell-like cells between the sheets of primitive small round cells. This finding raised the possibility of a subgroup of neuroblastoma with ganglion cell differentiation as another differential diagnosis in this case. Neuroblastoma also shares small round cell morphology with the tumours of the PNET/Ewing family and often contains the cells showing partial or even complete ganglionic differentiation or areas of a ganglioneuromatous element (ganglioneuroblastoma). However, neuroblastoma is characterized by a distinct cytogenetic background, usually 1p abnormalities such as deletion [6]. Regarding the cytogenetics in ganglioneuroblastoma, so far only a case with t(1; 13)(q22; q12) has been described [15]. In the present case, the tumour was distinguished from neuroblastoma or ganglioneuroblastoma by the characteristic immunohistochemical and molecular profiles of the PNET/Ewing family mentioned above. Entrapment of the spinal ganglion, which is usually located within the intervertebral foramina, by the tumour seems to be a remote possibility, because the tumour was confined to the intradural space without evidence of extradural extension and there were no satellite cells surrounding the ganglion cells. This assumption may also be supported by the distinctly positive immunostaining result of β2-microglobulin in the ganglion cell-like cells of this tumour, suggesting an aberrant expression of this HLA-related molecule, which is almost absent or only weakly expressed in normal peripheral ganglion cells or neurons in the central nervous system [7, 13, 18]. The presence of ganglion cells is considered to be sufficient morphological evidence of neural differentiation in pPNET. However, it appears to be a very rare phenomenon [4], and we found a paucity of documentation on ganglion cells or ganglion cell-like cells in previously reported pPNETs [3, 21]. Several in vitro culture studies have shown that Ewing's sarcoma of bone and of extraskeletal sites has a variable capacity to develop neural traits by the addition of neural inducers such as retinoic acid, nerve growth factor and dibutyryl cAMP [19]. Thus, the primitive pluripotential tumour cells of the PNET/Ewing family are presumed to be likely to respond to certain inductive factors leading then to differentiate further along a neural line in vivo. Several examples of pPNET with focal divergent differentiation, such as epithelial, glial, ependymal, cartilaginous and muscle differentiation, have also been described [8, 9, 23, 24].

In conclusion, the current case emphasizes that pPNET can develop as an intradural mass in the cauda equina and have a morphological appearance of ganglionic differentiation. In this study, we confirmed the diagnostic utility of a coupled ancillary immunohistochemical and molecular approach on an *MIC2* gene product, β2-microglobulin and an aberrant transcript generated by the *EWS/FLI-1* fusion gene in PNET/Ewing family tumours with such unusual clinicopathological features.

Acknowledgements The authors are grateful to Dr. Y. Hachitanda, Department of Pathology, Kitakyushu Municipal Medical Canter, Kitakyushu, Japan, for his comments on this case.

References

1. Adams V, Hany MA, Schmid M, Hassam S, Briner J, Niggli FK (1996) Detection of t(11; 22)(q24; q12) translocation breakpoint in paraffin-embedded tissue of the Ewing's sarcoma family by nested reverse transcription-polymerase chain reaction. Diagn Mol Pathol 5:107–113
2. Akeyson EW, McCutcheon IE, Pershouse MA, Steck PA, Fuller GN (1996) Primitive neuroectodermal tumor of the median nerve. Case report with cytogenetic analysis. J Neurosurg 85: 163–169
3. Bolen JW, Thorning D (1980) Peripheral neuroepithelioma: a light and electron microscopic study. Cancer 46:2456–2462
4. D'Amore ESG, Ninfo V (1996) Soft tissue small round cell tumors: morphological parameters. Semin Diagn Pathol 13: 184–203
5. Dehner LP (1993) Primitive neuroectodermal tumor and Ewing's sarcoma. Am J Surg Pathol 17:1–13
6. Enzinger FM, Weiss SW (1995) Primitive neuroectodermal tumors and related lesions. In: Enzinger FM, Weiss SW (eds) Soft tissue tumors, 3rd edn. Mosby, St Louis, pp 929–964
7. Funa K, Gazdar AF, Minna JD, Linnoila RI (1986) Paucity of β2-microglobulin expression on small cell lung cancer, bronchial carcinoids, and certain other neuroendocrine tumors. Lab Invest 55:186–193
8. Hachitanda Y, Tsuneyoshi M, Enjoji M, Nakagawara A, Ikeda K (1990) Congenital primitive neuroectodermal tumor with epithelial and glial differentiation. An ultrastructural and immunohistochemical study. Arch Pathol Lab Med 114:101–105
9. Hashimoto H, Kiryu H, Enjoji M, Daimaru Y, Nakajima T (1983) Malignant neuroepithelioma (peripheral neuroblastoma). A clinicopathologic study of 15 cases. Am J Surg Pathol 7:309–318
10. Jeon IS, Davis JN, Braun BS, Sublett JE, Roussel MF, Denny CT, Shapiro DN (1995) A variant Ewing's sarcoma transloca-

tion (7; 22) fuses the EWS gene to the ETS gene ETV1. Oncogene 10:1229–1234
11. Kaneko Y, Yoshida K, Handa M, Toyoda Y, Nishihira H, Tanaka Y, Sasaki Y, Ishida S, Higashino F, Fujinaga K (1996) Fusion of an ETS-family gene, E1AF, to EWS by t(17; 22)(q12; q12) chromosome translocation in an undifferentiated sarcoma of infancy. Genes Chromosom Cancer 15:115–121
12. Kepes JJ, Belton K, Roessmann U, Ketcherside WJ (1985) Primitive neuroectodermal tumors of the cauda equina in adults with no detectable primary intracranial neoplasm – three case studies. Clin Neuropathol 4:1–11
13. Lampson LA, Whelan JP, Fisher CA (1984) HLA-A,B,C and β2-microglobulin are expressed weakly by human cells of neuronal orgin, but can be induced in neuroblastoma cell lines by interferon. Prog Clin Biol Res 175:379–388
14. Liu H-M, Yang WC, Garcia RL, Noh JM, Malhotra V, Leeds NE (1987) Intraspinal primitive neuroectodermal tumor arising from the sacral spinal nerve root. J Comput Tomogr 11:350–354
15. McDermott VGM, El-Jabbour JN, Sellar RJ, Bell J (1994) Primitive neuroectodermal tumour of the cauda equina. Neuroradiology 36:228–230
16. Meis-Kindblom JM, Stenman G, Kindblom LG (1996) Differential diagnosis of small round cell tumors. Semin Diagn Pathol 13:213–241
17. Michalski AJ, Cotter FE, Cowell JK (1992) Isolation of chromosome-specific DNA sequences from an Alu polymerase chain reaction library to define the breakpoint in a patient with a constitutional translocation t(1; 13)(q22; q12) and ganglioneuroblastoma. Oncogene 7:1595–1602
18. Natali PG, Bigotti A, Nicotra MR, Viora M, Manfredi D, Ferrone S (1984) Distribution of human class I (HLA-A,B,C) histocompatibility antigens in normal and malignant tissues of nonlymphoid origin. Cancer Res 44:4679–4687
19. Noguera R (1996) Cytogenetics and tissue culture of small round cell tumors of bone and soft tissue. Semin Diagn Pathol 13:171–183
20. Pappo SA, Douglass EC, Meyer WH, Marina N, Parham DM (1993) Use of HBA71 and anti-β2-microglobulin to distinguish peripheral neuroepithelioma from neuroblastoma. Hum Pathol 24:880–885
21. Schmidt D, Harms D, Burdach S (1985) Malignant peripheral neuroectodermal tumors of childhood and adolescence. Virchows Arch [A] 406:351–365
22. Scotlandi K, Serra M, Manara MC, Benini S, Sarti M, Maurici D, Lollini P-L, Picci P, Bertoni F, Baldini N (1996) Immunostaining of the p30/32^{MCI2} antigen and molecular detection of EWS rearrangements for the diagnosis of Ewing's sarcoma and peripheral neuroectodermal tumor. Hum Pathol 27:408–416
23. Seemayer TA, Thelmo WL, Bolande RP, Wiglesworth FW (1975) Peripheral neuroectodermal tumors. Perspect Pediatr Pathol 2:151–172
24. Sinoda M, Tsutsumi Y, Hata J, Yokoyama S (1988) Peripheral neuroepithelioma in childhood: immunohistochemical demonstration of epithelial differentiation. Arch Pathol Lab Med 112: 1155–1158
25. Smith DR, Hardman JM, Earle KM (1969) Metastasizing neuroectodermal tumors of the central nervous system. J Neurosurg 31:50–51
26. Stout AP (1918) A tumor of the ulnar nerve. Proc NY Pathol Soc 18:2–12
27. Zucman J, Melot T, Desmaze C, Ghysdael J, Plougastel B, Peter M, Zucker JM, Triche TJ, Sheer D, Truc-Carel C, Ambros P, Combaret V, Lenoir G, Aurias A, Thomas G, Delattre O (1993) Combinational generation of variable fusion proteins in the Ewing family of tumors. EMBO J 12:4481–4487