Ewing’s Sarcoma in the Spinal Nerve Root: A Case Report and Review of the Literature

SHUJI ISEFUKU, MASAHIRO SEKI, TAKAHIRO TAJINO, MICHIYUKI HAKOZAKI, SHIGEYUKI ASANO, HIROSHI HOJO and MASAHITO HATORI

Department of Orthopedic Surgery, Iwaki Kyouritsu General Hospital, Iwaki, Japan, 1Department of Orthopedic Surgery, Fukushima Medical University, Fukushima, Japan, 2Department of Pathology, Iwaki Kyouritsu General Hospital, Iwaki, Japan, 3Department of First Pathology, Fukushima Medical University, Fukushima, Japan, and 4Department of Orthopaedic Surgery, Tohoku University School of Medicine, Sendai, Japan

ISEFUKU, S., SEKI, M., TAJINO, T., HAKOZAKI, M., ASANO, S., HOJO, H. and HATORI, M. Ewing’s Sarcoma in the Spinal Nerve Root: A Case Report and Review of the Literature. Tohoku J. Exp. Med., 2006, 209 (4), 369-377 ——— Ewing’s sarcoma (ES) is a highly malignant tumor composed of uniform small round cells. Recently, a single biologic entity, Ewing’s sarcoma family of tumors (ESFT) has been accepted. The entity includes ES, extraskeletal Ewing’s sarcoma (EES) and primitive neuroectodermal tumor (PNET). ESFT cells have immunoreactivity for CD99, an antigen determined by the MIC2 gene. Most ESFT has the (11;22) (q24;q12) translocation. The translocation results in the fusion of the EWS gene with the transcription factor gene FLI1 which has been considered a hallmark of ESFT. We present an extremely unusual case with ESFT in a spinal nerve root mimicking a neurogenic dumbbell tumor. A male aged 20 years noticed pain in his right buttock. Magnetic resonance imaging (MRI) revealed a mass in the right L5/S intervertebral foramen and the lesions in the sacrum. Surgery was performed with a presumptive diagnosis of a nerve sheath tumor. At surgery, the tumor was located in the right L5 nerve root sleeve. The sacral lesions were observed closely. At one month after surgery, radiologically multiple lesions were detected in the pelvic bones. Microscopically the lesions from the root and ilium were composed of small round cells immunoreactive for CD99. Reverse transcription-polymerase chain reaction detected transcripts resulting from the fusion of the EWS gene with FLI1 genes in the iliac lesion. Immunoreactivity for CD99 and detection of the EWS-FLI1 hybrid transcripts are important for the correct diagnosis of ESFT arising in an unusual location. ——— Ewing’s sarcoma; Ewing’s sarcoma family of tumors; extraskeletal Ewing’s sarcoma (EES); primitive neuroectodermal tumor (PNET); nerve root

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Ewing's sarcoma (ES) is a highly malignant bone tumor composed of uniform small round cells. It was originally described by James Ewing in 1921 as a malignant tumor of the shaft of the long bones in children and young adults (Ewing 1921). Later, malignant soft tissue tumors morphologically indistinguishable from ES were reported and termed Extraskeletal Ewing's sarcoma (EES). Recently, a single biologic entity, Ewing’s sarcoma family of tumors (ESFT) has been proposed and gradually accepted (Horowitz et al. 1993). The entity includes ES, EES and peripheral primitive neuroectodermal tumor (PNET), which shows more neural differentiation than ES. These tumors share identical morphological, immunohistochemical and cytogenetical features. EESs are very rare and represent 8% of all ESFT cases (Horowitz et al. 1993). There have been reports of EESs occurring in the epidural or the para-vertebral area (Angervall and Enzinger 1975; Fink and Meriwether 1979; Spaziante et al. 1983; Benesch et al. 1999; Hadfield et al. 2000), but EESs arising in the spinal cord or in the cauda equina are extremely rare. Metastasis of ES to the spinal nerve root is also very unusual. Only one case has been reported so far (Wald and Roland 1984). We present an unusual case with ESFT arising in a spinal nerve root sleeve mimicking a neurogenic dumbbell tumor in the lumbar spine.

A PATIENT AND METHODS

Case report

A male aged 20 years felt pain in his right buttock and lower extremity without any particular cause. The worsening pain made him visit a clinic four years later. Magnetic resonance imaging (MRI) revealed a mass lesion in the right L5/S intervertebral foramen and he was referred to us. His past and family histories were unremarkable. He had spontaneous pain, which was aggravated in flexion and extension of the lumbar spine, but tenderness on percussion was not detected in his low back region. He complained of numbness on the dorsal aspect of his right foot, but had no apparent sensory changes and no muscle weakness in the lower extremities. The patellar and Achilles tendon reflexes were symmetrical and brisk. The Babinski sign was absent bilaterally. The straight leg raising test elicited pain at 40 degrees on the right side, but no additional pain occurred on the femoral nerve stretch test. He did not have any difficulty in defecation or urination. The white blood cell count was 10,200 and C-reactive protein was 0.19 mg/dl. Blood chemistry findings were all within normal limits.

An antero-posterior radiograph of the lumbar spine showed scalloping with slight sclerosis on the caudal surface of the right pedicle of the fifth lumbar vertebra. An oblique radiograph showed enlargement of the right L5/S intervertebral foramen. CT scans revealed a well-marginated homogeneous lesion (3 cm × 1 cm) expanding from the spinal canal to the paravertebral area through the right L5/S intervertebral foramen (Fig. 1). T1-weighted axial MRI detected a well-demarcated lesion within the fifth nerve root sleeve extending from the spinal canal to the paravertebral area through the L5/S intervertebral foramen. The signal intensity of the lesion was almost the same as that of muscle. T2-weighted image revealed a high intensity lesion that had a small higher intensity area near the dural sac. T1-weighted image with gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) enhancement demonstrated an apparent enhancement effect in the lesion except in a small part (Fig. 2). T2-weighted sagittal MRI also revealed two intra-medullary high intensity lesions with sizes of 2 cm × 2 cm and 4 cm × 3 cm in the sacrum. The sacral lesions were observed closely (Fig. 3).

Fig. 1. CT scan of the lumbar spine. A well-marginated homogeneous lesion (arrow heads) expanded from the spinal canal to the paravertebral area through the right L5/S intervertebral foramen.
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The patient underwent an operation with a presumptive diagnosis of a benign nerve sheath tumor, such as neurilemoma or neurofibroma. The right L5 nerve root sleeve was exposed through a right side laminotomy at the L4/L5 level and a right foraminotomy between L5/S. A dark-red tumor appeared through a longitudinal incision on the enlarged nerve root sleeve. It was fragile, bled easily upon contact and was contiguous with one of the rootlets. The proximal part of the tumor extended into the subarachnoid space. After cutting the rootlet, it was resected in a piece-by-piece fashion. The tumor was temporarily diagnosed as poorly differentiated type ganglioneuroblastoma at that time. The patient was informed that close observation of clinical course was required because of the tentative histological diagnosis.

The pain in his right buttock and lower extremity subsided temporarily, but worsened one month after the operation. Radiographs of the pelvis revealed poorly demarcated osteolytic lesions around the right iliosacral joint. CT scans of the pelvis showed multiple lesions destroying cortical and cancellous bone of the sacrum and the right ilium. T1-weighted MRI showed intramedullary lesions in the sacrum and the iliac bones with iso-intensity to the muscle and extending to the surrounding soft tissue. The lesion showed high signal intensity on T2-weighted image and an apparent enhancement effect with Gd-DTPA on T1-weighted image (Fig. 4). MRI also revealed further enlargement of the previously operated right fifth lumbar nerve-root sleeve. When the patient complained of a persistent headache three months

Fig. 2. MRI of the lumbar spine. T1-weighted axial image detected a well-demarcated lesion (arrow heads) within the fifth nerve root sleeve extending from the spinal canal to the paravertebral area through the L5/S intervertebral foramen. The signal intensity of the lesion was almost the same as that of muscle. T2-weighted image revealed a high intensity (arrow heads) lesion that had a small higher intensity area near the dural sac (arrow). T1-weighted image with Gd-DTPA enhancement demonstrated an apparent enhancement effect in the lesion (arrow heads) except in a small part (arrow). A: T1-weighted image, B: T2-weighted image, C: T1-weighted image with enhancement.
after the operation, brain CT scans revealed a bony lesion in the basal area of the cranium but no intra-cranial lesions. The urinary excretion of vanillylmandelic acid was within normal limits.

Open biopsy specimens were obtained from the lesion in his right iliac wing. On the basis of morphological, immunohistochemical and cytogenetical findings, both the nerve root lesion and the pelvic lesions were diagnosed as ESFT. In spite of multi-agent chemotherapy with adriamycin, vincristine, cyclophosphamide ifosfamide and actinomycin D, the patient died of multiple bone metastases without other clinically detectable metastases 15 months after the first operation. Autopsy was not performed.

RNA preparation and Reverse Transcription-PCR analysis for specific fusion gene mRNA expression

Total RNA from tissue sample was isolated using ISOGEN reagent (Wako Pure Chemical Industries, Osaka). After priming of 1 μg of total RNA with 1 μl of 1,000 pmol random hexadeoxynucleotide primers (Takara Bio Inc., Shiga) and 1 μl of 10 mM dNTP mixture (Takara Bio Inc.), reverse transcription (RT) was performed by using Super Script III RNase H- Reverse Transcriptase (Invitrogen Co., Carlsbad, CA, USA), according to the manufacturer’s protocol. Aliquot of cDNA (0.5 μl) was amplified using Takara Ex Taq HS polymerase in a total volume of 50 μl. For detection of EWS-FLI1 fusion gene mRNA, PCR was performed in a thermal cycler. PCR for EWS-FLI1 fusion gene was performed as follows, denaturing at 94°C for 10 min followed by 40 cycles of amplification (95°C for 20 sec, 60°C for 20 sec,
72°C for 20 sec) and 10-min extension at 72°C. The sequencing primers were as follows; EWS exon7: 5’-TCCTACAGCCAGCTCAAGTC-3’ (sense), FLI1 exon6: 5’-TTCATGGTTATTGCCCAGCCTC-3’ (antisense). These primers were obtained from Sigma-Aldrich Japan K.K. (Hokkaido, Japan). PCR reaction products were electrophoresed through 2% agarose gels containing 0.2 mg/ml ethidium bromide. The PCR product of 1 μl was applied for PCR with the condition consisting of 1 cycle of 95°C for 5 min and 25 cycles of 95°C for 30 sec and 60°C for 30 sec by the direct sequence method. Then, oligonucleotide sequences of EWS-FLI1 fusion gene were analyzed using a sequencer and compared with the germline sequences recorded in the GenBank database.

**RESULTS**

Histological examination of biopsy specimens from the right iliac lesion showed hypercellular small round cells having scant cytoplasm and apparent nuclei with finely dispersed chromatin (Fig. 5). The tumor cells contained Periodic acid-Schiff staining-positive granules that disappeared after diastase digestion and occasionally formed pseudo-rosettes. The cells exhibited immunohistochemical staining for vimentin, neuronal specific enolase, chromogranin and CD99, but no reaction to S-100 protein, desmin and synaptophysin. Reverse-transcriptase polymerase chain reaction detected transcripts resulting from the fusion of the EWS gene with FLI1 gene in the specimens from the ilium (Fig. 6). Morphological, immunohistochemical and cytogenetic findings of the biopsy specimen were consistent with those of ESFT.

Histological examination of the surgical specimen from the nerve root lesion demonstrated that the tumor was composed of small round cells with hyperchromatic, round to oval nuclei and scant cytoplasm surrounded with fibrous tissue (Fig. 7). It also showed occasional abortive rosette formation and a few ganglion cells scattered within cluster of the small round cells. Ganglion cells were hardly identified in the biopsy specimens from the ilium, suggesting that the ganglion cells were not tumor components but pre-existing normal cells in the nerve root. Immunohistochemical examination demonstrated positive staining with vimentin, neuron specific enolase, S-100 protein and chromogranin, negative with epithelial membrane antigen. Examinations with either Periodic acid-Schiff staining or CD99 were not performed soon after the operation. After the diagnosis of the pelvic lesion, re-examination of the specimens from the nerve root demonstrated positive staining for CD99 (Fig. 8). The nerve root tumor was also diagnosed as ESFT, on the basis of similar morphological and immunohistochemical findings of the small round cells obtained from both the lesions.

**DISCUSSION**

Diagnosis of Ewing’s sarcoma family of tumors

Accurate diagnosis of small-round-cell tumors in bone and soft tissue is sometimes a challenge even to experienced pathologists. A group of small-round-cell tumors of soft tissue comprises several entities such as lymphoma,
rhabdomyosarcoma, PNET and EES (d’Amore and Ninio 1996). ES and PNET share immunohistochemical and cytogenetical features, therefore, they are considered to be closely related tumors at the opposite ends of a spectrum; the former has more evidence of neural differentiation (Dehner 1993; Horowitz et al. 1993; d’Amore and Ninio 1996). Furthermore, a single biologic entity, ESFT, which includes ES, EES and PNET, has been proposed and gradually accepted (Horowitz et al. 1993). Immunohistochemical analysis of ESFT demonstrate positive staining with some of the markers suggesting neural differentiation and with CD99, an antigen determined by the MIC2 gene. CD99 is expressed in almost all cases of ESFT but negative in other small-round-cell tumors (Horowitz et al 1993). Translocations involving band q12 of chromosome 22 to chromosome 11 have been frequently observed in ESFT. The breakpoints in the translocation are localized within the EWS gene on chromosome 22 and within the human homologue of the murine Fli-1 gene on chromosome 11. The t(11;22) translocation results in a transcript of the chimeric EWS-FLI-1 gene and the detection of the transcript has been reported as a specific and sensitive diagnostic test for ESFT (Delattre et al. 1994). In the present case, immu-
nohistochemical analysis of the specimens from the nerve root tumor and the ilium demonstrated positive staining with some of neural markers and with CD99. An EWS-FLI1 hybrid transcript was detected by the polymerase chain reaction from tumor-derived messenger RNA in the iliac lesion. Microscopic examination revealed few other findings indicating neural differentiation except occasional abortive rosette formation in the specimens from the nerve root tumor and the ilium. A universally accepted criterion for distinguishing between PNET and ES has not been proposed yet. However, some authors have advocated that PNET and ES should be distinguished because of their different clinical behaviors (Tsuneyoshi et al. 1989; Schmidt et al. 1991; Isotalo et al. 2000; Hadfield et al. 2000). The present case was diagnosed as ES because of fewer findings for neural differentiation.

Ewing’s sarcoma family of tumors arising in the spine

EES occurs less frequently than ES of bone. The compiled data including 1,505 patients of ESFT from United States, Europe and Japan showed that EES cases represented 8% compared to ES of bone with 87% (Horowitz et al. 1993). The remaining 5% of the cases were diagnosed as peripheral PNET. It has been reported that the extra-osseous cases occurred in the epidural or the para-vertebral area (Angervall and Enzinger 1975; Fink and Meriwether 1979; Spaziante et al. 1983; Benesch et al. 1999; Hadfield et al. 2000). However, EES or PNET arising in the spinal cord or in the cauda equina are extremely rare (Hisaoaka et al. 1997; McDermott et al. 1994; Isotalo et al. 2000; Weil et al. 2001; Mawrin et al. 2002). To the best of our knowledge, only two cases of PNET arising in the spinal nerve root have been described (Ishikawa et al. 1979; Liu et al. 1987).

Metastasis of Ewing’s sarcoma family of tumors

One fourth of ESFT occurs in the pelvis and metastases to other organs including the lungs and the bones remain common therapeutic problems (Horowitz et al. 1993). A case of epidural EES in the lumbar spinal canal presented with bone marrow metastasis in the ilium at diagnosis was reported (Benesch et al. 1999). In contrast,
Radiological characteristics of Ewing’s sarcoma family of tumors in the nerve root

In the present case, the preoperative radiological findings were interpreted as being consistent with benign nerve sheath tumors, such as neurilemoma or neurofibroma. In PNET in the cauda equina or in the nerve root, enlargement of the intervertebral foramen on plain radiographs (Ishikawa et al. 1979) and soft tissue mass extending through the intervertebral foramen on CT (Liu et al. 1987; McDermott et al. 1994) have been described. Such radiological findings were detected in the nerve root lesion of the present case as well. MR images in cases with PNET in the spinal cord or in the cauda equina have revealed lesions with high or iso-intensity to that of the intact spinal cord (Liu et al. 1987; Mawrin et al. 2002) and the lesions are well-enhanced with contrast medium (Hisaoka et al. 1997; Isotalo et al. 2000; Weil et al. 2001; Mawrin et al. 2002). It is noteworthy that, in spite of the highly malignant nature of such tumors, no destruction of adjacent bone has been detected in radiological examinations in the PNET cases as seen in the present case. Interestingly, similar findings have been reported on radiographs and CT in a case with cancer metastasis in the nerve root (Ng and Fehlings 1995).

CONCLUSION

Ewing’s sarcoma in the spinal nerve root is very rare. It is indistinguishable symptomatically and radiologically from benign nerve sheath tumors. The correct diagnosis is possible by immunoreactivity for CD99 and detection of the EWS-FLI1 hybrid transcripts in the surgical specimen.

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References


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