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CASE REPORT

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Peripheral primitive neuroectodermal tumour of the cervix

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Abstract Peripheral primitive neuro-ectodermal tumours (PNET) of the cervix are very rare. Here, we report the clinical, pathological, immunohistochemical and genetic features of a case of a PNET located in the cervix. Hysterectomy revealed a cervical tumour. On microscopic examination, a vaguely lobular arrangement of uniformly appearing neoplastic cells, with round to oval nuclei, distinct nuclear membranes and a clear, moderately glycogen-rich cytoplasm was seen. Cells stained positive for LEU 7, S 100, monoclonal NSE and particularly for MIC2. Neurogenic differentiation was also seen by electron microscopic examination. The genetic hallmark of PNET, a 22q12 rearrangement was demonstrated by fluorescence in situ hybridisation experiments, supporting the diagnosis. Awareness of the existence of primary PNET of the cervix is important to avoid confusion with other tumours of the cervix.

Key words Cervix · Peripheral primitive neuroectodermal tumour · Ewing's tumour pathology · Immunohistochemistry · Cytogenetics

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Introduction

Among the primary neoplasms arising in the cervix, sarcoma is the least common [14, 20]. Most of these sarcomas have been described as leiomyosarcoma [1, 3, 29]. Peripheral primitive neuroectodermal tumour (PNET) of the cervix seems to be extremely rare. Clinical and histopathological data have been published on only three cases of PNET [7, 17, 30], in addition to eight cases originating in the corpus uteri [7, 8, 19]. PNETs show, to varying degrees, morphological, immunohistological, ultrastructural or tissue culture evidence of neuroectodermal differentiation. They are cytogenetically characterised by a reciprocal translocation, t(11;22)(q24;12), or by a deletion, del (22)(q12) [4, 5, 33, 38], and immunohistochemically by a highly increased expression of the MIC2 gene [2]. The molecular events underlying the different rearrangements of 22q12 in these tumours have been elucidated. Most frequently, the EWS gene at 22q12 is fused with genes of the ETS proto-oncogene family, the FLI-1 gene at 11q24 [10, 38, 39], but also with other members of this gene family, such as the ERG gene at 21q22 [32, 41], the ETV1 gene at 7p22 [18], the E1AF gene at 17q12 [19] or the FEV gene at 2q [28]. Understanding these molecular events offers new diagnostic possibilities by using fluorescence in situ hybridisation (FISH) [24, 25, 26] or reverse-transcriptase polymerase chain reaction (RT-PCR) [16, 13].

In this report, a case of a PNET of the cervix is described along with the morphological differential diagnosis and interphase cytogenetic data.

Materials and methods

Tissue was formalin fixed, processed routinely and embedded in paraffin. Tissue sections 5 µm thick were cut and stained with haematoxylin and eosin and immunohistochemically with a variety of antibodies (Table 1) using the standard avidin biotin technique. Immunohistochemistry was also performed on sections from a snap frozen portion of the tumour.

Table 1 Immunohistochemical reagents. *MC* monoclonal; *PC* polyclonal; *EMA* epithelial membrane antigen; *NSE* neuron-specific enolase; *GFAP* glial fibrillary acid protein

Antibody	MC	Source	Dilution
Vimentin	MC	Dako	1:25
Cytokeratin (MAK-VI)	MC	Zymed	1:5
Cytokeratin (Lü-5)	MC	Biogenex	1:30
Epithelial membrane antigen	MC	Dako	1:50
S 100	PC	Dako	1:750
Desmin	MC	Dako	1:50
Neuron-specific enolase	PC	Signet LCA	
Chromogranin	MC	Biogenex	1:30
P30/32 MIC-2 (O-13;CD 99)	MC	Signet	1:20
Synaptophysin	PC	Dako	1:50
Leu 7	PC	Dako	1:1000
Estrogen R	MC	Immunotech	1:40
Progesterone R	MC	Abbott	1:100
GFAP	MC	Biogenex	1:100
MYO D1	MC	Novocastra	1:10

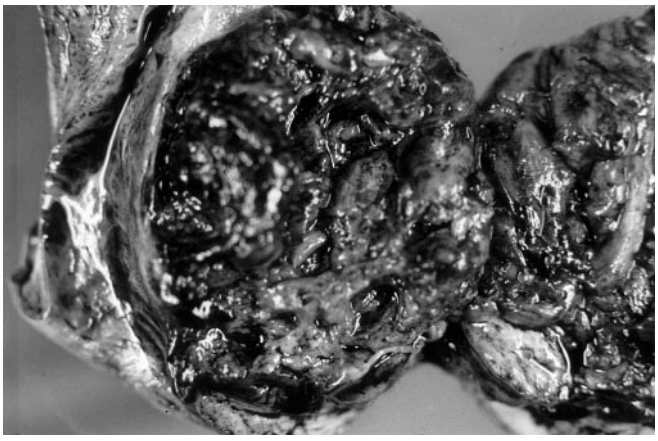

Fig. 1 A large, partially necrotic tumour located in the anterolateral wall of the cervix

Tissue for electron microscopy was fixed in Karnovsky II (2.5% glutaraldehyde, 2% formaldehyde and 0.025% calcium chloride) solution and buffered in sodium cacodylate. It was post-fixed with osmium tetroxide, stained en bloc with uranyl acetate and dehydrated with graded ethanol solutions. The tissue was then infiltrated with propylene oxide/epoxy resin and embedded in 100% resin. Semi-thin sections were cut and stained with toluidine blue. Representative areas were chosen for thin sectioning. Sections were stained with lead citrate and examined under a Philips 301 electron microscope.

Touch preparations were made for double-target FISH experiments. Cosmids located proximal (F10, G9) and distal (F7, E4) to the breakpoint region EWSR1 at 22q12 were used according to protocols described previously [22] to analyse a possible 22q12 rearrangement. Proximal and distal probes were detected using fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC) conjugated antibodies, respectively. To evaluate a possible presence of additional aberrations, which can frequently be seen in PNET, double-target FISH was performed with paracentromeric probes specific for chromosomes 1, 8, 12 and 16 and the subtelomeric probe p1.79 specific for 1p36.33 [6, 12, 23, 25, 26, 38, 39]. Images were taken using the software ISIS from MetaSystems, Altlusheim, Germany.

Results

Case report

A 45-year-old female without relevant clinical antecedents was first seen because of irregular vaginal blood loss. A large tumour was discovered to be located anteriorly in the cervix. No intra-uterine abnormalities showed up on ultrasound examination.

Although a mildly dyskaryotic cervical smear was found, colposcopy only revealed the typical aspect of a cervical fibroid. As neither needle biopsy nor a large-loop biopsy of the cervix showed any suspicion of malignancy, an abdominal hysterectomy was performed.

Based on routine morphological features and particularly on immunohistochemistry (O-13, NSE, Leu 7 and S-100 positivity), electron microscopy (dense core granules, glycogen, neuritic processes) and FISH studies, a diagnosis of a peripheral PNET (pPNET), was made. In order to improve local tumour control, the patient was irradiated afterwards. The patient is free of disease 42 months after hysterectomy.

Pathological findings

A uterus was received, weight 324 g. In the anterolateral wall of the cervix, a tumour with a maximum diameter of 8 cm was noted as pale and soft, with necrotic areas (Fig. 1). There was no extension outside the cervix. The overlying cervical mucosa showed no abnormalities. There was no connection with the uterine cavity.

Microscopic features

Histologically, sheets of uniformly appearing neoplastic cells with a vaguely lobular pattern were seen. Numerous rosettes were also found (Fig. 2). The tumour cells had round to oval nuclei with marginated chromatin, distinct nuclear membranes, small but clearly discernable nucleoli, and sparse clear cytoplasm. The cytoplasmic boundaries were indistinct so that the cytoplasm of several cells seemed to form a syncytium with the nuclei embedded in it. Some tumour cells contained small amounts of glycogen, varying in different portions of the tumour. A high mitotic activity was seen. No reticulin fibres were visible between the cells. The tumour was limited to the cervix. The cervical epithelium was normal. Only in one slide could tumour and cervical epithelium be seen together (Fig. 3). Endocervical glands were not involved. No expression of cytokeratins or leukocyte common antigen (LCA) was seen. Oestrogen and progesterone receptors were not detected.

The tumour cells were positive for Vimentin, S100, LEU 7 and monoclonal N.S.E. A uniform O-13 reactivi-

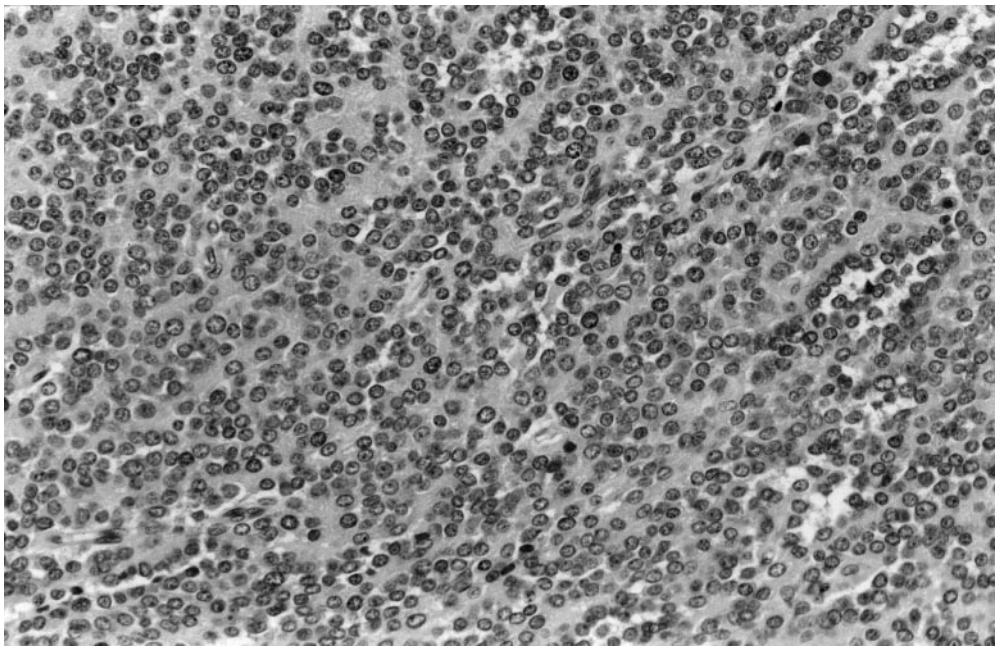

Fig. 2 Sheets of uniformly appearing neoplastic cells with rosette formation

Fig. 3 Tumour and cervical epithelium. The cervical epithelium remained intact

ty, which appeared typically as membrane staining was noted (Fig. 4).

Electron microscopic examination showed cells with long cytoplasmic projections, containing dense-core, neurosecretory-type granules. Except for the membrane-bound dense-core granules, few other cytoplasmic organelles were noted. Well-formed neuritic processes were also seen. Fairly small amounts of glycogen were present.

Cytogenetics and in situ hybridisation

Classical cytogenetic analysis failed due to the lack of metaphase chromosomes. Therefore, interphase cytogenetics has been performed to evaluate the presence of chromosomal aberrations, which could support the diagnosis of PNET. In our FISH experiments, all tumour cells showed the hybridisation pattern typical for Ewing's tumours, displaying one rearranged and one intact 22q12 region (Fig. 5). Numerical aberrations of chromosomes 8 and 12 were not found. None of the facultative structural aberrations, a der(16)t(1;16)(q12;q11.2) chromosome or a deletion at 1p36.33 were detected. All these probes revealed two signals in most of the nuclei analysed, indicating that this tumour had a DNA content in the diploid range. HPV DNA could not be detected, using in situ hybridisation.

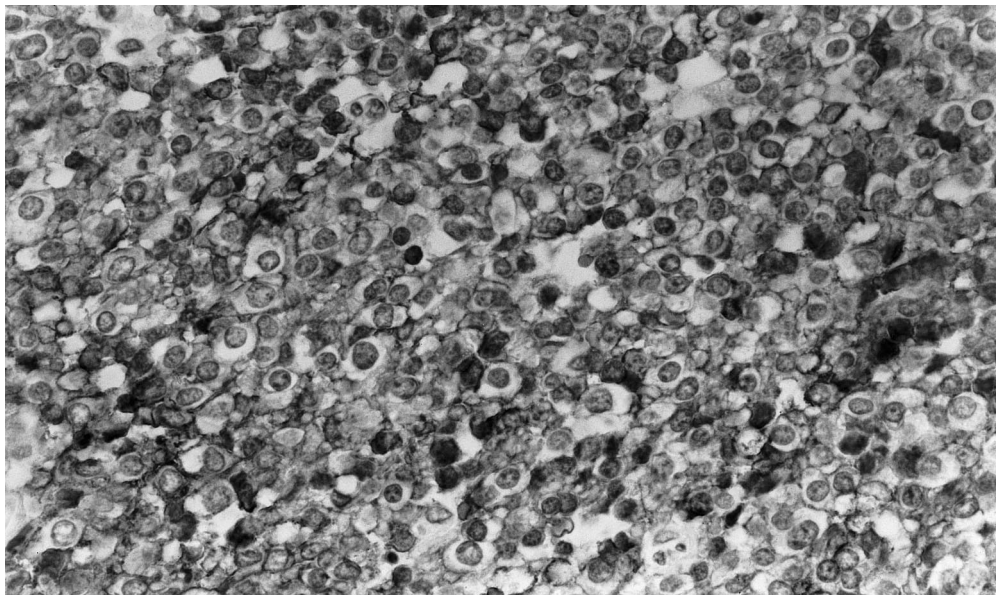

Fig. 4 O-13 (CD99) staining: typical membranous staining pattern

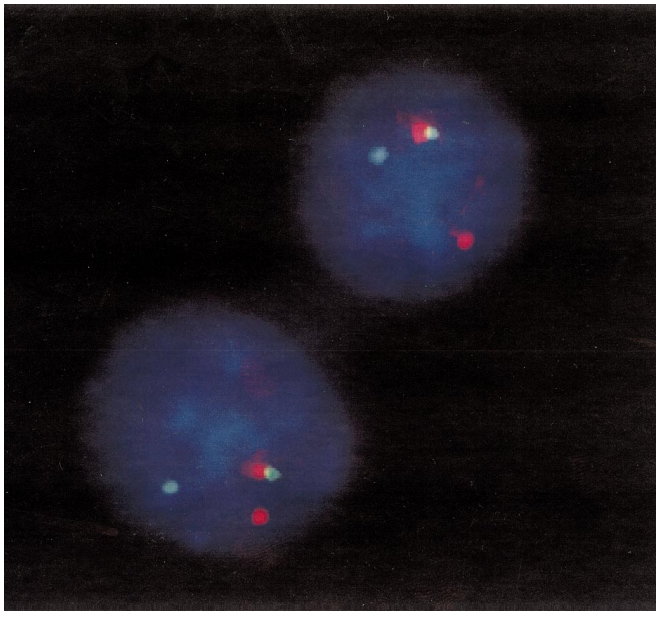

Fig. 5 Fluoresence in situ hybridisation (*FISH*): tumour cells display one rearranged and one intact 22q12 region

Discussion

We believe that this case is situated at the higher differentiated end within the spectrum of PNETs [9]. Homer–Wright-like rosettes were seen, and glycogen was not as abundant as would be expected in Ewing's sarcoma.

Primary sarcomas of the cervix are very rare The most common forms are leiomyosarcomas (botryoid type), alveolar soft-part sarcomas, stromal sarcomas and osteosarcomas [20]. A single reported case of Wilm's tumour has been reported [20]. Also, primary cervical mixed epithelial and mesenchymal tumours have been described [1]. Most of these tumours can be ruled out by routine histology and immunohistochemistry. Negative staining for desmin, Myo D1 and actin were helpful in ruling out rhabdomyosarcoma. Most stromal sarcomas contain cells that react with muscle-specific actin and a smooth muscle actin. Oestrogen and (particularly) progesterone receptors have also been demonstrated immunohistochemically in stromal sarcomas. Regarding this entity, it should be noted that so-called "endometrial" stromal sarcomas can arise in the cervix as a primary tumour [20].

In the reported case, a haematolymphoid neoplasm was also considered, but leucocyte common antigen was not demonstrated. The presence of glycogen can be misleading in this respect since glycogen has also been demonstrated in lymphomas. Recently, a large cell lymphoma with fibrillary matrix was reported [37]. Also, rosette formation in a follicular lymphoma has been described [15]. Therefore, a diagnosis of lymphoma must be ruled out by immunohistochemical means. In the reported case, small cell carcinoma of the cervix was ruled out by considering the architecture (no diffusely infiltrative growth pattern), the absence of hyperchromatic nuclei, pseudocrush artefact and nuclear moulding, and the strong O-13 positivity. Cytokeratin-negative staining also helped to rule out small cell carcinoma as well as other carcinomas (large cell neuroendocrine carcinoma, poorly differentiated squamous cell carcinoma) and carcinoid tumours [17].

The demonstration of O-13 antibody positivity was particularly helpful in establishing the diagnosis in this case. O-13 is a monoclonal antibody that detects the cell surface antigen defined by the cluster of CD-99, and is useful in distinguishing Ewing's tumours from other small round cell tumours [2, 34]. However, it has been shown that CD 99 is not specific for these tumours, since

it reacts with wide variety of other tumours [27, 34]. Small cell carcinomas can stain positively, but infrequently. The staining is less intense than in PNET [21]. O-13 is best used in a differential diagnostic panel of antibodies.

PNET is genetically characterised by different rearrangements involving the EWS gene band 22q12 [11, 38, 40, 41]. In the reported case, metaphase cytogenetic analysis could not be performed, but FISH analysis permitted us to detect a 22q12 rearrangement. However, the identification of a 22q12 rearrangement by means of RT-PCR or interphase cytogenetics should not be taken as an absolute proof for the diagnosis of PNET, in the absence of confirming histology including positive staining for MIC2 [36].

PNET was said to have a worse prognosis than Ewing's sarcoma. This viewpoint has been challenged [5, 14], but the results of the Kiel's group would still support the clinical relevance of differentiation in the ES/PNET group [31].

The overall percentage, however, of accurately diagnosed PNET patients who truly survive 5 years, does not exceed 20–30% at the present time [14].

However, no metastases have been detected in our patient 42 months after surgery. In another well-documented case report, a 36-year-old woman with a PNET of the cervix, measuring 7 cm in diameter (with necrosis) was alive and well 18 months after total abdominal hysterectomy [16]. More cases are needed in order to asses the biological behaviour of PNET in this particular location.

In summary, this case report shows that the differential diagnosis of a rare tumour entity can be very difficult. The use of different techniques, immunohistochemistry, electron micorscopy and FISH, finally allowed the diagnosis of a PNET of the cervix.

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