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# Ewing's sarcoma of the cervix: a case report and review of literature

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**Summary.** Ewing's sarcoma (ES) is a small cell malignant tumor that occurs in the bone of children or adolescents. ES can also occur in extraskeletal organs, such as the pancreas, thyroid, liver, proximal phalanx, and, rarely, cervix. Only 15 published case reports have discussed ES arising in the cervix. We report a 76-year-old woman who had groin mass. ES was diagnosed in accordance with morphological and immunohistochemical maps. Fluorescence in situ hybridization and RT-PCR (reverse transcription PCR) revealed *ESWR1* gene rearrangement and fusion gene formation (*EWS-FLI-1*), both of which confirmed the diagnosis of ES. Although the patient underwent surgical resection, the patient died without chemotherapy and radiotherapy. This case is the first one to involve a patient aged over 70 years and the fifth one to show metastasis occurrence.

**Key words:** Ewing's sarcoma (ES), Cervical, Gene arrangement

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## Introduction

Ewing's sarcoma (ES) was first described by James Ewing in 1921 (Chen et al., 2014); ES was also called undifferentiated reticulocyte sarcoma. Angervall and Enzinger (Angervall and Enzinger, 1975) first proposed the term of extraskeletal ES (E-ES) in 1975. ES is a representative type of extraosseous soft tissue small round cell tumor. Currently, E-ES, primitive neuroectodermal tumor, Askin tumor, and ES belong to the ES family of tumors (EFTs).

ES is characterized by low incidence, high degree of malignancy, short course of disease, early metastasis, and poor prognosis. ES often occurs in the bone; ES occurring in the cervix can be considered rare. Only 15 cases (Russin et al., 1982; Sato et al., 1996; Horn et al., 1997; Cenacchi et al., 1998; Pauwels et al., 2000; Tsao et al., 2001; Malpica and Moran, 2002; Snijders-Keilholz et al., 2005; Farzaneh et al., 2011; Arora et al., 2012; Masoura et al., 2012; Li et al., 2013; Khosla et al., 2014; Mashriqi et al., 2015) of ES occurrence in the cervix have been reported. In this report, a case of a 76-year-old female with ES arising in the cervix is described, and the literature on ES is reviewed.

## Materials and methods

### *Case report*

A 76-year-old woman came to the hospital in November 2016 because of a painful left inguinal mass that had been present for approximately 1 month. Vital

signs were normal, except for a painful left groin mass. Enhanced computed tomography scan showed signs of malignant tumors in the cervix region and metastasis of multiple lymph nodes in the bilateral groin. No past history on her cervix was noted.

The patient underwent surgical excision and bilateral lymph node dissection. Information acquired from the patient's clinical doctor indicates that the patient died of multiple organ failure after two weeks because the patient's family refused radiotherapy and chemotherapy treatment.

### *Methods*

Three tissues of no more than 0.5 cm were removed from the surgical specimen and fixed in formalin. The tissues were embedded in paraffin, cut into small pieces (2 μm thick), and stained with hematoxylin–eosin.

Immunohistochemistry (IHC) was performed on 4 μm-thick unstained sections. The antibodies used in the study included CD99 (ZM-0296, 1:200; Zsbio), FLI-1 (ZM-0108, 1:100; Zsbio), Vimentin (ZM-0260, 1:400; Zsbio), NSE (ZM-0203, 1:1000; Zsbio), S-100 (ZM-0224, 1:1600; Zsbio), CD56 (ZM-0057, 1:500; Zsbio), LCA (ZM-0183, 1:100; Zsbio), EMA (ZM-0095, 1:800; Zsbio), and AE1/AE3 (ZM-0068, 1:100; Zsbio). Appropriate positive and negative controls were performed simultaneously with all tested antibodies.

A fluorescence in situ hybridization (FISH) study was carried out on 4 μm-thick sections generated from the formalin-fixed, paraffin-embedded tissues to assess the EWSR1 or FLI-1 gene rearrangement. Briefly, the sections were incubated in a humidified chamber using dual-color break-apart probes of EWSR1 and FLI-1 (Vysis EWSR1 Break Apart Fish Probe Kit) in accordance with the manufacturer's protocol. The fluorescence signals were analyzed using a macroscope (Imagen A2, ZEISS, German). A total of 200 successive nuclei were assessed. The cutoff level for a positive score was when at least 15% of the nuclei showed a break-apart signal.

Total RNA was extracted from 4 μm-thick sections. Then, PCR was performed using primers 22.3 (5'CCAACAGAGCAGCAGCTACG3') and 11.3 (5'GGTGATACAGCAGCTGGCGTTGG3') for EWS/FLI1 fusion transcript analysis. All amplification products were fractionated through a 2% agarose gel and stained with ethidium bromide.

## Results

The macroscopic appearance of several pieces of cervical neoplasm and right inguinal lymph node tissue measured 3.0×1.0×1.2 cm and 5.0×3.0×2.0 cm, respectively. Seven lymph nodes were found.

Histopathological examination revealed that the tumor consisted of small round blue tumor cells surrounded by fibrous connective tissue (Fig. 1A). Some areas of the tumors had a glandular configuration (Fig. 1B). The tumor presented a conventional sheet-like growth pattern (Fig. 1C). Tumor cells were arranged in strips in some areas (Fig. 1D). Tumor stroma had abundant blood vessels (Fig. 1 E). Nucleus, round or oval, clearly possessed membrane and fine chromatin similar to dust (Fig. 1F). Nuclear division was considerably evident (Fig. 1F).

**Table 1.** Clinicopathologic features, treatment and outcome of ES of the cervix reported in the literature.

| | Age/y | Symptoms | CD99 | EWSR1 arrangement | Treatment | Outcome | Metastasis | Case |
|---|---|---|---|---|---|---|---|---|
| 1 | 60 | Vaginal bleeding | NED | NED | S+C+R | Alive at 16 months | NED | Russin et al., 1982 |
| 2 | 44 | Vaginal bleeding | NED | NED | S+C | Alive 6 months | No | Sato et al., 1996 |
| 3 | 26 | Suspect cervical smear | NED | NED | S+R | Died 4.2 years after diagnosis | Yes, 3 years after diagnosis | Horn et al., 1997 |
| 4 | 36 | Vaginal bleeding | + | + | S | Alive 18 months | No | Cenacchi et al., 1998 |
| 5 | 45 | Vaginal bleeding | + | + | S+R | Alive 42 months | NED | Pauwels et al., 2000 |
| 6 | 24 | Vaginal bleeding | + | NED | S+C | Alive 24 months | No | Tsao et al., 2001 |
| 7 | 35 | Vaginal bleeding | + | + | S+C | Alive 5 months | Yes, at diagnosis | Malpica and Moran, 2002 |
| 8 | 51 | Vaginal bleeding | + | NED | S+C | Alive 18 months | No | Malpica and Moran, 2002 |
| 9 | 21 | Vaginal bleeding | + | NED | S+C | Alive 27 months | No | Snijders-Keilholz et al., 2005 |
| 10 | 45 | Yellow purulent vaginal discharge | + | NED | S+C | Alive 4 months | No | Farzaneh et al., 2011 |
| 11 | 23 | Vaginal bleeding | + | NED | S+C+R | Alive 4 months | No | Arora et al., 2012 |
| 12 | 23 | Vaginal bleeding, abdominal pain | + | + | S+C | Died of MOF after 12 days | Yes, at diagnosis | Masoura et al., 2012 |
| 13 | 27 | Contact bleeding | + | NED | S+C+R | Alive at 6 months | No | Li et al., 2013 |
| 14 | 28 | 10 weeks pregnant with vaginal bleeding and pelvic pain | + | NED | C+R | Alive 33 months | No | Khosla et al., 2014 |
| 15 | 49 | Vaginal bleeding, abdominal pain | + | + | S+C+R | Died after 10 months | Yes, 4 months after diagnosis | Mashriqi et al., 2015 |
| 16 | 76 | Painful left inguinal mass | + | + | C | Died of MOF after 12 days | Yes, at diagnosis | Present case |

ES, Ewing's sarcoma; Y, years old; C, chemotherapy; R, radiation therapy; S, surgery; NED, no evidence of disease; MOF, multiple organ failure.

Immunohistochemical analysis demonstrated that the tumor cells underwent strong, diffused membranous staining for CD99 (Fig. 2A); nuclear staining for FLI-1 (Fig. 2B); cytoplasm staining for Vimentin (Fig. 2C); and negative staining for LCA (Fig. 2D), Desmin (Fig. 2E), and HMB 45 (Fig. 2F).

Owing to the strong, positive staining of CD99 and FLI-1, FISH was conducted. Two-color FISH with a split *EWSR1* probe showed the nucleus of isolated green and red signals due to the rearrangement of the *EWSR1* gene (Fig. 3A).

RT-PCR was then conducted to investigate the patient's tumor for *EWS-FLI1* fusion transcripts. The patient's sample was found positive for an *EWS-FLI1* product at the expected size *EWS-FLI*; thus, the diagnosis of ES was confirmed (Fig. 3B).

## Discussion

ES, which is a poorly differentiated malignant tumor of small round cells, usually occurs in children and adolescents. ES is common in the pelvis and lower extremities, whereas its occurrence in the cervix is rare. Since the introduction of the term E-ES, only 15 cases in the English literature have been reported (Table 1).

In 1999, Baldini et al. (1999) demonstrated that age

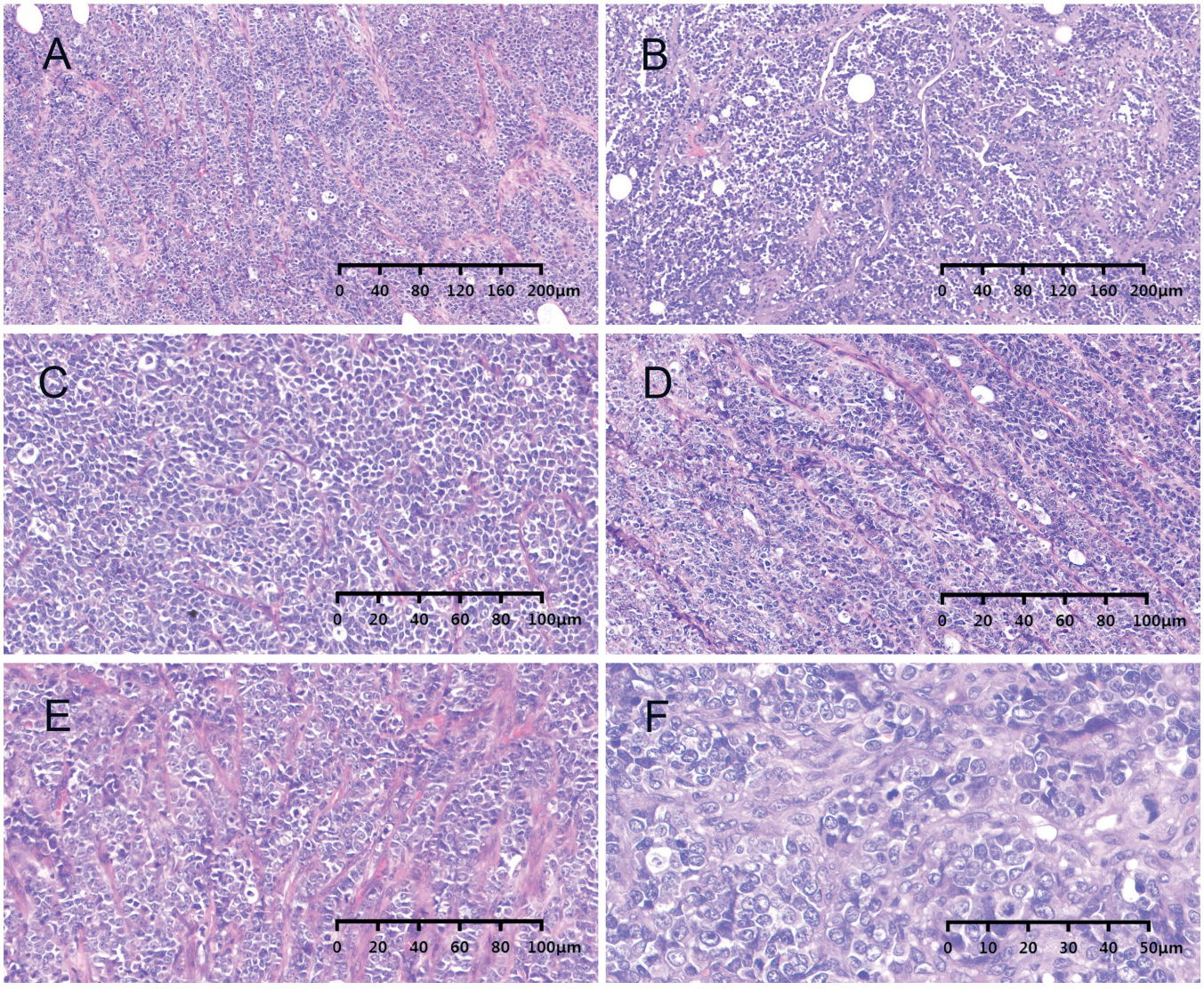


**Fig. 1.** Pathological features of E-ES revealed by hematoxylin and eosin staining. **A.** Sheets of small round tumor cells are surrounded by fibrous connective tissue. **B.** Glandular configuration is sometimes present. **C.** Tumor shows a sheet-like pattern of infiltration. **D.** Tumor has a cord-like growth pattern. **E.** Tumor stroma has abundant blood vessels. **F.** Nucleus, round or oval, clearly possesses membrane and fine chromatin similar to dust; nuclear division is common.

is an adverse prognostic factor. Despite the rarity of cases occurring in the cervix, the cases reported in the literature are summarized in Table 1. E-ES occurrence in the cervix is common in young females. This study is the first case in which the patient is over 70 years old and is the fifth case with metastasis occurrence, thus resulting in poor outcome. Owing to the metastasis, the main symptoms of the patient included painful inguinal mass instead of vaginal bleeding.

Although an increasing number of studies regarding the origin of ES from either mesenchymal or neuroectoderm are available, the origin and pathogenesis of ES remain unclear (Toomey et al., 2010). E-ES and ES share the same histopathological features, immunohistochemical expression, and cytogenetic changes. Both types have small round cells, round nucleus with fine granular chromatin, strong expression of CD99 and *FLI-1*, and a characteristic translocation at t (11;22) (q24; q12) in 85% of patients. Combined with clinical, pathological, immunophenotypic, and related fusion genes, making an ES diagnosis is easy. However, histological morphology is diverse and immunophenotypic, and the diagnosis of several cases is often difficult due to the low incidence. A differential diagnosis is required before providing a definitive diagnosis. Although 90% of ES is positive for CD99, CD99 can also be positive in other tumors, such as synovial sarcoma, rhabdomyosarcoma, and leiomyo-

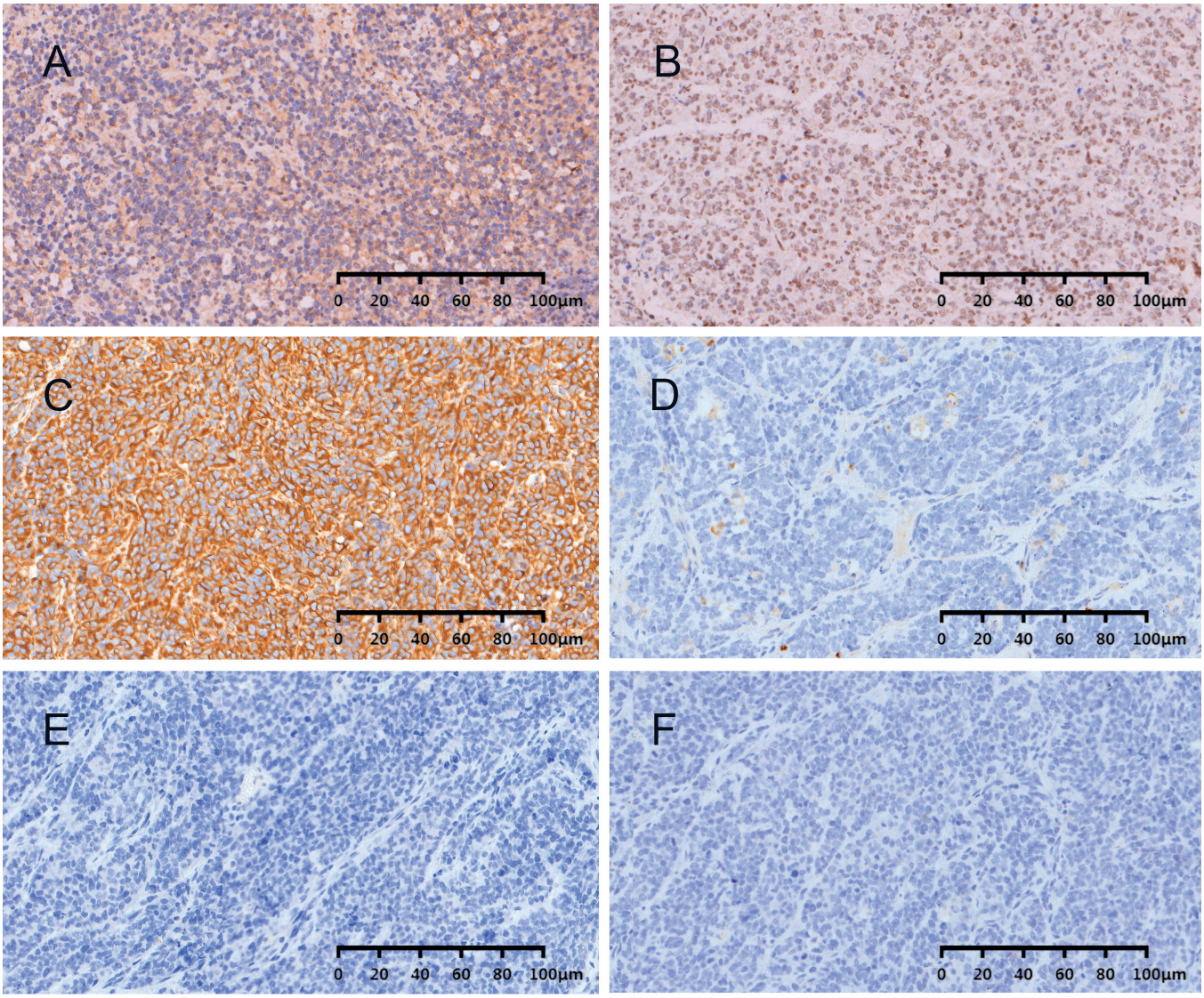


**Fig. 2.** Immunohistochemistry of the tumor. Immunoreactivities of E-ES with a characteristic of strong, diffused membranous staining for CD99 **(A)**, nuclear staining for FLI-1 **(B)**, and cytoplasm staining for Vimentin **(C)**. Tumor cells have negative staining for LCA **(D)**, Desmin **(E)**, and HMB 45 **(F).**

sarcoma. Therefore, CD99 can only help identify ES and small round blue cell tumor, but it is ineffective in differentiating ES from other CD99-positive diseases (Table 2).

The presence of specific translocations involving the *EWSR1* gene, which is usually fused to an E26 transformation-specific (ETS) family gene (*FLI-1, ERG*, or *ETS* variant 1), is common for ES (Mashriqi et al., 2015). A total of 90% to 95% of cases have t (11; 22) (q24; q12), resulting in the fusion of the *FLI-1* gene located at 11q24 with the *EWS* gene located at 22q12 and the fusion gene *EWS-FLI-1*. FISH has advantages in detecting gene fusions using dual-color break-apart probes. Given that the rearrangement of *EWSR1* could be observed in other tumors (Table 2), combining IHC with FISH may be effective to diagnose ES.

No standard treatment is available because E-ES in the cervix is limited. The treatment varies with patient conditions, such as location, stage, or metastasis. Surgery combined with chemotherapy and radiation therapy is presently considered an effective treatment. Thousands of genes deregulated by EWS/FLI in the ES cell line have been identified (Kauer et al., 2009). Although the relationship of these genes with the process of tumor development has not been confirmed, further research may find that new targeted therapies can greatly improve the prognosis of ES.

Overall, E-ES is a member of the EFTs. Except for location and symptom, E-ES has the same histopathology and IHC as ES. The occurrence of ES in

**Table 2.** The role of IHC and FISH test for the diagnosis of ES.

| | CD99 | EWSR1 rearrangement |
|---|---|---|
| Small round cell tumors | | |
| Fibroblastic connective tissue proliferative small round cell tumor | - | - |
| Small cell amelanotic melanoma | - | - |
| Mesenchymal chondrosarcoma | + | - |
| Lymphoblastic lymphoma | + | - |
| Metastatic small cell carcinoma | + | - |
| Rhabdomyosarcoma | + | - |
| Desmoplastic small round cell tumors | - | + |
| Spindle cell tumor | | |
| Synovial sarcoma | + | - |
| Solitary fibrous tumor | + | - |
| Ewing's sarcoma | + | 90-95% + |

ES, Ewing's sarcoma; IHC, immunohistochemic; FISH, fluorescence in situ hybridization.

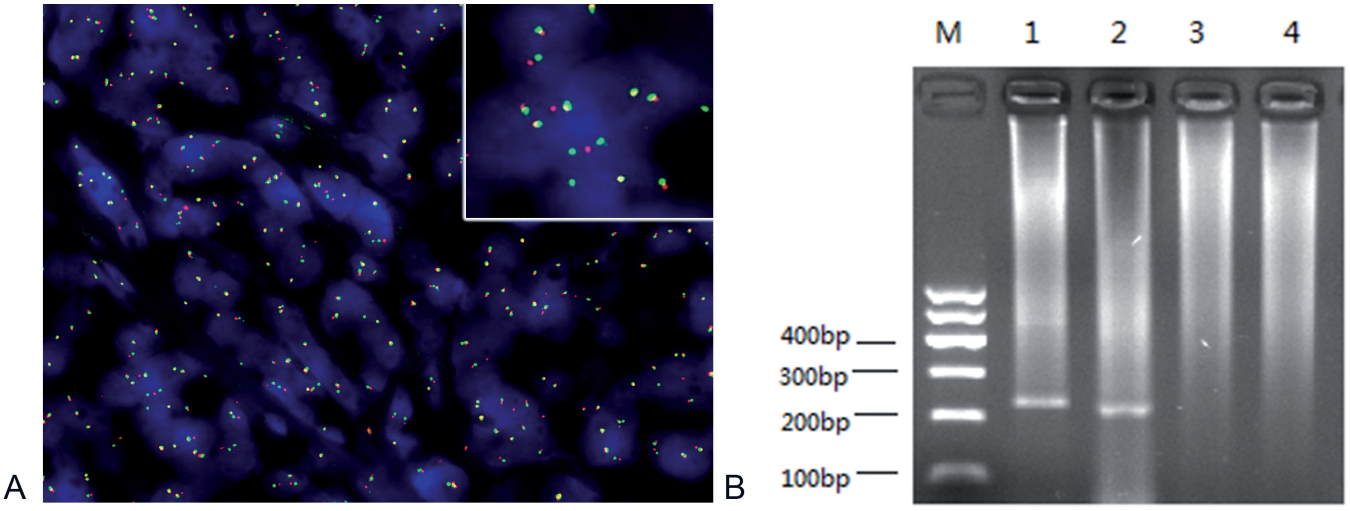


**Fig. 3. A.** Fluoresence in situ hybridization (FISH): tumor cells display some rearranged and some intact 22q12 region. **B.** Investigation of patient's tumor for EWS-FLI1 fusion transcripts. The patient's sample was found positive for an EWS-FLI1 product at the expected size (arrow). M: DNA Marker I; 1: Positive control; 2: Test sample; 3: Negative control; 4: Blank control.

extraskeletal organs, especially the cervix, is extremely rare. A case of ES of the cervix is presented in this paper. To our best knowledge, this case is the 16th one to show ES in the cervix and the first case to involve a patient aged over 70 years. The origin, pathogenesis, treatment, and outcome of ES are also discussed.

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*Competing Interests.* The authors declare no conflict of interest.

## References

Angervall L. and Enzinger F.M. (1975). Extraskeletal neoplasm resembling Ewing's sarcoma. Cancer 36, 240-251.

Arora N., Kalra A., Kausar H., Ghosh T.K. and Majumdar A. (2012). Primitive neuroectodermal tumour of uterine cervix - a diagnostic and therapeutic dilemma. J. Obstet. Gynaecol. 32, 711-713.

Baldini E.H., Demetri G.D., Fletcher C.D., Foran J., Marcus K.C. and Singer S. (1999). Adults with Ewing's sarcoma/primitive neuroectodermal tumor: adverse effect of older age and primary extraosseous disease on outcome. Ann. Surg. 230, 79-86.

Cenacchi G., Pasquinelli G., Montanaro L., Cerasoli S., Vici M., Bisceglia M., Giangaspero F., Martinelli G.N. and Derenzini M. (1998). Primary endocervical extraosseous Ewing's sarcoma/PNET. Int. J. Gynecol. Pathol. 17, 83-88.

Chen C., Borker R., Ewing J., Tseng W.Y., Hackshaw M.D., Saravanan S., Dhande R. and Nadler E. (2014). Epidemiology, treatment patterns, and outcomes of metastatic soft tissue sarcoma in a community-based oncology network. Sarcoma 2014, 145764.

Farzaneh F., Rezvani H., Boroujeni P.T. and Rahimi F. (2011). Primitive neuroectodermal tumor of the cervix: a case report. J. Med. Case Rep. 5, 489.

Horn L.C., Fischer U. and Bilek K. (1997). Primitive neuroectodermal tumor of the cervix uteri. A case report. Gen. Diagn. Pathol. 142, 227-230.

Kauer M., Ban J., Kofler R., Walker B., Davis S., Meltzer P. and Kovar, H. (2009). A molecular function map of Ewing's sarcoma. PLoS One 4, e5415.

Khosla D.R.B., Firuza P. and Sreedharanunni S. (2014). Primitive neuroectodermal tumor of the uterine cervix diagnosed during pregnacy: A rare case with review of literature. J. Obstet. Gynaecol. Res. 40, 878-882.

Li B., Ouyang L., Han X., Zhou Y., Tong X., Zhang S. and Zhang Q. (2013). Primary primitive neuroectodermal tumor of the cervix. Onco. Targets Ther. 6, 707-711.

Malpica A. and Moran C.A. (2002). Primitive neuroectodermal tumor of the cervix: a clinicopathologic and immunohistochemical study of two cases. Ann. Diagn. Pathol. 6, 281-287.

Mashriqi N., Gujjarlapudi J.K., Sidhu J., Zur M. and Yalamanchili M. (2015). Ewing's sarcoma of the cervix, a diagnostic dilemma: a case report and review of the literature. J. Med. Case Rep. 9, 255.

Masoura S., Kourtis A., Kalogiannidis I., Kotoula V., Anagnostou E., Angelidou S. and Agorastos, T. (2012). Primary primitive neuroectodermal tumor of the cervix confirmed with molecular analysis in a 23-year-old woman: A case report. Pathol. Res. Pract. 208, 245-249.

Pauwels P., Ambros P., Hattinger C., Lammens M., Dal Cin P., Ribot J. and van den Berghe H. (2000). Peripheral primitive neuroectodermal tumour of the cervix. Virchows Arch. 436, 68-73.

Russin L.A., Robinson M.J., Engle H.A. and Sonni A. (1982). Ewing's sarcoma of the lumbar spine: a case report of long-term survival. Clin. Orthop. Relat. Res. 126-129.

Sato S., Yajima A., Kimura N., Namiki T., Furuhashi N., and Sakuma H. (1996). Peripheral neuroepithelioma (peripheral primitive neuroectodermal tumor) of the uterine cervix. Tohoku J. Exp. Med. 180, 187-195.

Snijders-Keilholz A., Ewing P., Seynaeve C. and Burger C.W. (2005). Primitive neuroectodermal tumor of the cervix uteri: a case report -- changing concepts in therapy. Gynecol. Oncol. 98, 516-519.

Toomey E.C., Schiffman J.D. and Lessnick S.L. (2010). Recent advances in the molecular pathogenesis of Ewing's sarcoma. Oncogene 29, 4504-4516.

Tsao A.S., Roth L.M., Sandler A. and Hurteau J.A. (2001). Cervical primitive neuroectodermal tumor. Gynecol. Oncol. 83, 138-142.

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