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CASE REPORT

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Pulmonary metastases from a low-grade endometrial stromal sarcoma confirmed by chromosome aberration and fluorescence in-situ hybridization approaches: a case of recurrence 13 years after hysterectomy

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Abstract Pulmonary metastasis from low-grade endometrial stromal sarcomas (ESSs) occasionally are found after long, disease-free periods, mostly as incidental histological or radiological discoveries. We describe a case of low-grade ESS presenting as nodular pulmonary metastases finally diagnosed by estrogen-receptor staining, cytogenetic and fluorescence in situ hybridization (FISH) analyses, and perusal of the histology of hysterectomy material. An abnormal nodule in the lung field was discovered by means of chest X-ray of a 47-year-old woman. She had been disease free for 13 years after hysterectomy for an alleged leiomyoma. A computed tomographic scan revealed nodules, with fluctuation in size over the 2-year period, in both lungs. Finally the lesion in the left lung was resected, and pulmonary endometriosis was suspected because of the lack of stromal cell nuclear atypia and positive immunohistochemical reactions for estrogen and progesterone receptors. However, a characteristic karyotype was identified cytogenetically: 46, XX, t(7;17)(p15;q11), the translocation of which, specific to ESS, was confirmed by FISH analysis. A final diagnosis of pulmonary metastases from an ESS could be made by reviewing the histology of the previous uterine tumor. In this case, metastatic lesions from an ESS showed a decrease as well as an increase in size, despite the malignant potential. Immunostaining for estrogen and progesterone receptors and cytogenetic and FISH analyses, together with clinical information on the past gynecological history, are valuable diagnostic keys.

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Introduction

The endometrial stromal sarcoma (ESS) is a rare neoplasm comprising only 0.2% of all uterine malignancies and approximately 15–26% of primary uterine sarcomas [1, 5, 8, 13, 14, 18]. It is subdivided into two distinct subtypes, low-grade and high-grade, based on differences in morphological atypia and proliferative activity [17]. The high-grade ESS has an aggressive nature, whereas low-grade lesions, known as endolymphatic stromal myosis, occasionally recur locally in the pelvic cavity or show distant metastasis, sometimes long after (for example, more than 10 years) the initial diagnosis [12, 21]. With such low-grade ESSs, diagnostic difficulties may arise, because neoplastic cells usually show slight or even no nuclear atypia as well as lacking any specific structural pattern on histological assessment.

We experienced an unusual case of pulmonary metastases from a low-grade ESS, in which asymptomatic nodules in both lungs were discovered by imaging procedures in a patient who had been disease free for 13 years after hysterectomy for a suspected leiomyoma. Since the nodules were composed of cells resembling those of the normal endometrial stroma and showed peculiar growth characteristics, they were the initially diagnosed as reflecting pulmonary endometriosis because of the lack of detailed information on the actual nature of the uterine tumor. Here, we describe the entire features of the case, of which a final diagnosis could be made based on immunohistological, cytogenetic and fluorescence in situ hybridization (FISH) analyses.

Table 1 Changes in sizes of the bilateral lung nodules during follow-up. *CT* computed tomographic, – no record

Date	Left lower lobe		Right lower lobe	
	Roentgenogram	CT scan	Roentgenogram	CT scan
10/04/1997	15 mm	–	6 mm	–
10/19/1998	25 mm	–	8 mm	–
11/07/1998	–	25 m	–	9 mm
12/08/1998	26 mm	–	–	–
01/16/1999	–	26 mm	–	–
03/24/1999	20 mm	–	–	–
04/19/1999	–	20 mm	–	–
08/07/1999	28 mm	–	–	–
09/09/1999	20 mm	–	4 mm	–
09/21/1999	–	23 mm	–	–
11/11/1999	32 mm	–	–	7 mm
11/19/1999	Resected	Resected	6 mm	–

Case report

A 47-year-old Japanese woman was admitted to the Cancer Institute Hospital for further examination of nodules existing in both lungs. These had been found at a regular health check-up on a chest X-ray film 2 years previously and followed up at another hospital. They had decreased in size, then increased again during the course of observations (Table 1). The patient had a history of simple hysterectomy under the clinical diagnosis of leiomyoma uteri at a local hospital 13 years previously (at the age of 34 years in 1986). She was followed up at the same hospital for several months after the operation, then ceased showing up because she felt well. Thereafter, she remained healthy and had no pulmonary symptoms, now or in the past. She had never used any hormonal medications and had always been in good health.

A computed tomographic (CT) scan revealed one 23-mm, well-circumscribed nodule in the left lower lung field and one 7-mm, well-circumscribed nodule in the right lower lung field. Considering the patient's history of hysterectomy for "myoma uteri", differential diagnosis included a "benign" metastasizing leiomyoma derived from the leiomyoma uteri as well as other benign and malignant lesions.

Transbronchial biopsy failed to access the tumors, so video-assisted thoracoscopic surgery for the nodule in the left lung was performed. As intra-operative frozen sections showed proliferation of monotonous small-sized oval to spindle-shaped cells without atypia, a benign nonepithelial neoplastic lesion was highly suspected.

Hormonal treatment was not administered after surgery. There were no new pulmonary nodules and the right one showed a gradual decrease in size during 1 year of follow-up. She is now alive and well after 2 years of follow-up.

Materials and methods

For light microscopy, material from the surgical specimen was fixed in 15% buffered formalin and embedded in paraffin. Sections, 4 μm thick, were stained with hematoxylin and eosin. For immunohistochemistry, paraffin sections were stained using the SBC peroxidase method, with antibodies against the vimentin, actin (HHF35), desmin, S-100 protein, neuron-specific enolase (NSE), chromogranin A, synaptophysin, epithelial membrane antigen (EMA), cytokeratin AE1/AE3, estrogen and progesterone receptors (Nichirei), thyroid transcription factor-1 (TTF-1; Neomarkers) and surfactant apoprotein (PE-10; Dako).

Chromosome analysis was performed on fresh surgical material. Without collagenase dissociation, a cell suspension was seeded into tissue culture containing RPMI-1640 medium and antibiotics, supplemented with 10% fetal bovine serum, and incubated at 37°C in a 5% CO_2 atmosphere. When cultures entered exponential growth, they were harvested after Colcemid (0.01–0.05 μg/ml) treatment. Slides were prepared using conventional techniques and, after air drying, underwent quinaklin mustard staining. The standard International System of Human Cytogenetic Nomenclature (ISCN, 1995) was used to identify chromosomal abnormalities [16].

For FISH analysis, slides were prepared according to previously described protocols. FISH experiments were carried out using whole chromosome painting probes for chromosomes 7 and 17 labeled with Cy3 and FITC (fluorescein isothiocyanate), respectively (Cambio, Cambridge, UK), and chromosomes were counterstained with 4'-6'-diamidino-2-phenylindole (DAPI). Analyses were carried out using a fluorescence microscope with a charged-coupled device (CCD) camera. At least ten metaphase spreads were analyzed. Comparison of FISH signals and Q-banding was used to determine the composition of the derivative chromosomes.

Results

Histological findings

The resected material was an intra-parenchymal single, pale and soft nodule, 20 mm in diameter, found in the peripheral portion of the lower lobe. The nodule bulged above the covering pleura with rounded contours.

Microscopically, the nodule had an expansive non-infiltrative margin and was composed of cells identical to, or closely resembling, those of the normal endometrial stroma (Fig. 1a). The cells were uniform in appearance, size, shape and staining qualities. They were arranged in vaguely concentric patterns around vessels (Fig. 1b). Glandular structures were not evident within the lesion, and the proliferating cells had small amounts of eosinophilic cytoplasm, and the nuclei, with an oval to spindle shape, had finely granular chromatin with a thin nuclear membrane. Nucleoli were not easily identified. Mitotic activity was moderate (from 5 to 9 counts per 10 high-power fields), but no necrotic foci were identified. Some gland-like structures, thought to be residual alveoli with hyperplastic type-II pneumocytes, were embedded in the periphery of the tumor nest. No hemosiderin granules were observed within the lesion. No abnormalities were noted in the uninvolved pulmonary parenchyma around the lesion.

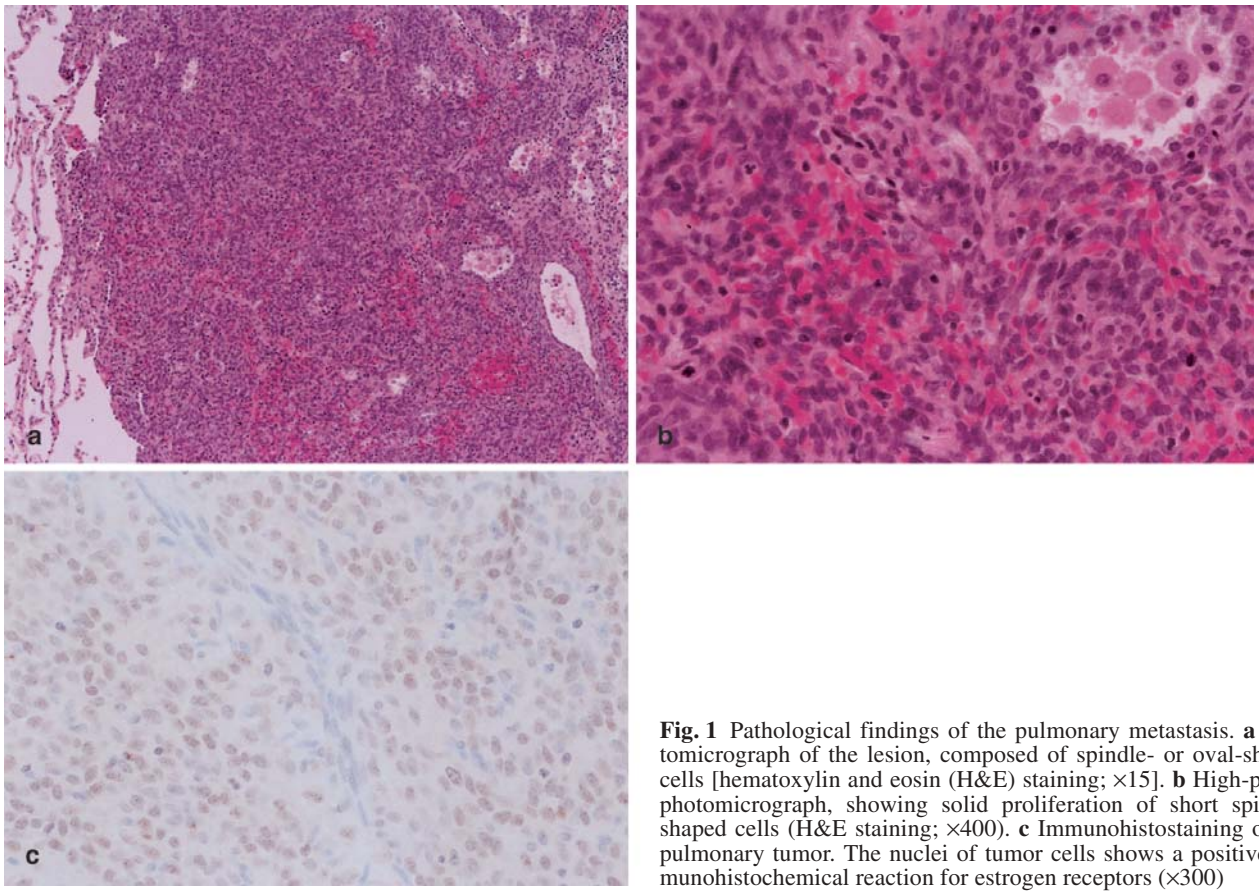

Fig. 1 Pathological findings of the pulmonary metastasis. **a** Photomicrograph of the lesion, composed of spindle- or oval-shaped cells [hematoxylin and eosin (H&E) staining; ×15]. **b** High-power photomicrograph, showing solid proliferation of short spindle-shaped cells (H&E staining; ×400). **c** Immunohistostaining of the pulmonary tumor. The nuclei of tumor cells shows a positive immunohistochemical reaction for estrogen receptors (×300)

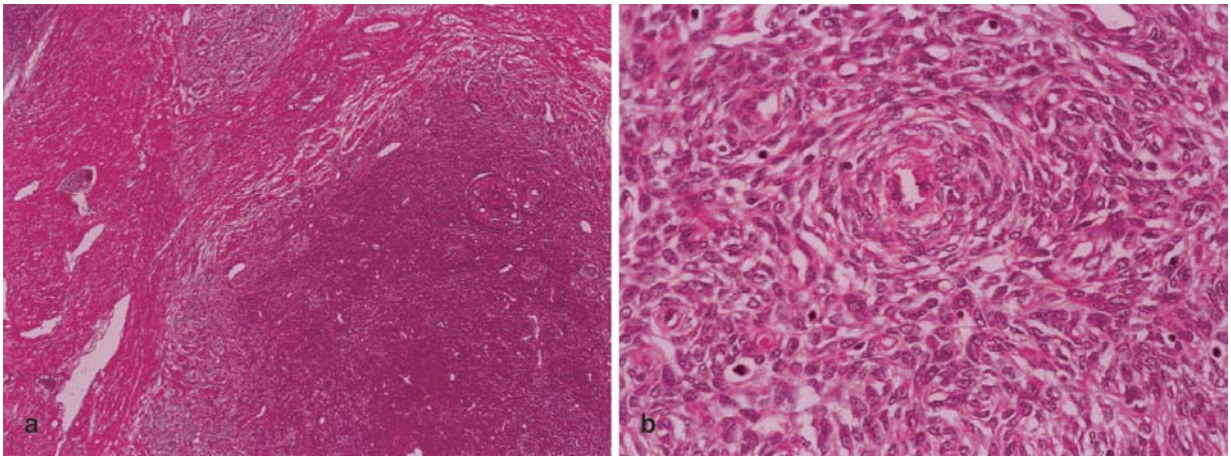

Fig. 2 Histology of the uterine tumor resected 13 years previously. **a** Neoplastic cells resembling endometrial stromal cells with dense proliferation [hematoxylin and eosin (H&E) staining; ×15]. **b** No mitotic figures are apparent, but nuclear atypia are evident (H&E staining; ×400)

Immunohistochemistry demonstrated the tumor cells to be positive for vimentin and highly positive for estrogen and progesterone receptors (more than 90%, Fig. 1c); they were negative for cytokeratin, actin (HHF35), NSE, EMA, desmin, S-100 protein, chromogranin A, surfactant apoprotein (PE-10), TTF-1 and synaptophysin. These findings are compatible with an endometrial stromal nature [2, 4, 6, 15, 18]. Therefore, the tentative diagnosis at this stage was pulmonary stromal endometriosis [11].

Fortunately, it was possible to review the corpus uterine tumor, resected in 1986, retrospectively. According to the pathology report made 13 years ago, the tumor was a solid mass, sized 100×70×50 mm, whitish to yellowish in color, and showing expansive growth with a partly irregular border. The histological findings were

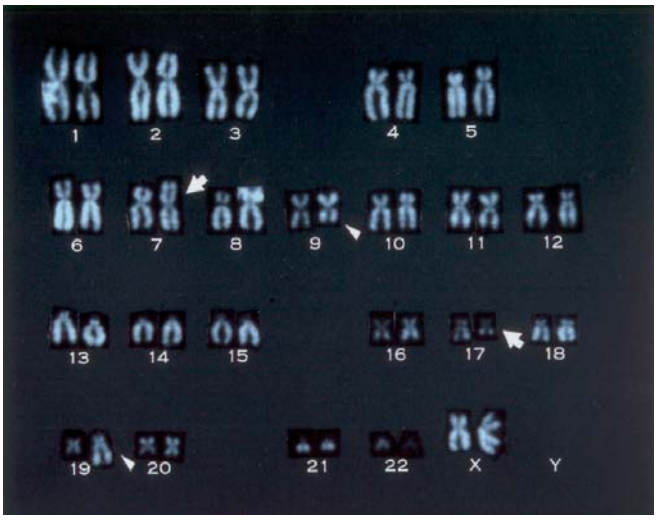

Fig. 3 Q-banded karyotype of the pulmonary tumor cells. *Arrowheads* indicate the del(9)(q22) and add(19)(q13). *Arrows* indicate the two derivative chromosomes, der(7) and der(17), resulting from a 7;17 translocation

fundamentally the same as those for the lung nodule except for nuclear atypia. The tumor was composed of sheets of densely packed, uniform, small to medium-sized cells with slight nuclear atypia, and there were sparse mitotic figures (about 0–1 cell per 10 HPFs, Fig. 2). Venous infiltration was evident at the periphery, but no serosal involvement was seen. Based on these findings, the uterine neoplasm was diagnosed as a low-grade ESS with low proliferative activity in 1986.

Chromosome analysis

For Q-banded analysis, ten metaphases were examined. The tumor cells were pseudo-diploid and showed monoclonal abnormalities, resulting in the following karyotype: 46,XX,t(7;17)(p15;q11),del(9)(q22), add(19)(q13) (Fig. 3). The translocation t(7;17) was confirmed by FISH analysis (Fig. 4).

Clinical course, hormonal examination and basal body temperature

Hormonal parameters, including serum estradiol levels over 8 weeks, showed irregularities. Clinical tests, including gynecological examination and pelvic and abdominal ultrasonography, revealed no abnormalities. A CT scan of the abdomen performed after application of contrast medium also demonstrated no adnexal or peritoneal abnormalities. The basal body temperature chart recorded over 8 weeks showed no regular cycle.

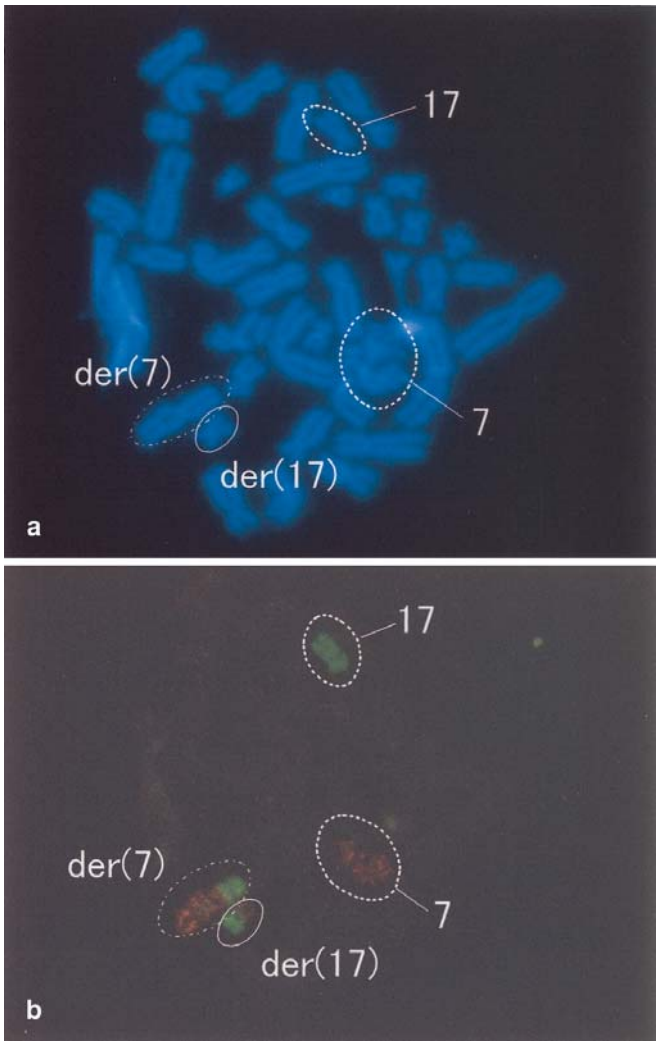

Fig. 4 Fluorescence in-situ hybridization (FISH) analysis. **a** 4'-6'-diamidino-2-phenylindole-stained metaphase cell. **b** The same chromosome spread hybridized with whole chromosome probes for chromosomes 7(red) and 17(green). Green/red fusion signals are present on both der(7) and der(17)

Discussion

When tumors or tumor-like conditions are found in the lungs of women who have a history of hysterectomy, even many years previously, clinicians and pathologists need to make a careful differential diagnosis [12]. Usually, the cases feature leiomyomatous lesions such as metastasizing leiomyomas of the uterus. In this report, we presented a case of multiple lesions composed of short spindle cells with little nuclear atypia and no other pathognomonic features in histology. Since the histology of the resected uterine tumor was unknown to either the surgeon or the pathologist at the time of the thoracotomy, the obvious question was whether these lesions were benign or malignant. Based on findings obtained from immunohistochemistry for estrogen and progesterone receptors and the characteristic fluctuation of size,

we diagnosed pulmonary endometriosis [1, 2, 4, 5, 14, 17, 18]. Further examinations, however, including chromosome analysis and FISH techniques, strongly suggested the lesions to be metastases from a low-grade ESS [4, 8, 9, 10, 20]. Subsequent examination of microscopic slides of the original uterine tumor confirmed this final diagnosis.

Low-grade ESS has been considered to be a hormone-sensitive neoplasm, as suggested by mRNA detection as well as by immunohistochemical evidence of estrogen and progesterone receptors [12, 19]. If sensitivity to hormones is identical to such immunohistochemical positivity to estrogen and progesterone receptors, the tumors in our case might have been dependent on serum concentration for growth. However, there was no apparent relationship between the tumor size and changes in serum gonadotropin levels or basal body temperature.

Cytogenetic analyses of pulmonary metastases of ESS have been limited, although several studies have demonstrated that primary lesions showed clonal structural abnormalities [4, 8, 9, 10, 20]. Rearrangements of chromosomes 6, 7 and 17 are the most consistent features [4, 8, 9, 10, 20]. In our case, a deletion of chromosome 9 at q22 band and rearrangement of chromosome 19 with material of unknown origin transferred to q13 with a t(7;17)(p15;q11) were present. A t(7;17) translocation seems to be a primary specific cytogenetic abnormality in the low-grade ESS [8]. The q13 band of chromosome 19 is often altered in malignant mixed mesodermal tumors of the uterus, arising mostly through the addition of material of unknown origin [7]. Aberrations of chromosome 19 have already been demonstrated in other low-grade ESSs, even if with different chromosomal change, so that such lesions, therefore, have relatively simple karyotypes, generally with rearrangements of two or three chromosomes. In contrast, multiple numerical and structural aberrations are typical of the high-grade ESS [8]. Therefore, application of cytogenetic techniques can provide useful evidence for diagnosis and subdivision of this type of lesion.

It is true that the lesion in our case metastasized from the uterus to the lungs, but the grade of malignancy was clearly very low. Although some mitotic figures were evident, stromal cell nuclear atypia such as enlargement, increased and/or coarse chromatin, and conspicuous nucleoli were absent in the pulmonary lesion. Furthermore, there were no hyaline sclerotic areas and no sex cord-like structures or cystic changes, strongly suggesting very low malignant potential [3, 4, 5]. The findings are thus in line with the well-differentiated nature, with fluctuation in size, presumably due to variation in response to female hormones.

When nonepithelial neoplastic lesions are found in the lungs of women with a history of gynecological disease, careful distinction between pulmonary metastases of ESS and other neoplasms is necessary. In such cases, it is suggested that immunostaining for estrogen or progesterone receptors and cytogenetic approaches for the pulmonary lesion, as well as the detailed investigation of history of gynecological disease, are very useful tools. Moreover, these could be applied for confirmation of the histomorphological diagnosis in our case for which pathological diagnosis of ESS was difficult due to the absence of stromal cell nuclear atypia.

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