Case Report of a Poorly Differentiated Uterine Tumour with t(10;17) Translocation and Neuroectodermal Phenotype

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Abstract. *Endometrial stromal sarcoma (ESS) with primitive neuroectodermal differentiation is a very uncommon entity. Such a case presenting as stage IIIc (International Federation of Gynaecology and Obstetrics (FIGO) 2010) disease in a 51-year-old female is described. Microscopy suggested a small blue round cell tumour. Cytogenetic and multicolour fluorescent in situ hybridisation (M-FISH) analysis revealed a complex karyotype with the presence of unbalanced t(10;17)(q22;p13) translocation, indicating ESS. Peripheral Ewing´s sarcoma was excluded based on FISH and RT-PCR fusion transcripts analysis. After surgical staging, the patient received bleomycin-etoposide-cisplatin combination chemotherapy. A detailed analysis of the histopathology and genetic findings forms the basis of this report.*

Uterine mesenchymal tumours, other than uterine fibroids, are uncommon since sarcomas of the uterus comprise only 3% of malignancies (1). Most common pure mesenchymal tumours are of muscular or endometrial stromal origin. The latter are exclusively composed of cells resembling the endometrial stroma in its proliferative phase. The stromal nodule is the benign variant; it has well circumscribed borders and is rare (2, 3). Endometrial stromal sarcoma (ESS) has infiltrating borders and behaves like a low-grade sarcoma, with the potential for recurrence and metastasis (4). Microscopic findings that unequivocally correspond to ESS include a uniform population of endometrial stromal-type cells invading the myometrium and myometrial vessels. Historically, ESSs were subdivided into low-grade and high-grade tumours. However, high-grade tumours lack the typical growth pattern and vascularity of low-grade ESS and show destructive myometrial invasion rather than the lymphatic permeation of a low-grade ESS. Moreover, they demonstrate marked cellular pleomorphism and brisk mitotic activity. As a result, ESS is now considered best restricted to malignancies that were formally referred to as low-grade ESS (3). Immunohistochemical staining for CD10, h-caldesmon and the oxytocin receptor can help to differentiate ESS from highly cellular leiomyomas and other smooth muscle cell tumours (5-7). Endometrial sarcomas without recognisable evidence of a definite endometrial stromal phenotype, designated as poorly-differentiated endometrial sarcomas, are almost invariably high grade (3, 8, 9) and termed poorly-differentiated or undifferentiated uterine sarcoma.

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Case Report

A 51-year old woman complained of three months of abnormal uterine bleeding. Her medical history was not significant. She had no family history of cancer. Endometrial biopsy was suggestive of small blue round cell tumour and she was subsequently referred to the University Hospitals, Leuven. Clinical examination and standard blood tests were within normal limits. Cancer antigen 125 (CA125) was 87 kU/L (normal <35 kU/L) and thrombocytosis (667×10^9/L) was observed. Sonography of the uterus was suggestive of a 34×48×43 mm lesion, probably arising from the posterior fundal wall and with possible growth through the serosa. Computed tomography of lungs and abdomen suggested pathological nodes at the level of the external iliac left, iliac communis right and interaortocaval lymph nodes. Based on a diagnosis of high-grade and high-stage endometrial cancer, a staging laparotomy was performed, consisting of a total hysterectomy, bilateral salpingo-oophorectomy and para-aortic lymphadenectomy up to the renal vessels. Stage IIIc

(International Federation of Gynaecology and Obstetrics, FIGO 2010) disease was diagnosed. Based on the pathology conclusion, six cycles of adjuvant combination chemotherapy, consisting of bleomycin, etoposide and cisplatin were initiated. Unfortunately, the disease recurred 8 months after initial diagnosis and the patient died 3 months later.

Histopathological analysis. Haematoxylin and eosin staining was performed on 4-μm formalin-fixed paraffin-embedded tissue sections mounted on charged glass slides. Representative slides were used for immunohistochemical evaluation using antibodies directed against vimentin, cytokeratin, CD99, proto-oncogene c-KIT/CD117, synaptophysin, chromogranin, glial fibrillary acidic protein (GFAP), alfa-smooth muscle actin, desmin, caldesmon, CD10, Wilms' tumour protein (WT1), progesterone receptor (all from Dako, Heverlee, Belgium), neuron-specific enolase (NSE; Biogenix, Fremont, CA, USA) and estrogen receptor (Klinipath, Olen, Belgium), following the manufacturers' instructions.

Fluorescence in situ hybridization. Chromosome metaphases were obtained after short-term culture of a primary tumour sample, utilizing standard procedures. G-Banded chromosomes were evaluated and classified according to the International System for Human Cytogenetics Nomenclature (ISCN) 2009. Multicolour fluorescent *in situ* hybridization (M-FISH) analysis was performed on the metaphases (MetaSystem GmgH, Altlussheim, Germany), according to the manufacturers' recommendations. Ewing sarcoma (*EWS*) gene integrity was investigated by FISH using dual-colour, split-apart EWS probe (Vysis Inc., Downers Grove, IL, USA) and standard procedures. To exclude possible cryptic rearrangements, the presence of *FLI1-EWS* and *ERG-EWS* fusion genes was explored by RT-PCR techniques, as previously described (10). In addition, for the evaluation of the integrity of genes previously identified in specific cytogenetic subgroups of ESS, bacterial artificial chromosome (BAC) DNA probes differentially labelled with SpectrumGreen (SG) or SpectrumOrange (SO) flanking suppressor of zeste 12 homolog (*SUZ12*) (RP11-42I19 and RP11-241P14) or PHD finger protein 1 (*PHF1*) (RP11-602P21 and RP11-175A4) genes were evaluated by FISH on metaphases obtained from the primary tumour specimens.

Results

The specimen weighed 220 grams. The *cavum uteri* was dilated and filled with a polypoid tumour (T1), with a diameter of 4.5 cm, arising from the posterior fundic wall (Figure 1 A-C). The tumour had a haemorrhagic surface appearance and on section a white 'encephaloid' aspect and a soft consistency. On the dorsal side of the corpus, there was a second, intramyometrial subserosal tumour nodule, with a diameter of 2.5 cm (T2), with a similar macroscopic appearance to T1, but separated from T1 by a tumour-free zone of 1 cm. The anterior side of the uterus and adnexae were normal.

Histopathology. Microscopically, the tumour consisted of a very dense, monotonous small cell proliferation extending from an atrophic endometrium to deep into the myometrium. The tumour cells showed no architectural differentiation, were sensitive to crush artefact and had a high nucleo-cytoplasmic index, with hyperchromatic nuclei and inconspicuous nucleoli. Evenly spaced small blood vessels were present between the tumour cells. There were sporadic Flexner-Wintersteiner rosettes and numerous Homer Wright pseudo-rosettes (Figure 2 A-B). A high mitotic index (29 mitotic figures/10 high power fields) and numerous apoptotic bodies were evident. Lymphovascular invasion was obvious and therefore T2, which had a similar microscopic appearance to T1, was interpreted as an intramyometrial subserosal lymphogenic metastasis. Lymphadenectomy revealed several lymph node metastases (6/34) in the right and left external iliacal areas and pre-sacral area.

The tumour cells expressed vimentin, CD99, c-KIT/CD117 (Figure 2 C-D), WT-1 and synaptophysine, but lacked the expression of broad-spectrum cytokeratin, chromogranin, NSE, GFAP and estrogen and progesterone receptors. The muscle markers alfa-smooth muscle actin, desmin, caldesmon and the endometrial stromal marker CD10 were not expressed in the tumour.

It was concluded that the tumour consisted of a uterine small blue round cell tumour, with a neuroectodermal phenotype, with lymph node and intra-myometrial sub-serosal lymphogenic metastasis, corresponding to FIGO stage IIIc.

Cytogenetic and molecular analysis. Chromosome analysis revealed a complex karyotype with the presence of der(17)t(10;17)(q22;p13) chromosome (Figure 3). The M-FISH analysis confirmed the presence of unbalanced t(10;17) translocation, and identified the marker chromosome as originating from chromosome 9 (Figure 4). The subsequent and final tumour karyotype description was 47,XX,der(9)del(9)(p11)del(9)(q12),del(10)(q22),der(11)t(9;11)(p12;q12),der(17)t(10;17)(q22;p13),+19[20]. By FISH, the tumour cells were negative for rearrangements of the *EWS, SUZ12* and *PHF1* genes, as evidenced by non-split hybridization signals using differentially labelled flanking probes (data not shown).

In the search for *FLI1-EWS* and *ERG-EWS* fusion transcripts, the RT-PCR analysis yielded negative results (data not shown), thus excluding the most common variants of Ewing sarcoma.

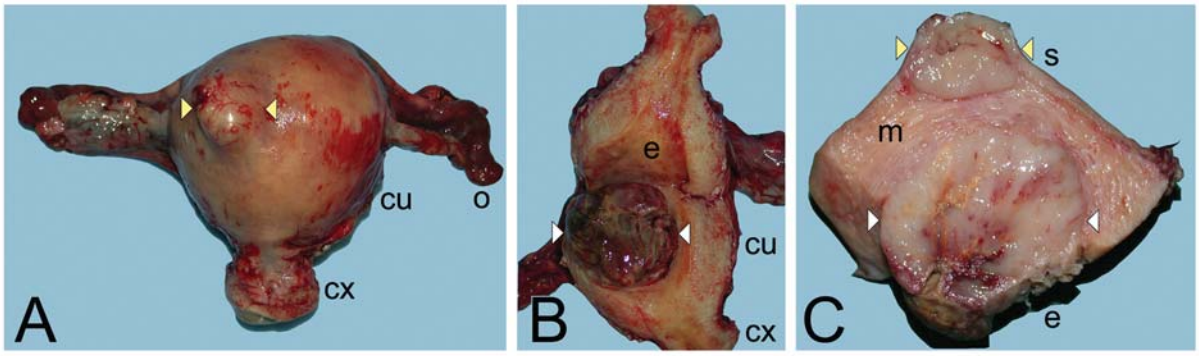

Figure 1. *Macroscopic appearance. A: Posterior view of the uterus, with a tumour nodule (T2; between triangles) extending into, but not breaking through the posterior serosal surface. (CX: Cervix uteri, CU: corpus uteri, o: ovary). B: Sagittal transection of the uterus with a large tumour nodule (T1; between triangles) extending into the endometrial cavity (e). C: Transverse section through the posterior half of the corpus uteri, showing 2 tumor nodules within the myometrium (m). The largest tumour nodule (T1; between white triangles) is not connected physically to the second smaller nodule (T2; indicated with yellow triangles). The latter was interpreted as an intramural subserosal metastatic localisation of the primary lesion.*

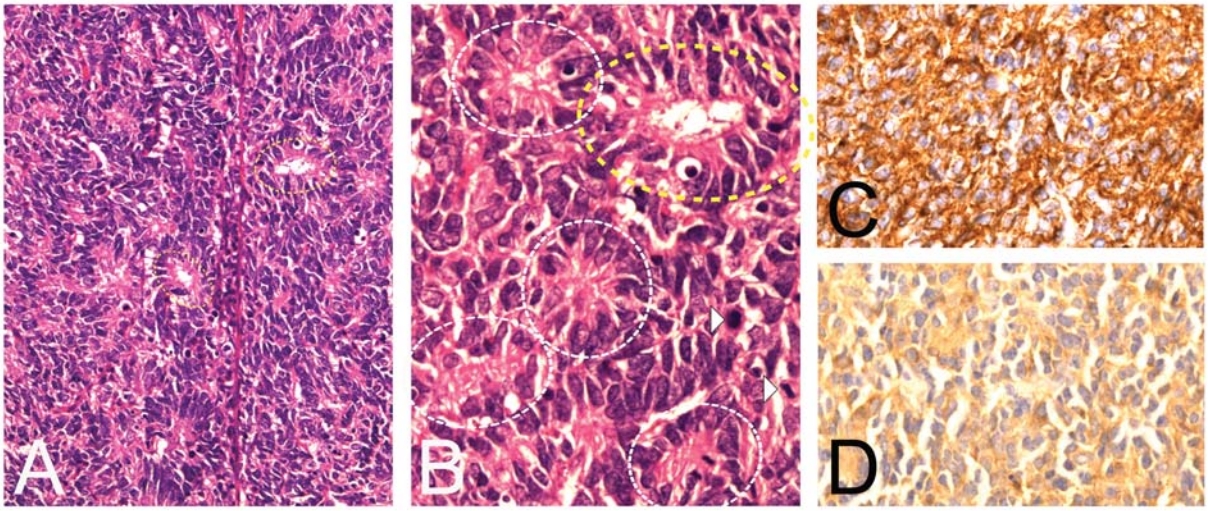

Figure 2. *Histopathological features. Low (A) and high (B) magnification images of haematoxylin-eosin staining showing sporadic Flexner-Wintersteiner rosettes (yellow dashed line) and numerous Homer-Wright pseudorosettes (white dashed line). High mitotic index is indicated by arrowheads. The tumour expressed CD99 (C) and c-KIT (D).*

Discussion

Uterine tumours with neuroectodermal differentiation are uncommon, tend to occur in postmenopausal women and frequently present with vaginal bleeding (11). Although, typical for ESS, expression of CD10, caldesmon and estrogen and progesterone receptors was absent, the presence of the distinctive t(10;17)(q22;p13) translocation in the tumour cells led us to consider ESS diagnosis. This was further supported by the exclusion of peripheral Ewing's sarcoma, as evidenced by negative results for *EWS* rearrangement by FISH and RT-PCR analysis. From the molecular point of view, to date at least three distinct and seemingly disease-specific fusion transcripts have been identified in ESS (for review see Micci and Heim (12)). Approximately one-half of these tumours have been shown to contain a specific recurrent chromosomal translocation, the t(7;17)(p15;q21), resulting in the fusion of two zinc finger genes, *JAZF1* (Juxtaposed with Another Zinc Finger) and *SUZ12* (previously named *JJAZ1*) (13). In addition, Micci *et al*. (14) showed that the *PHF1* gene in 6p21 was recombined with two different partners: with the *JAZF1* gene in two ESSs showing a 6p;7p rearrangement and

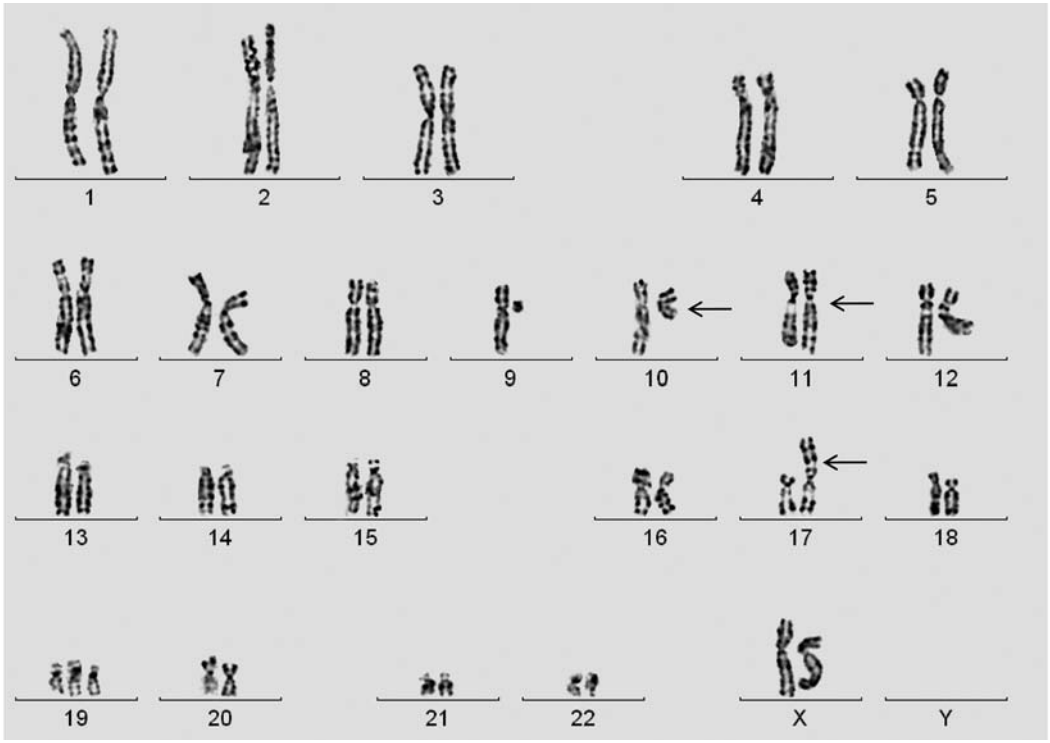

Figure 3. *The GTG-banded metaphases of the tumour cells revealed 47,XX,-9,del(10)(q22),der(11)t(9;11)(p12;q12),der(17)t(10;17)(q22;p13),+19, +mar karyotype. Arrows indicate chromosome breakpoints.*

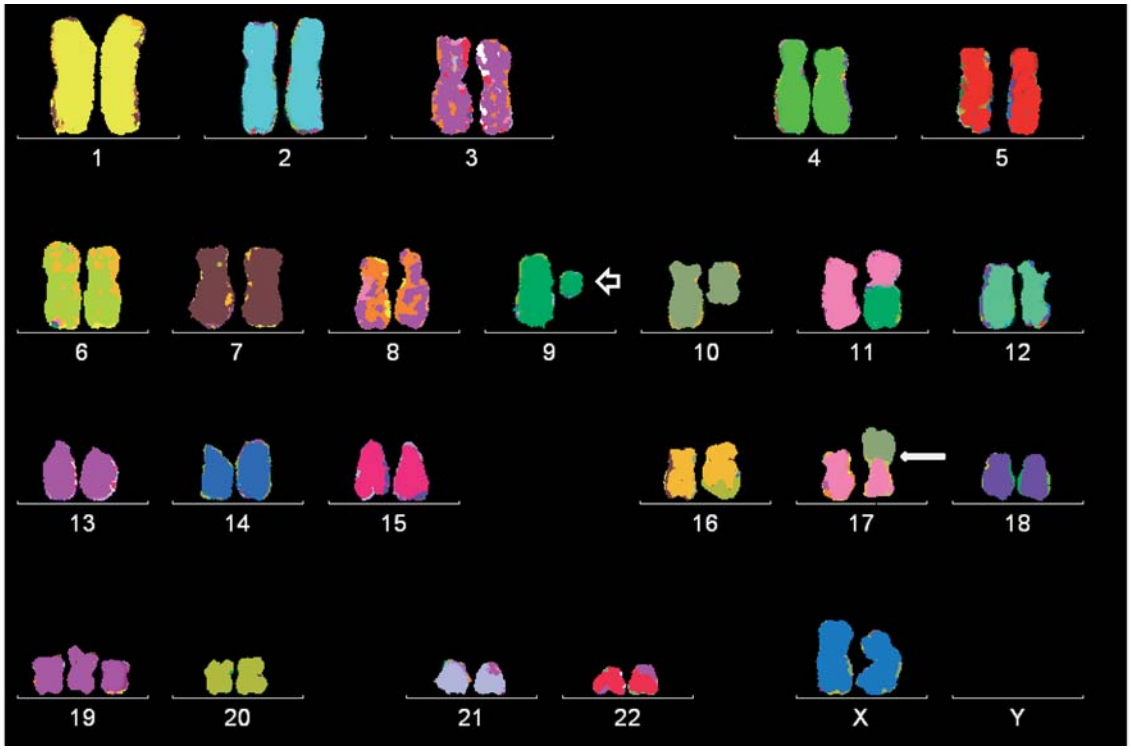

Figure 4. *M-FISH analysis using multicolour chromosomal painting probe confirming the presence of unbalanced t(10;17) translocation (solid arrow), and identifying the marker chromosome as originating from chromosome 9 (open arrow).*

with the enhancer of polycomb 1 (*EPC1*) gene on 10p11 in one tumour that had a 6;10;10 translocation. Recently, a new recurrent t(10;17)(q22;p13) translocation has been described in three ESS cases (15-17). The present case showed seemingly the same translocation, but in an unbalanced form. Importantly, the integrity of the *JJAZ1* and *PHF1* genes in this case was intact (at least by FISH analysis), supporting the existence of a distinct cytogenetic subgroup of ESS with t(10;17) translocation. Of note, however, all previously reported ESS cases with this translocation presented the histologically classical or rare fibrous variant of the entity, while the present case demonstrated the features of a poorly differentiated neuroendocrine tumour. Apparently, the histopathology of uterine sarcomas carrying t(10;17) might be variable, representing classical histological variants from one end of the spectrum to poorly-differentiated sarcomas at the other end. Based on the cytogenetic analysis identifying t(10;17), we opted to classify this case as an ESS with neuroectodermal differentiation. A larger series of uterine stromal tumours needs to be investigated to better understand the specificity of t(10;17) translocation and its possible association with explicit tumour clinical behaviour. The t(10;17)(q22;p13) translocation is extremely rare in other neoplasms, being reported also in clear cell sarcomas (CCS) of the kidney in children and young adults (Mitelman database (18)). The molecular basis of this translocation in both entities, ESS and CCS of kidney, is still unknown. Therefore, whether they result in the same genes fusion or involve different genes awaits further elaboration.

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