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Immunohistochemical Expression of Different Sub-Types of Cytokeratins by Endometrial Stromal Sarcoma

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Abstract

Endometrial stromal sarcomas (ESS) are rare and understudied gynaecological mesenchymal neoplasms. These tumours can be confused with many other gynaecological and not-gynaecological tumours due to their variegated morphological appearance and non-specific immunohistochemical profile.

ESS can express cytokeratin and, therefore, may be misdiagnosed as carcinoma especially in extra-uterine locations and when recurrence/metastasis is present.
In this study, we investigated the expression of a wide spectrum of cytokeratins consisting of AE1/3, CAM5.2, HMCK, MNF116, CK5, CK6, CK7, CK8/18, CK14, CK17, CK19, and CK20 in six low grade (LG) and five high-grade (HG) ESS. Additionally, staining for Oestrogen Receptor (ER), Progesterone Receptor (PR), CD10 and Cyclin D1 was performed.
Our results showed that CKs AE1/3, CM 5.2, MNF116 and CK8/18 are more expressed in LGESS while HGESS express more AE1/3 and CM 5.2.
In problematic cases, especially in recurrences or metastases, the immunohistochemical panel of antibodies AE1/3, MNF116, CAM 5.2 and CK8/18, together with other classic immunohistochemical markers CD10, Cyclin D1, ER and PR, may be helpful in the differential diagnosis between ESS and other gynaecological and non-gynaecological malignancies.

Keywords: endometrial; stroma; sarcoma; cytokeratins; immunohistochemistry.
Conflicts of interest: none.

Introduction

Endometrial stromal sarcomas (ESS) are rare mesenchymal neoplasms of the uterus and constitute approximately 10% of uterine mesenchymal tumours (1).

Approximately 50% of ESSs occur in premenopausal women and the majority, according to the International Federation of Gynaecology and Obstetrics (FIGO), are stage I disease (2). Grading of ESS is of paramount importance because it can protect patients with low-grade ESS against overtreatment with radiotherapy and inappropriate, potentially dangerous systemic chemotherapy.

The latest WHO classification divides ESS into low-grade (LG), high grade (HG) and undifferentiated uterine sarcoma (UUS) sub-types (3).

Morphologically LGESS consists of cells, which resemble those of the normal proliferative phase of endometrial stroma. HGESSs are neoplasms consisting of atypical cells reminiscent of endometrial stromal cells, but lacking the degree of pleomorphism necessary for a diagnosis of UUS (1).

The expression of some sub-types of cytokeratins (CKs) in ESS has been reported (4, 5). However, only one study investigated the expression of a relatively wide spectrum of CKs in ESS (6).

The aim of this study was to analyse the expression of a broad spectrum of common CKs antibodies in a series of LG and HG ESS in order to evaluate whether these express the same subtypes of CKs. The relationship of CKs expression with CD10, Oestrogen Receptor (ER), Progesterone Receptor (PR) and cyclin D1 was also investigated.

Methods

The pathology database of a general community hospital were searched for all cases of uterine LG and HG ESS between 2010 and 2013 and eleven cases were retrieved.

All cases arose in corpus uteri. Haematoxylin and eosin stained (H&E) slides were concomitantly reviewed by two authors (SR&CM). The cases were classified as LG and HG according to 2014 WHO classification. Histological features for differentiating LG from HG neoplasms were a grade of nuclear atypia, necrosis and pattern of myometrial invasion. Both LG and HG lesions showed resemblance to the proliferative phase of endometrial stroma.

Based on morphology and immunohistochemistry LGESS were differentiated from HGESS, gland-poor adenomyosis, cellular leiomyoma, intravascular leiomyomatosis, leiomyosarcoma with extensive intravascular component, uterine tumours resembling ovarian sex cord tumour (UTROSCT), adenosarcoma and perivascular epithelioid cell tumour (PEComa).

HGESS were distinguished from LGESS, carcinosarcoma, leiomyosarcoma and undifferentiated uterine sarcoma (UUS). No case showed a biphasic pattern of growth.

Uterine tumours resembling ovarian sex cord tumour (UTROSCT) were not included in this study.

Clinical data were reviewed from all patients and consisted of age, surgical treatment and adjuvant therapies. The Stage at disease presentation was determined according to FIGO and by reviewing pathology reports of a primary tumour. The pathological stage was reported for all patients.

The study was approved by the Proportionate Review Sub-Committee of the Wales REC 6 (REC reference 16/WA/0079).

Immunohistochemistry

Serial sections at 3.5 μm were cut from Formalin Fixed Paraffin Embedded (FFPE) tissue blocks, mounted onto adhesive slides (Surgipath Snowcoat) and dried at 65ºC for 15 minutes. Each series of sections were stained on Bond III Automated Immunohistochemistry System Leica Stainer using Leica Bond Polymer Refine detection with a DAB chromogen (0.5% Copper Sulphate in PBS buffer). All antigen retrieval methods were performed on-board, following Queen Alexandra Hospital laboratory protocols, using antigen-unmasking solutions: Epitope Retrieval Solution 1 (ER1) at pH 6.0 (Leica, AR9961) and Epitope Retrieval Solution 2 (ER2) at pH 9.0 (Leica, AR9640) for 20 minutes. Each run included a positive control (normal endometrium) and a negative control where the primary antibody was omitted.

Cytokeratin markers tested were, as follows: AE1/3, CAM5.2, HMWCK, MNF116, CK5, CK6, CK7, CK8/18, CK14, CK17, CK19 and CK20. Additionally, staining for ER, PR, CD10 and Cyclin D1 was performed. Details of antibodies (sources, clones, working dilutions and antigen retrieval pre-treatment) are summarised in Table 2.

The staining was assessed with a semi-quantitative method as shown in Table 3.

Results

The mean age of patients in both groups was 63 years (median 62 years, range 35 years). Six patients had LGESS (Fig. 1a, case 3) and five patients had HGESS (Fig. 1b, case 11). Two patients with LGESS were FIGO STAGE IA, one was stage IB, one was stage IIB, one was IIIA and one patient showed pelvic recurrence, after 49 years, of uterine “malignant haemangiopericytoma”.

One patient with HGESS was FIGO stage IA, one was stage IB, one was IIIB and two were stage IVA.

The clinicopathological data are summarised in Table 1.

Immunohistochemistry

CKs expression in LGESS:

All cases except one (case 4) were positive with AE1/3 (Fig. 2a) and CAM 5.2. Four cases (cases 1, 2, 5 and 6) were positive with MNF116 (Fig. 2b) and two cases (cases 3 and 4) were negative.

All cases except one (case 1) were negative with CK6. Three cases (cases 1, 5 and 6) were positive with CK 8/18 (Fig. 2c) and three cases (cases 2, 3 and 4) were negative.

CKs expression in HGESS:

Three cases (cases 7, 9 and 11) were positive with AE1/3 and two cases (cases 8 and 10) were negative. All cases except one (case 10) were positive with CAM 5.2 (fig. 3).

All cases except one (case 11) were negative with MNF116 and all cases except one (case 9) were negative with CK6 and CK 8/18.

All LG and HG ESSs were negative with CKs 5, 7, 14, 17, 19, 20 and HMWCK.

In all cases of LG and HG ESSs, the staining of CKs was cytoplasmic. The extension and intensity were different among cases.

CD10 was positive in all LG and HG ESSs. Nevertheless, not all cases showed diffuse staining.

All LGESSs demonstrated positive immunostaining with ER and PR except case 4 which was negative for ER. Interestingly this case showed also negative staining with all CKs.

Three HGESSs (cases 7, 9 and 10) were negative with ER and PR while two HGESSs (cases 8 and 11) were positive.

Cyclin D1 was positive in all HGESSs and negative in all LGESSs.

No relationship was found between the expression of CKs and ER, PR and Cyclin D1.

The results of immunohistochemical findings are shown in Table 4.

Discussion

In this study, we showed that, among the broad spectrum of CKs, the most diagnostically useful for both LG and HG ESS were AE1/3, MNF116, CAM 5.2 and CK8/18. There was no a significant difference between LG and HG ESS in terms of expression of CKs subtypes.

Immunohistochemically, CD10 is routinely used for diagnosis of endometrial stromal tumours, but it is well known that this antibody is not specific for the diagnosis of ESS (8, 9). Furthermore, some ESS may also show no immunoreactivity with CD10 (10).

Although the histological diagnosis of the classic type of LGESS is usually simple and straightforward, problematic cases also exist. Some ESS may show epithelioid morphology (11) with the presence of glands, pseudo-papillae (12) and cord-like structures (13).

LGESS may present different genetic rearrangements such as JAZF1-SUZ12, JAZF1-PHF1, EPC1-PHF1, MEAF6-PHF1, ZC3H7B-BCOR and MBTD1-CXorf67 (14-16), but a sub-group of LGESS, with classic morphology and immunoprofile, shows no known and definite genetic aberration (17). In contrast to LGESS, the diagnosis of HGESS is more challenging. Some HGESS can show intratumoural morphological variability with areas of LGESS which, contrary to HGESS, can show

positive immunostaining for ER and negative staining for Cyclin D1. None of the cases of HGESS reported herein showed convincing evidence of the presence of LGESS.

The relatively recent recognition of a subgroup of ESS with YWHAE-NUTM2 gene rearrangement and complex morphology supports the existence of a category of ESS which is morphologically HG and seems to have an intermediate prognosis between LGESS and UUS (18). It is worth mentioning that none of the HGESS in this study showed the morphology of YWHAE-NUTM2 positive HGESS such as the presence of round cells arranged in nests with a delicate stromal capillary network and cytologically bland and mitotically active spindle cell component with a fibrous/fibromyxoid stroma.

In surgical resection specimens, when a sufficient amount of tissue is available for histological analysis, the differential diagnosis between ESS and leiomyoma (cellular variant), leiomyosarcoma, adenocarcinoma with spindle cell morphology, and endometrioid adenocarcinoma arising in atypical polypoid adenomyoma should usually not be problematic. One should bear in mind that some morphological features of spindle cell endometrioid adenocarcinoma such as corded and hyalinised areas can also be present in ESS. In the above mentioned malignancies, for the differential diagnosis with ESS, immunohistochemical analysis of cytokeratins would not be as useful as morphological characteristics and possible molecular investigation findings. The classical vascular architecture and the pattern of invasion of ESS are important clues for making correct diagnosis.

In bioptic tissue when the classical morphology of ESS such as typical vascular architecture and the pattern of invasion is not present, diagnosis could be difficult.

The diagnosis of metastatic or recurrent ESS is even more complex, because of the occurrence of different morphology from an original tumour (19).

In addition, due to its non-specificity, positive immunostaining for CD10 is not entirely reliable. In these cases the immunohistochemical panel of cytokeratins AE1/3, MNF116, CAM5.2, CK8-18 could be useful for rendering correct diagnosis of ESS.

The molecular investigation is an expensive and time-consuming method, however, when suitable, should be performed. The data from the literature show that not all cases of ESS (LG and HG) harbour genetic fusion.

Our findings partly confirm the previous results by Adegboyega et al (6). However, none of our cases expressed CK19. This may be due to the different clone of an antibody or technical process used in the study of Adegboyega et al.

ESS is a very rare neoplasm, and although, theoretically, eleven cases could represent a reasonable number, the findings reported herein are still insufficient for definite conclusions and other studies with a large number of cases are warranted to confirm our data.

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Figures legend

Fig. 1a LG ESS, case 3

Fig. 1b HG ESS, case 11

Fig. 2a Expression of AE1/3 in LG ESS (case 6)

Fig. 2b Expression of MNF116 in LG ESS (case 5)

Fig. 2c Expression of CK8-18 in LG ESS (case 5)

Fig. 3 Expression of CAM 5.2 in HG ESS (case 11)