Title	Mixed low grade and high grade endometrial stromal sarcoma of uterus: Differences on immunohistochemistry and chromosome in situ hybridisation
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in 10 risk of intestinal lymphoma in a newly diagnosed patient with coeliac disease.[10] Exactly how and why coeliac disease is associated with an increased risk of lymphomatous and carcinomatous change remains unknown. Several explanations have been put forward. The mucosal damage in coeliac disease may make the small intestine more permeable to environmental carcinogens which, with associated malabsorptive vitamin A deficiency and increased vulnerability to oxidative injury, would enhance carcinogenic change. Known predisposing factors for the development of small intestinal adenocarcinoma include Crohn's disease, adenomatous polyps and Peutz-Jeugher's syndrome.[4] Perzin *et al*[11] reported that 23 of 51 cases of small intestinal adenocarcinoma had evidence of adenoma and carcinoma in the same lesion. Survival is dependent on the presence or absence of nodal involvement at presentation. Five year survival with node negative disease is 68%, while the overall figure varies from 15 to 20%.[12] Thus, as the prognosis is not uniformly poor and some patients are potentially curable by total resection (as in patient 2 in the present paper), limiting postoperative morbidity and recognising potential complications becomes very important. Clinically unmasked coeliac disease complicating Whipple's resection has not been described before and these two cases illustrate the importance of early diagnosis and dietary intervention prior to surgery. Previous cases of failure to thrive and weight loss following Whipple's resection may be simply put down to the effects of small bowel resection, pancreatic insufficiency, tumour progression, or early recurrence. Underlying coeliac disease should also be considered and the tumour-free mucosal margins carefully inspected.

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Mixed low grade and high grade endometrial stromal sarcoma of uterus: differences on immunohistochemistry and chromosome in situ hybridisation

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Abstract

A case of a 64 year old woman with a tumour of the uterus is reported. The patient presented with postmenopausal bleeding and subsequently underwent total hysterectomy and bilateral salpingo-oophorectomy. Sections of the tumour showed a low grade endometrial stromal sarcoma coexisting with areas consistent with high grade sarcoma. The sarcoma cells, in both the low and high grade areas, were positive for vimentin and negative for desmin and cytokeratin on immunohistochemistry. While the sarcoma cells in the low grade region showed immunoreactivity for oestrogen and progestogen receptors, those in the high grade region did not. Using chromosome in situ hybridisation, the low grade portion of the sarcoma was diploid for chromosomes X, 11, 12, and 17, whereas the more anaplastic areas were aneuploid for these chromosomes. This case may represent an example of high grade endometrial stromal sarcoma arising by dedifferentiation from a low grade stromal sarcoma. Adequate sampling is important in identifying such anaplastic changes as the origin of the tumour will affect patient management.

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Keywords: endometrial stromal sarcoma, dedifferentiation.

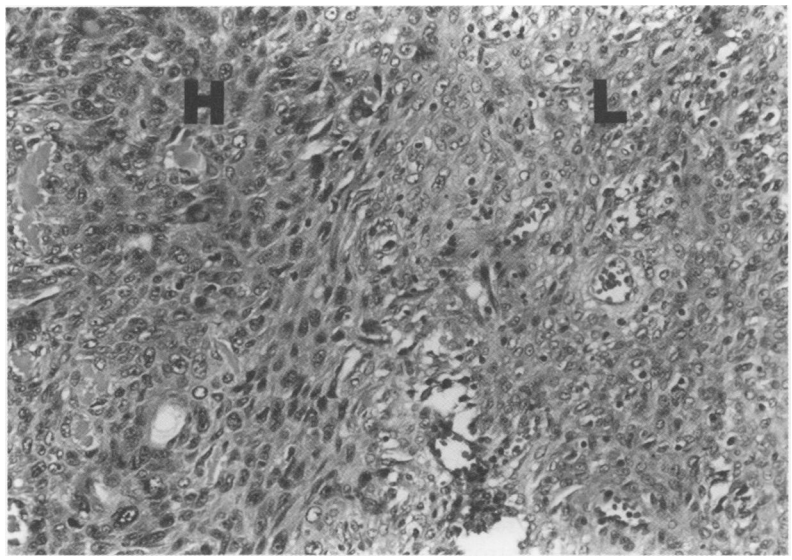

Figure 1 Photomicrograph showing the interface between the low grade (L) and high grade (H) endometrial stromal sarcoma.

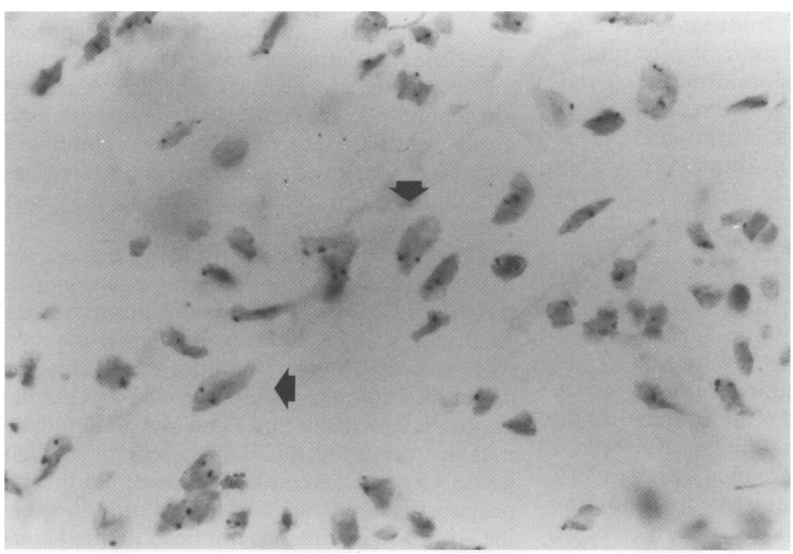

Figure 2 Two in situ hybridisation signals were detected in the low grade sarcoma area after hybridisation with a DNA probe specific for chromosome 12 (arrows).

Endometrial stromal neoplasms, including stromal nodules and low and high grade stromal sarcomas, have different architectural and cytological features and clinical behaviour.[1 2] They also vary with respect to their response to treatment. Here, we report a case of uterine high grade stromal sarcoma coexisting with, and probably arising from, adjacent low grade endometrial stromal sarcoma. Unlike soft tissue tumours,[3] tumour dedifferentiation has rarely been reported in uterine stromal sarcomas.[4 5] Comparative studies of steroid hormone receptor expression and cytogenetic analysis have not been described previously in such cases.

Case report

A 57 year old woman, with a long history of primary infertility, presented with postmenopausal bleeding of two months' duration. The patient's uterus was enlarged on physical examination. An endometrial aspirate yielded insufficient material for diagnosis. A total hysterectomy and bilateral salpingo-oophorectomy were carried out subsequently.

The uterus weighed 240 g and its anterior wall was thickened by an ill defined tumour mass, 4 cm diameter, which completely obliterated the endometrial cavity. Sectioning revealed whitish tumour tissue with patchy necrosis. The patient's ovaries and fallopian tubes were unaffected.

Most sections of the tumour mass showed monotonous sheets of cells traversed by ramifying small vessels. The tumour cells had scanty cytoplasm and ovoid nuclei resembling those of endometrial stromal cells. The mitotic count in these areas was about three per 10 high power fields (hpf). Vascular permeation was present. Epithelioid differentiation in the form of fine trabecular cords was present focally. Therefore, the features of the major portion of the tumour were those of a low grade endometrial sarcoma. Tumour close to the endometrial surface showed foci with obvious anaplastic changes. The tumour cells in these more anaplastic foci were large and pleomorphic with large hyperchromatic nuclei and prominent nucleoli. The mitotic count was about 18/10 hpf. Heterologous elements could not be detected. The features in these foci were consistent with a high grade stromal sarcoma.

An endometrial stromal sarcoma with both low and high grade features was diagnosed (fig 1). The patient received postoperative pelvic irradiation. She is well 18 months after treatment.

IMMUNOHISTOCHEMISTRY

Standard immunohistochemical studies were performed on formalin fixed, paraffin wax embedded tissue sections using the Streptavidin biotin and diaminobenzidine peroxidase antiperoxidase technique (Dako, Glostrup, Denmark). Antibodies used were directed against desmin (Dako), vimentin (Dako), CAM 5.2 (Becton Dickinson, USA), Mak 6 cytokeratin (Triton, USA), AE1/3 (BioGenex, USA), oestrogen receptor (Dako), and progestogen receptor (Abbott Laboratories, UK). Paraffin wax sections were pretreated in a microwave oven (Bio-Rad H2500, USA) before being stained for oestrogen and progestogen receptors.

The tumour cells in both the low and high grade regions were positive for vimentin but negative for the cytokeratins and desmin, supporting the histological diagnosis of endometrial stromal sarcoma. The low grade portion of the sarcoma showed immunoreactivity for both oestrogen and progestogen receptors while the high grade sarcoma was negative for both hormone receptors.

CHROMOSOME IN SITU HYBRIDISATION

The chromosome in situ hybridisation method used in the present study was a modification of a protocol used for detecting chromosome copy numbers in routinely processed, paraffin wax tissue sections.[6 7] Briefly, the DNA probes specific for chromosomes 11 (D11Z1), 12 (D12Z3), 17 (D17Z1) (American Type Cul-

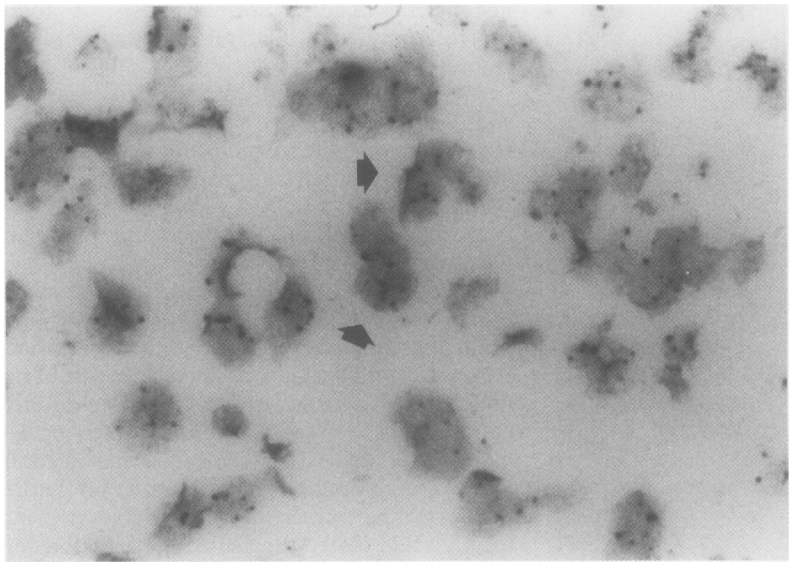

Figure 3 Multiple in situ hybridisation signals were observed in the high grade sarcoma after hybridisation with a DNA probe specific for chromosome 12 (arrows).

ture Collection, USA), and the X chromosome (pBamX5) (generous gift from Dr AHN Hopman) were labelled with biotin via nick translation (Boehringer, USA). Immunohistochemistry was carried out using avidin, biotinylated mouse anti-avidin, rabbit anti-mouse peroxidase, and 3,3'-diaminobenzidine (Dako, High Wycombe, UK) to visualise peroxidase activity. The slides were then counterstained with haematoxylin and mounted.

At least 500 nuclei were assessed in the low grade and high grade portions of the sarcoma. The number of chromosome specific in situ hybridisation signals was evaluated as described previously.[6 7] Uterine smooth muscle cells present in the tissue sections acted as the internal control for normal diploid XX cells.

In areas of low grade sarcoma, less than 15% of the cells had more than two copies of chromosomes X, 11, 12, and 17 (fig 2). In contrast, over 60% of cells in areas of high grade sarcoma contained more than two copies of these four chromosomes. Some cells contained up to eight or nine signals (fig 3).

Discussion

Dedifferentiation is a well recognised phenomenon in bone and soft tissue tumours, where a high grade tumour coexists with a relatively low grade and well differentiated malignant tumour of the same origin. It is regarded as a histological indicator of tumour progression when a low grade tumour transforms into a poorly differentiated and more malignant tumour.[3] However, dedifferentiation has rarely been described in uterine stromal sarcoma. Review of literature revealed that only two cases of coexisting low grade and high grade uterine stromal sarcomas have been reported.[4 5] Two cases of high grade stromal sarcoma have been reported, one arising from the sarcomatous overgrowth of a low grade mullerian adenosarcoma and the other following radiotherapy for low grade endometrial stromal sarcoma.[9] Details of oestrogen and progestogen receptor expression and the karyotype in these cases were not given. In the case reported here, the uterine tumour was a low grade stromal sarcoma, with transition to high grade stromal sarcoma at the peripheral portion of the tumour lining the endometrium.

Immunoreactivity for both oestrogen and progesterone receptors was noted in the part of the tumour with appearances histologically diagnostic of low grade stromal sarcoma. The high grade tumour was negative for both steroid hormone receptors. This is consistent with previous immunohistochemical studies by other investigators.[10] Such differential expression of hormone receptors may explain differences in response to hormone therapy. It has been reported that complete or partial resolution of recurrent or metastatic low grade stromal sarcoma can occur after treatment with progestogens.[1 2 4] These reports have led to the recommendation that women with low grade stromal sarcoma should be treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by long term progestogen therapy. Alternative treatment such as radiotherapy should only be considered for women whose neoplasms do not contain significant amounts of progestogen receptors or are resistant to progestogens. Progestogens are generally of little effect in patients with high grade stromal sarcoma. Surgery combined with preoperative or postoperative pelvic radiation is currently recommended as the most effective mode of treatment.

Using chromosome in situ hybridisation, we demonstrated that the low grade sarcoma in the present case was diploid for chromosomes X, 11, 12, and 17, whereas the high grade area was aneuploid. These findings are consistent with flow cytometry studies reported in the literature[11]: stromal nodules and low grade sarcomas are generally diploid whereas most high grade stromal sarcomas are aneuploid. The prognostic significance of aneuploidy in uterine stromal sarcoma has not been defined conclusively as yet.[2 11]

This case report also emphasises that adequate sampling is important in detecting anaplastic differentiation of an apparently low grade tumour, especially if the high grade tumour comprises a relatively small part of the tumour mass. Recognition of dedifferentiation significantly affects treatment and prognosis as the high grade portion of the stromal sarcoma is unlikely to respond to progestogen treatment.

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Iododeoxyuridine labelling of S-phase fraction in fine needle aspirates from breast carcinomas

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Abstract

The suitability of measuring the S-phase fraction in human breast cancer by labelling tumour cells from fine needle aspirates (FNAs) in vitro with iododeoxyuridine (IdU) was studied in 11 patients. The S-phase fraction was measured both in preoperative FNAs labelled in vitro with IdU, and in FNAs taken from the same tumour when surgically removed after intravenous administration of IdU. Frozen sections were also immunostained for incorporated IdU. The mean S-phase fraction measured in FNAs after in vitro or in vivo labelling and in sections after in vivo labelling was 4.0, 3.6, and 3.1, respectively. Results of in vitro and in vivo labelling of FNAs with IdU were similar. However, as the S-phase fraction in breast cancer is generally low, the variation between the different measurements is too large; therefore, the S-phase fraction is not a suitable indicator of response to treatment.

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Keywords: breast cancer, fine needle aspirates, S-phase fraction.

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Decreasing proliferative activity in tumours could be used as an early indication of response to systemic treatment. Changes in S-phase fraction as a measure of the proliferative activity could be monitored in sequentially obtained fine needle aspirates (FNAs). Immunostaining of iododeoxyuridine (IdU) incorporated into the DNA in the S-phase of the cell cycle offers a sensitive and specific method to assess S-phase fraction in FNAs from breast tumours. In vivo labelling of tumour cells requires the intravenous administration of IdU. Apart from the discomfort to the patient and costs involved, IdU administration may in turn cause further mutation. Moreover, the optimal timing of IdU administration varies from patient to patient. In vitro labelling with IdU is an attractive alternative.

Methods

IdU LABELLING

The S-phase fraction was measured in 17 patients with primary breast cancer. FNAs were taken from the primary breast carcinomas on the day before surgical removal of the tumour. Viable carcinoma cells were counted using the trypan blue exclusion test. The tumour cells were labelled in vitro by being incubated at 37°C for two hours in DMEM culture medium (Gibco BRL, Breda, The Netherlands) containing 10% fetal calf serum (Gibco BRL) and 10 μM IdU (Sigma, Axel, The Netherlands). Approximately six hours before surgery the patients received an intravenous infusion of 100 mg IdU in 50 ml. Directly after surgical removal of the tumour a second FNA was taken for the analysis of in vivo labelling. FNAs were washed in phosphate buffered saline (PBS), resuspended in 70% alcohol and stored at 4°C pending analysis. Tumour tissue was snap frozen in liquid nitrogen until further processing.

Permission to administer IdU was obtained from the Medical Ethical Committee of the Antoni van Leeuwenhoekhuis. Informed consent was obtained from each patient.

IMMUNOCYTOCHEMICAL STAINING FOR IdU

Cytospin preparations were prepared, dried and washed in PBS. After being washed in PBS, frozen tumour sections were treated in the same way as the cytospin preparations. All samples were incubated in 95% formamide in PBS at 70°C for 45 minutes, washed three times for five minutes in 0.1 M Tris/HCl (pH 7.6) supplemented with 5% Tween 20, followed by a 10 minute wash in Tris/HCl (pH 7.6). After preincubation for 15 minutes in PBS supplemented with 0.5% Tween 20, 0.1% bovine serum albumin (BSA) and 10% normal rabbit serum, the preparations were incubated at room temperature with anti-IdU murine