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ORIGINAL ARTICLE

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# Expression of androgen receptors in benign and malignant endometrial stromal neoplasms

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**Abstract** Several studies have shown that endometrial stromal neoplasms express estrogen and progesterone receptors (ER, PR). To our knowledge, the presence or absence of androgen receptors (AR) in these rare uterine neoplasms has not been investigated. Tumors ($n$=20)—3 endometrial stromal nodules, 14 low-grade endometrial stromal sarcomas (ESS, low grade), and 3 high-grade endometrial sarcomas (undifferentiated endometrial sarcoma, UES)—were studied. Immunohistochemical analyses for ER, PR, and AR were performed on formalin-fixed, paraffin-embedded archival material. Positive immunoreactions for ER and PR were observed in 14 (70%) and 17 (85%) cases, respectively. Furthermore, 9 cases (45%) were positive for AR. Among 17 ESS and UES cases, 7 (41%) revealed positivity for AR. Two of three benign stromal nodules were also positive for AR. Moreover, one of the three high-grade sarcomas (undifferentiated endometrial sarcoma) was negative for both ER and PR, but showed positive reaction for AR. In summary, ARs are expressed in 45% of endometrial stromal neoplasms. In addition to determination of ER and PR, the results of immunohistochemical examination of AR in these rare uterine tumors may have some impact on the postoperative management of the patients.

**Keywords** Endometrial stromal tumors · Androgen receptor · Estrogen receptor · Progesterone receptor · Immunohistochemistry

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## Introduction

Uterine endometrial stromal tumors are among the rarest neoplasms in the female genital tract. Malignant mesenchymal tumors comprise less than 5% of primary uterine cancers, with endometrial stromal neoplasms accounting for less than 10% thereof [1, 11]. Endometrial stromal tumors are composed of cells resembling those of proliferative phase endometrial stroma. These tumors are subdivided into benign and malignant categories based on the type of tumor margin. Uterine endometrial stromal tumors with pushing margins are benign and designated endometrial stromal nodules (ESN). In contrast, endometrial stromal sarcomas (ESS) infiltrate the myometrium; they have been traditionally divided into low and high grades mainly based on mitotic count [16]. However, since high-grade endometrial sarcomas largely lack specific differentiation and hardly display histological resemblance to endometrial stroma, it has been proposed that they should be designated undifferentiated endometrial or uterine sarcomas [5]. Thus, the recent World Health Organization Classification of Tumors of the Breast and Female Genital Organs divides the uterine stromal neoplasms into three groups: (i) benign ESN, (ii) low-grade ESS, and (iii) undifferentiated endometrial sarcoma [27].

While low-grade ESS is a clinically indolent malignant neoplasm, which shows minimal cytological atypia, infrequent mitotic figures, and numerous thin-walled small arteriolar type (plexiform) vessels, the undifferentiated endometrial sarcoma is a highly aggressive tumor that lacks a plexiform vasculature, features severe cytological atypia, and has frequent and often atypical mitotic figures [4, 27].

Several previous biochemical and immunohistochemical studies [13, 21, 22, 29] have shown that uterine stromal neoplasms, particularly low-grade ESS, often express estrogen and progesterone receptors (ER, PR). Studies have also revealed that therapy with progestational agents is an important adjunct to surgical treatment in cases of low-grade ESS [7]. Many of the tumors that

contain PR will, at least partially, respond to hormonal manipulation using progestins [10, 15]. In contrast to ER and PR, the status of androgen receptors (AR) in benign and malignant uterine stromal neoplasms (ESN, ESS) has not yet been investigated. In the present study, we analyzed the immunoexpressions of AR as well as ER and PR in a series of 20 primary endometrial stromal tumors.

## Materials and methods

Cases ($n$=20) comprising 3 benign ESN, 14 low-grade ESS, and 3 undifferentiated endometrial sarcomas were retrieved from the files of the Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology, Washington, DC, and the Department of Pathology, Medical University of Graz, Austria. Determination of tumor type and histopathological grade was performed according to the new World Health Organization classification on Tumors of the Breast and Female Genital Organs [27].

Formalin-fixed, paraffin-embedded tissue blocks were cut into 4-μm-thick serial sections, which were mounted on pre-coated slides. The sections were deparaffinized, rehydrated, and rinsed in distilled water. Immunohistochemical assays for AR, ER, and PR were performed on consecutive paraffin sections using standardized automated procedures (Ventana Medical System, Tucson, AZ and Dako, Glostrup, Denmark) (Table 1). Monoclonal mouse antihuman antibody clones 6F11 and 1A6 (Ventana Medical Systems) were used as primary antibodies for ER and PR, respectively. For determination of AR expression, the monoclonal mouse antihuman androgen receptor antibody (clone: AR441; Dako) was used. In brief, antigen retrieval was achieved with microwave treatment (ER, PR) or heating in a water bath (AR) (Table 1). A Ventana ES (Ventana Medical Systems) or Chem-Mate (Dako) autostainer was used in conjunction with an indirect streptavidin-biotin method. After incubation with the primary antibody, incubation with the secondary (link) biotinylated antibody was performed for 30 min. After washing, sections were incubated with streptavidin-peroxidase for 30 min. Finally, the enzyme was visualized with diaminobenzidine. Counterstaining was performed with hematoxylin. In each case, the intensity of immunoreaction (negative, 1+, 2+, 3+) and the percentage of tumor cells with positive nuclear reaction were evaluated. Samples were scored as positive when at least 10% of nuclei were immunoreactive. Internal positive controls included normal endomyometrial tissue surrounding the tumors (Fig. 1A). Negative controls included substitution of the primary antibody with normal sera or phosphate-buffered saline, omission of the secondary antibody, and incubation of the primary antibody solution with lymphoid tissue. All slides were evaluated independently by at least two investigators (F.M. and A.D.T.). The rare cases in which disagreement occurred were reevaluated using a multi-headed microscope; a final agreement was reached in all cases.

## Results

Results are summarized in Table 2. Positive immunoreactions for ER and PR in benign and malignant stromal tumors were observed in 14 (70%) and 17 (85%) cases, respectively. Androgen receptor positivity was found in 9

**Table 1** Summary of antibodies and methods used in the current study. *DAB* diaminobenzidine

| Antigen | Antibody (manufacturer) | Method (autostainer) | Dilution | Incubation time | Antigen retrieval |
|---|---|---|---|---|---|
| Androgen receptor | AR441 (Dako) | ChemMate-DAB (Dako ChemMate) | 1:100 | 20 min | Water bath 100°C for 40 min, then cooling in Epitope Retrieval solution (Dako) |
| Estrogen receptor | 6F11 (Ventana) | Biotin-DAB (Ventana ES) | Ready to use | 32 min | Microwave at 160 W in sodium-citrate, pH 6.0, for 30 min |
| Progesteron receptor | 1A6 (Ventana) | Biotin-DAB (Ventana ES) | Ready to use | 32 min | Microwave at 160 W in sodium-citrate, pH 6.0, for 30 min |

**Table 2** Expression of androgen receptors, estrogen receptors, and progesterone receptors in benign and malignant endometrial stromal tumors. Less than 10% of tumor cells positive was designated as negative in the results. *ESN* endometrial stromal nodule, *ESS, low* endometrial stroma sarcoma, low grade, *UES* undifferentiated endometrial sarcoma

| Case no. | Diagnosis | Androgen receptor | | Estrogen receptor | | Progesterone receptor | |
|---|---|---|---|---|---|---|---|
| | | Percentage | Intensity | Percentage | Intensity | Percentage | Intensity |
| 1 | ESN | <10% | 1+ | 70% | 2+ | 90% | 3+ |
| 2 | ESN | 40% | 1+ | 60% | 2+ | 80% | 2+ |
| 3 | ESN | 20% | 1+ | <10% | 1+ | 50% | 2+ |
| 4 | ESS, low | 90% | 3+ | 90% | 3+ | 70% | 2+ |
| 5 | ESS, low | 40% | 1+ | 20% | 1+ | 80% | 2+ |
| 6 | ESS, low | 0 | – | 50% | 1+ | 0 | – |
| 7 | ESS, low | 20% | 1+ | 60% | 2+ | 80% | 3+ |
| 8 | ESS, low | 20% | 2+ | 0 | – | 90% | 3+ |
| 9 | ESS, low | <10% | 1+ | <10% | 1+ | 70% | 2+ |
| 10 | ESS, low | 0 | – | 40% | 1+ | 90% | 3+ |
| 11 | ESS, low | 0 | – | 60% | 2+ | 20% | 1+ |
| 12 | ESS, low | 90% | 3+ | 60% | 2+ | 90% | 3+ |
| 13 | ESS, low | 0 | – | 30% | 1+ | 70% | 2+ |
| 14 | ESS, low | <10% | 1+ | 80% | 3+ | 20% | 1+ |
| 15 | ESS, low | 0 | – | 60% | 2+ | 70% | 3+ |
| 16 | ESS, low | 0 | – | 30% | 2+ | 30% | 2+ |
| 17 | ESS, low | 0 | – | 0 | – | 70% | 2+ |
| 18 | UES | 40% | 2+ | 0 | – | 0 | – |
| 19 | UES | 30% | 2+ | 50% | 2+ | 80% | 3+ |
| 20 | UES | 0 | – | 0 | – | 0 | – |

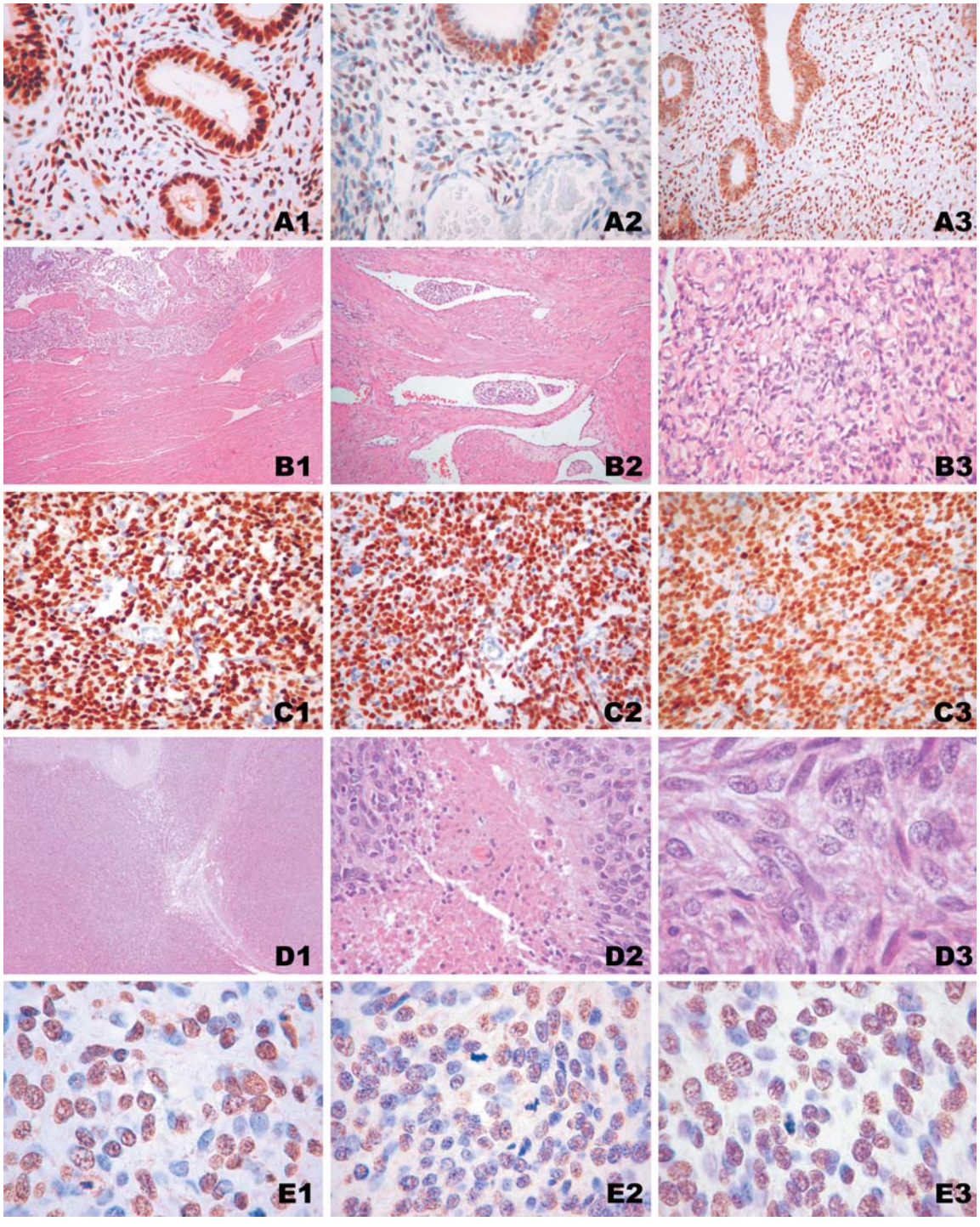


**Fig. 1** **A** Expressions of estrogen receptors (ER) (**A1**), progesterone receptors (PR) (**A2**), and androgen receptors (AR) (**A3**) in non-neoplastic, normal endometrium (proliferative phase). **B** A low-grade endometrial stromal sarcoma (ESS) with typical infiltrative margins (**B1**), multiple lymphatic invasions (**B2**), and numerous thin-walled, small blood vessels (**B3**). **C** Expressions of ER (**C1**), PR (**C2**), and AR (**C3**) in a low-grade ESS. **D**, **E** A high-grade endometrial sarcoma (undifferentiated endometrial sarcoma) showing infiltrative pattern (**D1**), tumor cell necrosis (**D2**), and significant nuclear atypia (**D3**). Although the malignant stromal cells were completely negative for ER and PR (data not shown), a positive immunoreaction (1+ to 2+) for AR could be identified in many areas of this high-grade (undifferentiated) sarcoma (**E1**, **E2**, **E3**)

(45%) cases. While all three ESN cases were PR positive, two of them were positive for ER and AR. In 17 sarcomas (14 ESS, low grade and 3 undifferentiated endometrial sarcoma), positive reactions for ER, PR, and AR were observed in 12 (75%), 14 (83%), and 7 (41%) cases, respectively. In low-grade ESS, positive immunoreactions for ER, PR, and AR were found in 11 (79%), 13 (93%), and 5 (36%) cases, respectively (Fig. 1B, C). Although

two of the three undifferentiated endometrial sarcomas were negative for both ER and PR, a positive reaction of highly atypical tumor cells for AR was observed in one of the ER and PR negative cases (Fig. 1D, E) (Table 2).

## Discussion

Previous studies demonstrated ER and PR in normal proliferative and secretory phase endometrium, both in endometrial glands and in stromal cells [19, 26]. A previous study also showed high ER and PR content in epithelium and stromal cells of simple and complex hyperplasia [2]. In atypical complex hyperplasia and endometrioid adenocarcinoma, however, the receptor content was significantly lower compared with that of normal proliferative or hyperplastic endometrium [2, 17, 19]. A few studies have demonstrated nuclear staining for AR in normal glands, endometrial stromal cells, and in endometrioid adenocarcinomas [3].

Several previous studies revealed expression of ER and PR in a high percentage of endometrial stromal sarcoma [13, 21, 22, 29]. However, to the best of our knowledge, the issue of AR expression in benign and malignant endometrial stromal neoplasms has not been examined previously.

According to our study, 45% of endometrial stromal neoplasms were AR positive. In sarcomas (low and high grades), a positive immunoreaction for AR was observed in 41% of examined cases. In all cases, the distribution of nuclear positivity for ER, PR, and AR among the tumor cells was quite heterogeneous (Table 2).

At least a partial response of low-grade ESS to hormonal therapy with progestins has been observed [8, 12, 18]. Recurrent or metastatic low-grade ESS have also been reported to be stabilized or suppressed with progestational agents in more than 50% of patients [5, 9, 20]. Tumors with a high level of progesterone receptors are most likely to respond to progestin therapy [20, 23, 28]. Treatment with gonadotropin-releasing hormone agonists has also been used in a few studies [14, 24]. However, although the vast majority of low-grade ESS have been shown to express ER and PR, some of the tumors, even with high ER and PR content, did not respond to adjuvant hormonal treatment [6, 25, 30]. This could be, at least in part, due to the heterogeneity of tumor cells in terms of expression of ER and PR and the presence or absence of other steroid receptors, such as AR. It is possible that the proportional distribution and concentration of each steroid receptor (ER, PR, and AR) among tumor cells influences the response to hormonal treatments. The biological significance of AR (stimulation versus inhibition of the growth of neoplastic endometrial stromal cells) and its interaction(s) with ER and PR in ESS requires further investigation.

In summary, our study shows for the first time that ARs are expressed in 45% of endometrial stromal neoplasms. Thus, in addition to ER and PR, the immunohistochemical examination of AR in endometrial stromal neoplasms, particularly in low-grade ESS, may have impact on the postoperative management of the patients.

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