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# Genomic characterization of endometrial stromal sarcomas with array comparative genomic hybridization

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ABSTRACT

*Introduction:* The endometrial stromal sarcoma (ESS) is a very rare uterine sarcoma, counting for 1–3% of all gynecologic malignancies. ESS represents 0.2–8% of all uterine malignant tumors and accounts for about 10% of all uterine sarcomas. With regard to chromosomal aberrations, very little is known about benign and malignant endometrial stromal tumors.
*Methods:* 30 tumors, consisting of 4 cases of benign endometrial stromal nodule (ESN), 22 cases of low-grade ESS and 4 cases of undifferentiated endometrial sarcoma (UES), were analyzed by array-comparative genomic hybridization (aCGH).
*Results:* ESN did not show many copy number changes (CNCs) by aCGH. Frequent losses could be identified on chromosomes 7p and 19, and gains on chromosomes 1q, 6p and 8q. Low-grade ESS presented as a very heterogeneous group. 90% (20/22) of cases displayed aberrations. Most frequent changes were losses on chromosomes 7 and 22, and gains on chromosome 1q or 11. UES showed a high number of chromosomal aberrations and on every chromosome CNCs were detected. Most frequent changes were losses on chromosomes 1q, 2q (3/4, 75%) and 13, and gains on chromosomes 1q and 17p.
*Conclusion:* Our data shows an increasing number of CNCs from ESN to low-grade ESS and to UES. However, the chromosomal aberrations differ considerably between the investigated ESN-, low-grade ESS- and UES cases and thus, a linear tumor progression seems to be unlikely.



## 1. Introduction

The endometrial stromal sarcoma (ESS) belongs to uterine sarcomas, which account for 1–3% of all gynecologic malignancies. ESS forms 0.2–8% of all uterine malignancies (Ashraf-Ganjoei et al., 2006a; Brooks et al., 2004; D'Angelo and Prat, 2010; Sharma et al., 2011) and about 10% of all uterine sarcomas. ESS constitutes approximately 3% of all malignant uterine corpus tumors (Ashraf-Ganjoei et al., 2006b; Fresia et al., 1992). Uterine sarcomas usually occur in women aged between 40 and 60 years (D'Angelo and Prat, 2010; Livi et al., 2003). According to the most recent World Health Organization (WHO, 2014) classification there are four categories of endometrial stromal tumors, corresponding to their mitotic activity, vascular invasion and prognosis: the benign endometrial stromal nodule (ESN), low-grade ESS, high-grade ESS and undifferentiated endometrial/uterine sarcoma (UES) (Ashraf-Ganjoei et al., 2006b; Kempson and Bari, 1970; Kurman et al., 2014; Puliyath and Nair, 2012). The prevalence of ESS is approximately 2 cases per 1,000,000 women, while the prevalence of endometrial cancer is 700 cases per 1,000,000 women (Amin et al., 2004). The annual incidence of ESS is 1–2 per million women (Puliyath and Nair, 2012). Women aged around 45–50 years are predominantly affected by ESS, yet, young women and girls can also be affected (Berek et al., 1999). Most women affected by ESS are pre-menopausal. ESS is an indolent tumor with a tendency to local recurrence, and distant metastases can occur even 20 years after the first diagnosis (Tavassoli and Devilee, 2003). The pathogenesis of ESS is not clear, but there is evidence that exposure to tamoxifen and unopposed estrogens, and polycystic disease of the ovary are associated with ESS (Ashraf-Ganjoei et al., 2006b; Cohen, 2004; Feeley et al., 2000; Sandberg, 2007).

The differentiation between ESN and ESS is mainly based on the character of the tumor margin and the presence or absence of vascular intrusion. Infiltrating margins and vascular intrusion only occur in low- or high-grade ESS and UES, while clearly circumscribed margins and the absence of vascular invasion are found in the ESN (Norris and Taylor, 1966; Tavassoli and Devilee, 2003).

Low-grade ESS is a tumor with less aggressive clinical behavior and a favorable prognosis (Sharma et al., 2011). However, late recurrence and distant metastases in low-grade ESS may occur (Bodner et al., 2001; Fekete and Vellios, 1984; Maluf et al., 2001; Schilder et al., 1999). In

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fact, the risk for recurrence in ESS is estimated to be around 50%, but the tumor is usually slow-growing and recurrence happens relatively late (Fekete and Vellios, 1984; Schilder et al., 1999). The stage of disease has been found to be the most important prognostic factor in uterine sarcomas (Sharma et al., 2011). A high mitotic count has been proven to be associated with a worse prognosis (Liokumovich et al., 1999).

Moreover, prolonged survival is common and even cure is possible when recurrent ESS or metastatic lesions are surgically removed (Berkowitz and Goldstein, 2005; Livi et al., 2003).

In previous studies, 27 ESS cases have been analyzed using classical cytogenetics. ESSs were found to be a highly heterogeneous group of tumors, according to these investigations (Amant et al., 2003; Dal Cin et al., 1992; Fletcher et al., 1991; Fresia et al., 1992; Fuzesi et al., 1995; Gil-Benso et al., 1999; Hrynchak et al., 1994; Iliszko et al., 1998; Koontz et al., 2001; Laxman et al., 1993; Micci et al., 2003; Pauwels et al., 1996; Sonobe et al., 1999; Sreekantaiah et al., 1991). In a study by our group in 2005, conventional CGH analysis of low-grade ESS, as well as UES cases has been performed (Halbwedl et al., 2005). Our previous data demonstrated a complex and very heterogeneous pattern of genomic alterations in the investigated tumor samples, suggesting a possible impact of chromosomes 6 and 7 on the development and progression of ESS (Halbwedl et al., 2005).

We have now performed an array-comparative genomic hybridization (aCGH)-analysis on an Agilent microarray platform to investigate not only the chromosomal aberrations in ESS and UES, but also the benign ESN phenotype. Moreover, the investigated sample cohort is larger in our current analysis, comprising 30 tumor samples. No samples from our previous cohort have been used. The main aim of our study was to assess, by means of copy number abnormalities, whether a linear tumor progression from ESN to low-grade ESS and to UES is likely or not. We also tried to outline specific aberrations which might play a key role in tumor development.

Notably, in our cohort none of the samples could be classified as high-grade ESS. Therefore we have analyzed three of the four tumor categories: ESN, low-grade ESS and UES.

## 2. Methods

Our intention was to evaluate copy number abnormalities in ESN, low-grade ESS and UES, respectively.

### 2.1. Tumor samples and DNA isolation

The tumor samples were formalin fixed, paraffin embedded archival tissue samples from the Institute of Pathology, Medical University of Graz. For DNA extraction, 10 μm sections were cut and tumor tissue was manually needle-microdissected. DNA extraction was performed with a FFPE DNA isolation kit (Qiagen, #56404) according to the manufacturer's instructions. The quality of the extracted DNA was evaluated using a multiplex PCR approach (Solcia et al., 1993). Samples with three or four bands on a 1.5% agarose gel were used for further aCGH analysis. In total 30 tumors, consisting of 4 cases ESN, 22 cases low-grade ESS and 4 cases UES, were analyzed by aCGH. No high-grade ESS samples were investigated in this study.

### 2.2. aCGH

aCGH detects copy number changes (CNCs) in a test DNA compared to a reference DNA on a high resolution. Here 44k oligonucleotide arrays from Agilent Technologies were used. Samples were labeled with the Bioprime Array CGH Genomic Labeling System (#18095-12, Invitrogen, Carlsberg, CA) according to the manufacturer's instructions. Briefly, 500 ng test DNA and female reference DNA (Promega, #G152A) were differentially labeled with dCTP-Cy5 or dCTP-Cy3 (#PA53021 and #PA55021, GE Healthcare, Piscataway, NJ). Samples were hybridized rotating for 48 h at 65 °C. Slides were scanned using a microarray scanner (#G2505B; Agilent Technologies, Santa Clara, CA). Data normalization and calculation of ratio values were conducted employing Feature Extraction software 10.5 from Agilent Technologies.

### 2.3. Data analysis

Data analysis was performed using the software Genomic Workbench 5.0.14 from Agilent Technologies with the following settings: statistical algorithm: ADM-2; aberration threshold: 7.0; moving average window: 2 MB; Fuzzy zero: off; and consecutive clone filter: 10. Log ratios higher/lower 0.27 were considered as CNC.

Further analysis concerning the location of genes in a certain region was done online by the help of the 'UCSC genome browser' (http://genome.ucsc.edu/cgi-bin/hgGateway?org=Human&db=hg18&hgsid=126007020), the query oriented data management system 'BioMart' (http://www.biomart.org/) using the BioMart Central Portal with Ensembl gene database, and the online tool 'Cancer Genes' (http://cbio.mskcc.org/CancerGenes/Select.action). Data conversion from hg18 to hg19 was done using the UCSC genome browser.

## 3. Results

### 3.1. Results in ESN samples

The benign phenotype ESN revealed only few copy number changes (CNCs) by aCGH. All four samples showed aberrations, but they did not have many gains or losses in common. Losses could be identified on chromosomes 7p (3/4, 75%), 18p (1/4, 25%) and 19 (2/4, 50%) and gains on chromosomes 1q (1/4, 25%), 6p (1/4, 25%), 8q (1/4, 25%) and 11p (1/4, 25%) (Fig. 4).

### 3.2. Results in low-grade ESS samples

Low-grade ESS presents as a very heterogeneous group. 90% (20/22) of cases displayed chromosomal aberrations, and two cases were balanced. Two of the aberrant cases had only very small CNCs, while the remaining 18 samples had various gains and losses involving almost every chromosome. Most frequent changes were losses on

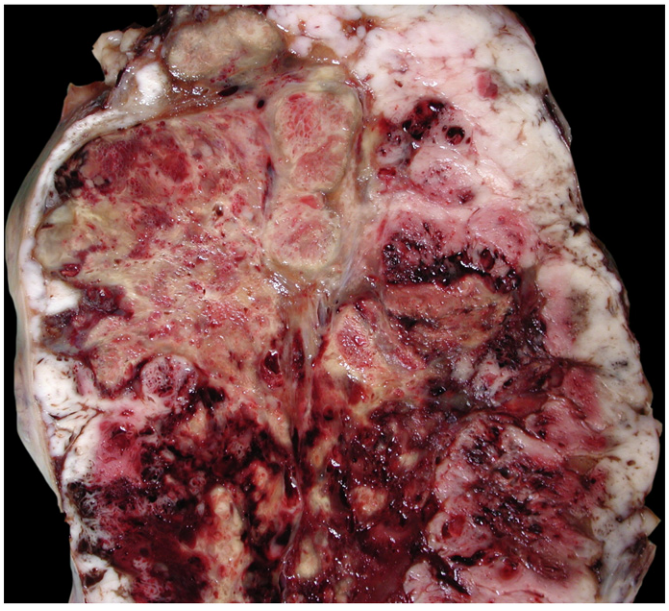

**Fig. 1.** Macroscopic image of UES. The cut surface shows a heterogeneous tumor with grayish, white and yellowish areas of necrotic tissue and with red and black-brown areas of hemorrhage.