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High mRNA levels of 17β-hydroxysteroid dehydrogenase type 1 correlate with poor prognosis in endometrial cancer☆

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ABSTRACT

Most endometrial cancers (ECs) are diagnosed at an early stage and have a good prognosis. However, 20–30% develop recurrence and have poor survival. Recurrence-risk prediction at diagnosis is hampered by the scarcity of prognostic markers.

Most ECs are estrogen related, and recent studies show that estrogen exposure in EC is controlled intracrinally. We aim at assessing any association between patient prognosis and the pathways controlling the intracrine estrogen generation in EC:

(a) the balance between 17β-hydroxysteroid-dehydrogenase-type 1 (HSD17B1), that generates active estrogens, and HSD17B2, converting active into poorly active compounds;

(b) the balance between steroid sulphatase (STS, that activates estrogens) and estrogen-sulphotransferase (SULT1E1, that deactivates estrogens);

(c) the levels of aromatase (ARO), that converts androgen into estrogens.

mRNA levels of HSD17B1, HSD17B2, STS, SULT1E1 and ARO were determined among 175 ECs using cDNA microarray. Proteins were explored by immunohistochemistry.

Patients with high mRNA of HSD17B1 had a poorer prognosis compared with those with low levels. Combining the expression of HSD17B1 and HSD17B2, patients with high tumour expression of HSD17B1 and low levels of HSD17B2 had the poorest prognosis. Contrarily, women that had high tumour levels of HSD17B2 and low of HSD17B1 had the best outcome. No differences were seen between mRNA level of other the genes analysed and prognosis. At the protein level, HSD17B2, STS and SULT1E1 were highly expressed, whereas HSD17B1 was low and ARO was almost absent.

In conclusion, HSD17B1 is a promising marker to predict EC prognosis. Immunohistochemical detection of this protein in ECs has low sensitivity and should be improved for future clinical applications.

1. Introduction

Endometrial cancer (EC) is the most common gynaecological cancer in the Western World. The majority of cases are diagnosed at an early stage, resulting in a good prognosis. Nevertheless, 20–30% of women diagnosed with early stage EC develop regional and/or distant recurrence for which there are limited therapeutic options and the 5 year survival rates are low (Morice et al., 2015). Recurrent disease could be prevented by the use of post-surgical adjuvant

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[1] ENITEC: European Network Individualized Treatment Endometrial Cancer (within the European Society of Gynaecological Oncology: http://www.esgo.org).

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treatments. However, this type of care is not yet standardised due to the lack of prognostic markers that would allow stratify patients with high risk of recurrence after primary surgical treatment (Kong et al., 2012).

Large clinical trials such as the MoMaTEC trial (Molecular Markers in Treatment in Endometrial Cancer, NCT00598845; www.clinicaltrials.gov) and large consortia like ENITEC (European Network Individualized Treatment Endometrial Cancer) have identified markers that have prognostic potential, i.e., p53, CA-125, steroid hormone receptors, DNA-ploidy, L1-CAM and Stathmin (Morice et al., 2015; Werner and Salvesen, 2014; Zeimet et al., 2013; and references therein). However, at present a phase 4 implementation trial (MoMaTEC2, NCT02543710; www.clinicaltrials.gov) is still exploring the utility of steroid hormone receptor status as stratification for lymphadenectomy among EC patients (Trovik et al., 2013) and no other marker is implemented in clinical practice.

Of the two types of EC classified today (type 1 and type 2), type 1 with endometrioid histology (EEC) is the most common and is associated with estrogen (over)exposure. Recent studies indicate that 17β-estradiol (the most potent form of estrogen) is produced at numerous extra-ovarian sites, including endometrial and fat tissue. Here, estrogens are generated locally (or intracrinally), from precursors in the serum (Labrie, 2015). Normal endometrial cells, as well as EEC cells, possess the machinery to create an intracellular estrogenic environment that is favourable for cell growth. This involves three main reactions: (a) the inter-conversion of estrone (poorly active) and the potent 17β-estradiol, which is catalysed by the enzymes 17β-hydroxysteroid-dehydrogenase-type 1 (HSD17B1, converts estrone to 17β-estradiol) and HSD17B2 (deactivates 17β-estradiol to estrone); (b) the inter-conversion of estrone and estrone-sulphate (inactive), which is catalysed by steroid sulphatase (STS) and estrogen-sulphotransferase (SULT1E1), respectively; (c) the conversion of androgens to estrogens, which is mediated by the enzyme CYP19A1 aromatase (ARO).

Several studies report the role of locally generated 17β-estradiol in the development of estrogen-dependent benign endometrial disorders and EEC. Although contrasting results have been reported and controversies remain, recent studies show that HSD17B1 is elevated in both endometriosis and EC (Cornel et al., 2012; Delvoux et al., 2014) and STS and ARO are elevated in EC (Jarzabek et al., 2013; Smuc et al., 2006; and references therein).

Besides the fact that estrogen overexposure is a risk factor for EC development, persistent exposure of cancerous cells to these steroids could worsen the prognosis of patients with EC. To test this hypothesis, we assessed the expression of enzymes that control the local generation of 17β-estradiol in a population of EC patients available within the ENITEC consortium. We examined whether high levels of the enzymes involved in the final generation of 17β-estradiol (HSD17B1, STS and ARO), and low levels of the enzymes that deactivate estrogens (HSD17B2 and SULT1E1), are associated with a poor prognosis in EC patients.

2. Materials and methods

2.1. Ethical statement

All procedures and analyses were conducted in accordance with ethical standards and national and international guidelines according to the Declaration of Helsinki and were approved by the local ethics authority (Krakstad et al., 2012).

2.2. Patient clinical specimens

In total, 175 tumour samples from patients diagnosed with EC (including all histological subtypes) in Hordaland County (Norway) between 2001 and 2009 were collected from hysterectomy specimens. All patients were treated with hysterectomy and bilateral salpingo-oophorectomy. Follow-up data spanning up to 9 years (mean, 7 years) was collected from patient records. The clinical-pathological characteristics of the patients are summarised in Table 1 and include age at diagnosis, histological type, grade, estrogen receptor alpha (ERα) and FIGO stage (according to 2009 criteria). This population was described earlier (Krakstad et al., 2012).

A second population based series, also collected in Hordaland County (Norway) between 2001 and 2009, consisted of 625 formalin-fixed paraffin-embedded tissues (FFPE; described in: Krakstad et al., 2012) mounted on tissue-micro-array (TMA) and was used for immunohistochemistry of HSD17B1. These samples included the 175 EC specimens used for RNA analyses. Additional EC FFPE samples were obtained from a tissue bank available at the Maastricht University Medical Centre and used for additional immunohistochemistry (local medical ethical committee tissue-bank protocol approval: METC-14-04-003).

2.3. mRNA analyses

RNA was extracted from fresh frozen tumour tissue and hybridised to Agilent Whole Human Genome Microarray Kit, 44K (catalogue number G4 112F), according to the manufacturer instructions (www.agilent.com) and as described previously (Krakstad et al., 2015; Krakstad et al., 2012; Wik et al., 2013). Arrays were scanned using the Agilent Microarray Scanner Bundle. Median spot intensity was used to define the intensity signal and expression data were quantile-normalised and log2-transformed. The software J-express (www.molmine.com; Dysvik and Jonassen, 2001) was used for microarray analysis. The expression levels of HSD17B1, HSD17B2, STS, SULT1E1 and ARO were determined based on the microarray probe signals corresponding to the mRNA entries indicated in Table 2.

Among the 14 types of hydroxysteroid dehydrogenases, types 5, 6, 7 and 12 are described as being able to use estrogens as a substrate (Huhtinen et al., 2012a; Huhtinen et al., 2012b; Prehn et al., 2009), at least using *in-vitro* systems or cell-free assay. Therefore, the levels of the genes encoding for these enzymes were analysed as well (AKR1C3, HSD17B6, HSD17B7 and HSD17B12; see Table 2).

Enzyme levels were categorised in quartiles, each consisting of

Table 1
Patient clinical characteristics.

	Number	%
Total study subjects	175	100.0
Histology		
Endometrioid - Grade I	49	28.0
Endometrioid - Grade II	53	30.3
Endometrioid - Grade III	39	22.3
Non-endometrioid	34	19.4
Age		
<66	84	48.0
≥66	91	52.0
FIGO stage[a]		
I-II	138	78.9
III-IV	37	21.1
Estrogen receptor alpha (ERα)[b] **(n = 168)**		
positive	126	75.0
negative	42	25.0
BMI (n = 174)		
≤25	60	34.5
25–30	59	33.9
≥30	55	31.6

[a] FIGO stage was determined according to 2009 criteria.
[b] Expression of the estrogen receptor was determined by immunohistochemistry in a previously published study (Krakstad et al., 2012).