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High mRNA levels of 17 β -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 17 β -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.

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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

[☆] We dedicate this study to Helga Brigitte Salvesen. She was a tremendously generous and insightful colleague and an inspiration for all of us.

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

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 17 β -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 17 β -estradiol, which is catalysed by the enzymes 17 β -hydroxysteroid-dehydrogenase-type 1 (HSD17B1, converts estrone to 17 β -estradiol) and HSD17B2 (deactivates 17 β -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 17 β -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 17 β -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 17 β -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 (ER α) 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).

Table 2
Gene IDs, Nucleotide entries and URLs.

Gene name	Gene ID ^a	Full name	Nucleotide entry ^b
HSD17B1 ^c	3292	17 β -hydroxysteroid dehydrogenase type 1	NM_000413
HSD17B2	3294	17 β -hydroxysteroid dehydrogenase type 2	NM_002153
AKR1C3	8644	17 β -hydroxysteroid dehydrogenase type 5	NM_003739
HSD17B6	8630	17 β -hydroxysteroid dehydrogenase type 6	NM_003725
HSD17B7	51,478	17 β -hydroxysteroid dehydrogenase type 7	NM_016371
HSD17B12	51,144	17 β -hydroxysteroid dehydrogenase type 12	NM_016142
ARO	1588	CYP19A1 aromatase	NM_031226
STS	412	steroid sulphatase	NM_000351
SULT1E1	6783	estrogen-sulphotransferase	NM_005420

List of genes whose expression levels were analysed. The microarray probes examined correspond to mRNA given in the nucleotide entry.

^a GENE IDs <http://www.ncbi.nlm.nih.gov/gene>.

^b Nucleotide entry: <http://www.ncbi.nlm.nih.gov/nucleotide>.

^c Two independent array probes that showed a significant direct correlation were used.

equal number of patients (see [Supplemental Fig. 1](#)). For statistical analyses, patients with high and patients with low levels of each enzyme were compared. To this end, for those enzymes generating intratumoural 17 β -estradiol (HSD17B1, STS and ARO), high mRNA levels consisted of all patients in the upper 4th quartile, whereas low levels consisted of patients in the 1st, 2nd and 3rd quartiles. For HSD17B2 and SULT1E1, an opposite classification was used: patients with low levels of expression consisted of all subjects in the 1st quartile only, whereas high levels were classified as all patients in the 2nd, 3rd and 4th quartiles.

2.4. Histology and immunohistochemical stainings

Haematoxylin & eosin staining was used for histologic evaluation of the tumour tissue and was examined by the local qualified pathologist (LFSK). Standard protocols ([Cornel et al., 2012](#); [Dassen et al., 2010](#)) and antibody manufacturer's instructions were used for immunohistochemistry. Antigens were retrieved with tris-EDTA buffer for HSD17B2 (1:100 Sigma-Aldrich, St. Louis, MO) and ARO (clone H4, 1:100; Serotec Ltd. Oxford UK). Heat-induced epitope retrieval in Citrate buffer (pH 6) and antibodies anti-SULT1E1 (1:100 Sigma-Aldrich, St. Louis, MO) or anti-STS (1:100 Sigma-Aldrich, St. Louis, MO) were used for STS and SULT1E1. For HSD17B1 detection, antigen was retrieved using 1 mg/ml proteinase k (Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands), tris-EDTA or citrate buffer as described in the text and supplemental materials. Any of these treatments was followed by incubation with rabbit monoclonal antibody EP1682-Y (1:100; Epitomics, California). The Chemate Envision and 3,3'-diaminobenzidine (Dako, Glostrup, Denmark) were used to visualize antibody binding.

For all stainings, the omission of the primary antibody and placental tissue were used as negative and positive controls, respectively.

2.5. Statistics

For statistical analysis, SPSS version 21 (IBM Chicago, IL, USA) was used. Associations between patients/tumour characteristics and enzyme mRNA level were explored using 2-sided Person Chi Square test. Survival analyses were conducted by the Kaplan-Meier method and differences were estimated by log-rank (Mantel-Cox) test for statistical significance.

3. Results

In total 175 EC tissues were used for mRNA analysis and clinical features are given in [Table 1](#). The majority (141 cases) were

endometrioid (49 Grade I, 53 Grade II and 39 Grade III) whereas 34 were non-endometrioid EC type. Estrogen receptor- α (ER α) was present in 75% of the tumours and most patients were diagnosed with low stage disease (FIGO I-II, 78.9%).

The distribution and spread of the expression levels of the various enzymes among patients were assessed by clustering the mRNA levels in quartiles. Each mRNA quartile included an equal number of patients (43–44). The mRNA levels of HSD17B1, HSD17B2 and STS showed large variability among patients and quartile clustering resulted in clear increases of the corresponding mRNA expression levels ([Supplemental Fig. 1](#)). Contrarily, SULT1E1 and ARO showed less variability among patients and the mRNA level distribution per quartile resulted relatively homogenous ([Supplemental Fig. 1](#)).

3.1. Enzyme mRNA levels and patient characteristics

[Tables 3 and 4](#) show the distribution of the patient characteristics for all enzymes according to their level of expression. Patients with high levels of HSD17B1 tended to be diagnosed with a higher FIGO stage (stages III and IV), whereas patients with high levels of HSD17B2 had more frequently an endometrioid histology, early stage but also high grade tumours ([Table 3](#)). EC specimens with high STS levels were more frequently of endometrioid histology and with high grade ([Table 4](#)). The levels of SULT1E1 ([Table 4](#)), ARO and the other enzymes tested did not show significant correlations with patient features (not shown).

Next, the enzyme levels were analysed in relation with patient survival. Patients with high levels of HSD17B1 had a significantly poorer prognosis than the remaining patients ([Fig. 1a](#)). Patients having low HSD17B2 levels had a statistically non-significant poorer prognosis compared with the remaining patients ([Fig. 1b](#)). Because HSD17B1 and HSD17B2 control the inter-conversion and balance between estrone and 17 β -estradiol, we next examined the combination of these enzymes. As shown in [Fig. 1c](#), patients with a tumour expressing high levels of HSD17B1 mRNA and low levels of HSD17B2 mRNA had the worst prognosis. By contrast, patients with low tumour levels of HSD17B1 mRNA and high levels of HSD17B2 mRNA had the most favourable prognosis.

The mRNAs for the other enzymes analysed (ARO, STS, SULT1E1, AKR1C3, HSD17B6, HSD17B7 and HSD17B12) was detectable in all specimens. However, there was no significant association between the expression levels and patient prognosis (data not shown).

3.2. Enzyme immunohistochemical analyses

The expression of HSD17B1, HSD17B2, STS, SULT1E1 and ARO was further explored by immunohistochemistry ([Fig. 2](#)). HSD17B2,

Table 3
Patient characteristics in relation to enzyme levels of HSD17B1 and HSD17B2.

	HSD17B1		p	HSD17B2		p
	Low ^a n (%)	High ^a n (%)		Low ^a n (%)	High ^a n (%)	
Histology (number of specimens analysed; n = 175)						
endometrioid	110 (78)	31 (22)	0.05	27 (19)	114 (81)	0.001
non-endometrioid	21 (62)	13 (38)		16 (47)	18 (53)	
Grade (number of specimens analysed; n = 173)						
I (low)	49 (68)	23 (32)	ns	26 (36)	46 (64)	0.001
II-III (med-high)	81 (80)	20 (20)		16 (16)	85 (84)	
Age (number of specimens analysed; n = 175)						
<66	69 (82)	15 (18)	0.03	17 (20)	67 (80)	ns
≥66	62 (68)	29 (32)		26 (29)	65 (71)	
FIGO stage (number of specimens analysed; n = 175)						
I-II	111 (80)	27 (20)	0.001	25 (18)	113 (82)	>0.001
III-IV	20 (54)	17 (46)		18 (49)	19 (51)	
ERα (number of specimens analysed; n = 168)						
positive	99 (79)	27 (21)	ns	27 (21)	99 (79)	ns
negative	29 (69)	13 (31)		14 (33)	28 (67)	

p = p-value.

ns = non significant.

^a As described in materials and methods, enzyme levels were categorised in quartiles. For HSD17B1, that generates intratumoural 17β-estradiol high mRNA levels consisted of the upper 4th quartile only, whereas low levels consisted of the 1st, 2nd and 3rd quartiles. For HSD17B2 low levels consisted of all subjects in the 1st quartile only, whereas high levels were classified as all patients in the 2nd, 3rd and 4th quartiles.

Table 4
Patient characteristics in relation to enzyme levels of STS and SULT1E1.

	STS		p	SULT1E1		p
	Low ^a n (%)	High ^a n (%)		Low ^a n (%)	High ^a n (%)	
Histology (n = 172)						
endometrioid	101 (72)	40 (28)	0.02	36 (26)	105 (74)	ns
non-endometrioid	28 (90)	3 (10)		7 (23)	24 (77)	
Grade (n = 171)						
I (low)	58 (83)	12 (17)	0.03	14 (20)	56 (80)	ns
II-III (med-high)	70 (69)	31 (31)		29 (29)	72 (71)	
Age (n = 172)						
<66	59 (72)	23 (28)	ns	21 (26)	61 (74)	ns
≥66	70 (78)	20 (22)		22 (24)	68 (76)	
FIGO stage (n = 172 for STS; n = 175 for SULT1E1)						
I-II	104 (75)	34 (25)	ns	37 (27)	101 (73)	ns
III-IV	25 (74)	9 (26)		18 (49)	19 (51)	
ERα (n = 166)						
positive	92 (73)	34 (27)	ns	36 (29)	90 (71)	ns
negative	33 (82)	7 (18)		5 (12)	35 (88)	

p = p-value.

ns = non significant.

^a As described in materials and methods, enzyme levels were categorised in quartiles. For STS, that generates intratumoural 17β-estradiol high mRNA levels consisted of the upper 4th quartile only, whereas low levels consisted of the 1st, 2nd and 3rd quartiles. For SULT1E1 low levels consisted of all subjects in the 1st quartile only, whereas high levels were classified as all patients in the 2nd, 3rd and 4th quartiles.

STS and SULT1E1 showed strong staining intensities, whereas HSD17B1 and ARO stained very weakly.

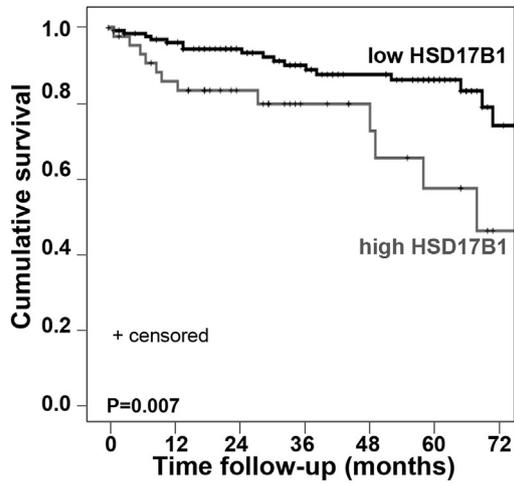
HSD17B1 and HSD17B2 exhibited cytoplasmic localisation, with predominant expression in glandular endometrial epithelium. STS and SULT1E1 were expressed at high levels with stronger staining in the glandular epithelium compared with the stroma. STS was mostly membrane associated, with also cytoplasmic staining, whereas SULT1E1 was cytoplasmic. ARO was barely detectable and only few samples showed some immunoreaction in the stroma cytoplasm.

For clinical practice, it is desirable to have prognostic markers that perform on FFPE tissue. Therefore, a large EC series with prognostic information consisting of FFPE material mounted on TMAs (625 samples, described in materials and methods as second population based series) was stained for HSD17B1 protein. However, quantification of protein expression resulted unreliable, probably due to the very low expression of HSD17B1 in tumours (see discussion). In an attempt to increase the limit of immune detection, different methods to retrieve the HSD17B1 epitope were

tested, either based on mild approaches (i.e. Tris EDTA or citrate) or based on a strong epitope retrieval protocol using proteinase k (Supplemental Fig. 2). In the first case, although a weak correlation between protein and mRNA levels of HSD17B1 could be seen (Supplemental Fig. 3), the majority of the samples were negative or very low at immunohistochemistry. Using the second method, the ratio between specific signal and background noise became unfavourable and it was difficult to distinguish between protein expression and unspecific staining (Supplemental Fig. 2). Although this immunostaining represents a negative result, it may still underlie an intrinsic biological characteristic of endometrial tissue, and not a simple technical failure, hence we consider it worth to report (see discussion).

4. Discussion

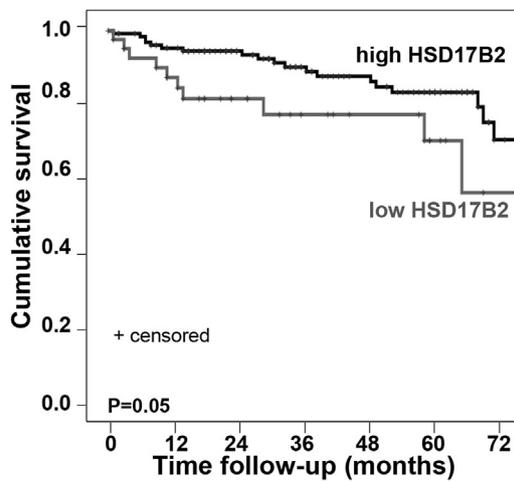
In this study, 175 EC samples were analysed to assess any association between the mRNA levels of the major enzymes controlling the local (intracrine) estrogen metabolism and patient



A

HSD17B1 mRNA level & patient prognosis

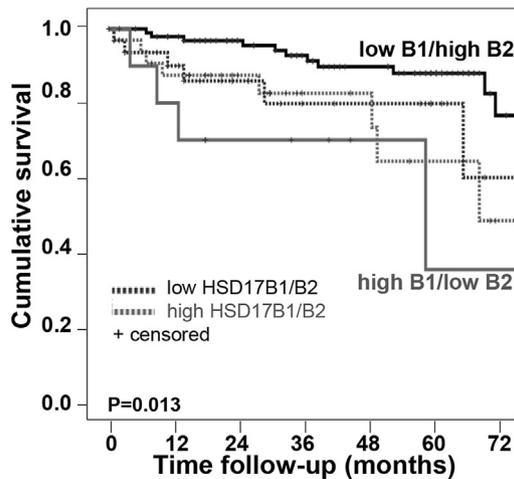
Quartiles	* No. (174)	Events (%)	expression level class	Events (%)	p-value
1 st	43	7 (16.3)	Low	19 (14.5)	0.007
2 nd	44	6 (13.3)			
3 rd	44	6 (13.3)			
4 th	43	12 (27.9)	High	12 (27.9)	



B

HSD17B2 mRNA level & patient prognosis

Quartiles	* No. (174)	Events (%)	expression level class	Events (%)	p-value
1 st	43	10 (23.3)	Low	10 (23.3)	0.05
2 nd	44	5 (11.4)	High	21 (16.0)	
3 rd	44	12 (27.3)			
4 th	43	4 (9.3)			



C

Combined HSD17B1 and HSD17B2 levels & patient prognosis

HSD17B levels type 1 - type 2	predicted estrogen generation	* No. (174)	Events (%)	p-value
low - high	low	99	13 (13.1)	0.013
high - high	medium	33	8 (24.3)	
low - low	medium	32	6 (18.8)	
high - low	high	10	4 (40.0)	

Figure 1. mRNA expression of HSD17B1, HSD17B2 and patient prognosis. Kaplan-Meier curves (left of each panel) for disease specific survival and expression levels of HSD17B1 (A), HSD17B2 (B) and HSD17B1 and HSD17B2 combined (C). Total number of patients in each group, number of events and p-value for statistical significance are shown at the right of each panel. For statistical analysis, survival analyses were conducted by the Kaplan-Meier method and differences were estimated by log-rank test (Mantel-Cox, SPSS version 21; IBM Chicago, IL, USA). The analyses shown in figure were performed on the complete specimen collection, irrespective of FIGO stage. Results for HSD17B1 and HSD17B2 did not change significantly when only EEC (n = 141) or only grade I and II EEC (n = 102) were analysed. *one specimen was excluded due to low quality RNA for microarray.

clinical characteristics, including survival.

High mRNA levels of HSD17B1 in EC patients were associated with a diagnosis at an advanced stage, which is a marker of poor

prognosis. Less clear was the scenario for HSD17B2 and STS mRNA levels that were associated with both good (i.e., endometrioid histology) and poor prognostic characteristics (high grade lesions).

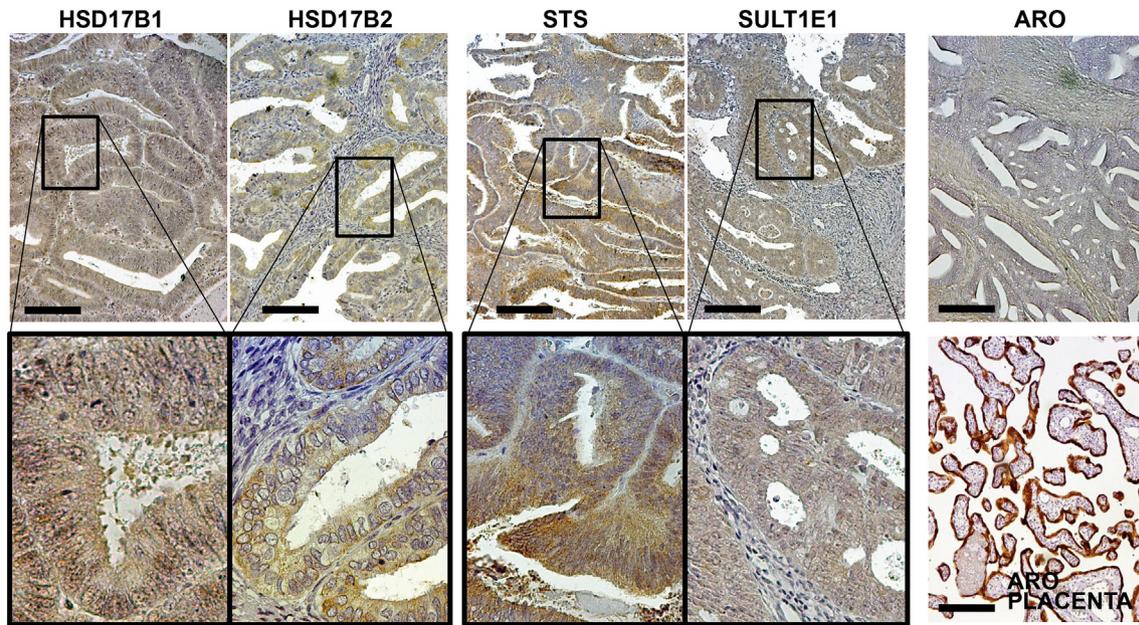


Figure 2. Protein expression of HSD17B1, HSD17B2, STS, SULT1E1 and ARO. Representative immunohistochemistry for HSD17B1, HSD17B2, SULT1E1, STS and ARO in EC (and placenta as positive for ARO). Bas scale: 100 μ m.

When survival analyses were performed, it was confirmed that patients with high mRNA levels of HSD17B1 had a significantly poorer prognosis than the remaining women. In this analysis, all EC samples, irrespective of the FIGO stage, were included. Therefore, it is relevant to determine whether HSD17B1 is a prognostic marker among patients diagnosed with early stage EC. Indeed, for this group of patients the prediction of the risk of recurrence is particularly difficult and there is need of additional prognostic markers. The low number of events among early stage ECs in our population did not allow answering this question.

Our RNA analyses showed that HSD17B1 has a potential utility for predicting the prognosis in EC. Ideally, for future implementation in clinical practice, these mRNA data should be confirmed by immunohistochemistry. However, although different antigen retrieval methods were tested and antigen-antibody association was visualised using amplification signal methods based on an enzyme-conjugated polymer backbone (Dako Envision System), staining intensity for HSD17B1 obtained in EC tissue resulted too low or unsuitable for accurate quantification. This lack of immune sensitivity was observed in spite of the fact that a clear enzyme activity is routinely measured in our laboratory by chromatography and is inhibited by inhibitors specific for HSD17B1 (Cornel et al., 2012; Delvoux et al., 2014; unpublished data). It is interesting to note that both HSD17B1 and ARO showed extremely low protein levels in EC (and other extra-ovarian tissues) whereas they are very strongly expressed in classical endocrine tissues like placenta and ovary (Supplemental Fig. 2 and <http://www.proteinatlas.org>). In contrast, HSD17B2, STS and SULT1E1 showed strong immunoreactivity in endometrial tissues. The difference between these enzymes consists in the fact that HSD17B1 and ARO are involved in the local but also in the ovarian steroidogenesis (Miller and Auchus, 2011), whereas HSD17B2, STS and SULT1E1 control almost exclusively the peripheral internalisation, activation and deactivation of steroids. It is expected that steroid synthesis in a central endocrine organ like the ovary needs to be very vigorous to maintain the estrogen levels systemically in the circulation, explaining the high protein levels in the ovaries. In contrast, very low enzyme levels are sufficient to maintain specific estrogen concentrations locally on in

the cell cytoplasm.

Our investigation is in line with previous studies that underscore the prominent role of HSD17B1 in controlling redox metabolism (HSD17B1/HSD17B2), and suggests that the contribution of other HSD17B types may be considered negligible (Cornel et al., 2012; Delvoux et al., 2014).

In addition, we observed that a combined analysis of HSD17B1 and HSD17B2 mRNA levels provided a more accurate distinction between patients with good versus poor prognosis. Since complex pathways control the final generation of 17β -estradiol and involve both enzyme levels and the availability of serum precursors (Labrie, 2015), we envisage that measuring the levels of all other enzymes (STS, SULT1E1, ARO, additional enzymes involved in estrogen clearance and excretion like glucuronosyltransferases, COMT and drug phase I/II metabolic cytochrome P450 oxidases, CYP1A1 and 1B1), together with the assessment of the availability of the corresponding substrates in serum, will allow accurate prediction of both estrogen production by cancer cells and patient prognosis. In this context, Audet-Walsh and colleagues showed that an increased level of estrone-sulphate (i.e., the substrate of STS), but not of the STS enzyme itself, correlated with EC relapse (Audet-Walsh et al., 2011).

The mechanism by which estrogens worsen the prognosis of patients is unclear but implies a role of estrogens that exceeds the classical mitogenic action. A number of recent investigations suggest that estrogens play a role in genotoxicity and epithelial-mesenchymal transition (EMT; Sun et al., 2014). In a recent study on advanced stage EC, STS mRNA was detected in circulating tumour cells that also co-expressed several EMT markers involved in tumour cell spreading and metastases (Alonso-Alconada et al., 2014).

In conclusion, HSD17B1 plays a central role in the local exposure of the endometrium to estrogens, which has physiological and pathological implications. Our data extend to EC results that recent studies show for breast cancer, where high levels of HSD17B1 are also associated with poor prognosis (Gunnarsson et al., 2008). The inability to detect this protein immunologically in tumour specimens is a limitation of the present study and improvements in its

detection for future clinical use are mandatory. Modelling the cellular exposure to estrogen by integrating data regarding the levels of all enzymes that control the activation and deactivation of 17 β -estradiol, in combination with data regarding the serum substrate availability, may be an important step toward a personalised prognosis for EC patients.

Declaration of interest

The authors have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.mce.2016.11.030>.

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