Journal of Steroid Biochemistry & Molecular Biology 150 (2015) 54–63

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Local estrogen metabolism in epithelial ovarian cancer suggests novel targets for therapy

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ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 9 March 2015
Accepted 22 March 2015
Available online 24 March 2015

Keywords:
Estrogen
Steroid sulfatase
Estrogen sulfotransferase
Epithelial ovarian cancer
Ovarian surface epithelium

ABSTRACT

Epithelial ovarian cancer (EOC) accounts for about 90% of malignant ovarian tumors, and estrogen is often implicated in disease progression. We therefore compared the potential for gating of estrogen action *via* pre-receptor metabolism in normal human ovarian surface epithelium (OSE), EOC and selected EOC cell lines (SKOV3 and PEO1). Steroid sulphatase (STS), estrogen sulfotransferase (EST), 17β-hydroxysteroid dehydrogenases 2 (17BHSD2) and 5 (17BHSD5) mRNAs, proteins and enzymatic activities were all detectable in primary cell cultures of OSE and EOC, whereas aromatase and 17BHSD1 expression was negligible. qRT-PCR assay on total mRNA revealed significantly higher EST mRNA expression in OSE compared to EOC ($P<0.05$). Radioenzymatic measurements confirmed reduced sulfoconjugation (neutralization) of free estrogen in EOC relative to OSE. OSE cells were more effective at converting free [^{3}H]-E_1 to [^{3}H]-E_1S or [^{3}H]-E_2S, while EOC cell lines mainly converted [^{3}H]-E_1 to [^{3}H]-E_2 with minimal formation of [^{3}H]-E_1S or [^{3}H]-E_2S. IL1α treatment suppressed EST ($P<0.01$) and 17BHSD2 ($P<0.001$) mRNA levels in OSE and stimulated STS mRNA levels ($P<0.001$) in cancer (SKOV3) cells. These results show that estrogen is differentially metabolized in OSE and EOC cells, with E_2 'activation' from conjugated estrogen predominating in EOC. Inflammatory cytokines may further augment the local production of E_2 by stimulating STS and suppressing EST. We conclude that local estrogen metabolism may be a target for EOC treatment.

1. Introduction

Estrogen is implicated in the progression of ovarian cancer, which is the most lethal of all gynecological malignancies. Epithelial ovarian cancer accounts for about 90% of malignant ovarian tumors [1]. Epidemiological data are suggestive that estrogen-only hormone replacement treatment (HRT) users have a higher risk of ovarian cancer [2,3]. In addition, anti-estrogen intervention inhibits the growth of ovarian carcinoma *in vitro* and *in vivo* [4,5]. Furthermore, clinical trials proved the aromatase inhibitor letrozole to benefit a sub-group of ovarian cancer patients [6,7].

Estrogen action in most cells is transduced by the nuclear estrogen receptor (ER) isoforms ERα and/or ERβ. Most ovarian cancers are ER positive [8]. ERα predominates in EOC, whereas ERβ expression is higher in normal ovarian surface epithelium (OSE) [9]. Thus EOC is likely estrogen-responsive. Paradoxically, ovarian cancer generally occurs in post-menopausal women when the ovary no longer actively secretes estrogen. This raises the question: if estrogen is involved, how is it produced?

Many tissues in the body that are incapable of *de novo* estrogen biosynthesis can still generate estrogen through the hydrolysis of sulfoconjugated steroids reaching them from blood. Free E_2, capable of activating ER signaling can be formed from circulating E_1S through the hydrolytic activity of STS and the 17-oxoreductase activity of 17BHSD5. Conversely, the oxidative function of 17BHSD2 produces the weak estrogen E_1 from E_2 and EST can sulfoconjugate E_1 to further minimize estrogen action. Intracellular steroid activation through the STS pathway is involved in estrogen-dependent epithelial cancers, such as breast and endometrial carcinomas [10], and single nucleotide polymorphisms in SULT1E1 lead to increased risk of breast [11] and endometrial [12] cancers, together with reduced survival. A study of Jewish women predisposed to breast and ovarian cancer found a link to a missense mutation (His224Gln) in the SULT1E1 gene [13]. Together, these observations suggest that if these mutations

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http://dx.doi.org/10.1016/j.jsbmb.2015.03.010

Table 1
Sequences of primer/probe sets for qRT-PCR. Sequences of assay-on-demand primers and probes were unavailable but were pre-validated by ABI. Probe number referred to the number of the probe in the Universal Probe Library and sequences were unavailable.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Probe (5′FAM-TAMRA/MGB 3′)	NCBI accession or reference number
Aromatase	Assay-on-demand	Assay-on-demand	Assay-on-demand	Hs00240671_m1
STS	Assay-on-demand	Assay-on-demand	Assay-on-demand	Hs00165853_m1
EST	AAGACTCATTTGCCACCTGAA	GCATTCCGGCAAAGATAGAT	Roche Probe library number: 4	NM_005420.2
HSD17B1	Assay-on-demand	Assay-on-demand	Assay-on-demand	Hs00166219_m1
HSD17B2	TGTCAGCAGCATGGGAGGA	GGTCACAGCCGCCTTTGAT	CCCCAATGGAAAGGCTGGCATCTT	NM_002153.2
HSD17B5	Assay-on-demand	Assay-on-demand	Assay-on-demand	Hs00366267_m1
ERα	TGATTGGTCTCGTCTGGCG	CATGCCCTCTACACATTTTCCC	TGCTCCTAACTTGCTCTTGGACAGGAACC	NM_000125.3
ERβ	GGTCCATCGCCAGTTATCACAT	GATGCGTAATCGCTGCAGACAG	TGTGAAGCAAGATCGCTAGAACACACCT	NM_001437.2

affected enzyme activity, they might be candidates for cancer promotion. Furthermore, the already substantial levels of E_1S that circulate in postmenopausal women are increased by hormone replacement therapy (HRT) [14].

We therefore hypothesize that E_2, is produced locally from circulating E_1S *via* the STS pathway in EOC cells. Additionally, since inflammatory cytokines such as IL1α secreted by OSE [15] are implicated in oncogenesis [16], they could have a role in activating estrogen formation within ovarian tumors. Here we demonstrate that EOC and normal OSE cells do indeed have distinct estrogen metabolizing signatures compatible with increased local generation of estrogen in ovarian cancer.

2. Materials and methods

2.1. Ovarian tissues

Non-pathalogical ovarian tissue was donated by pre-menopausal patients undergoing surgery for benign gynecological conditions (see Supplementary Tables 1 and 2 for

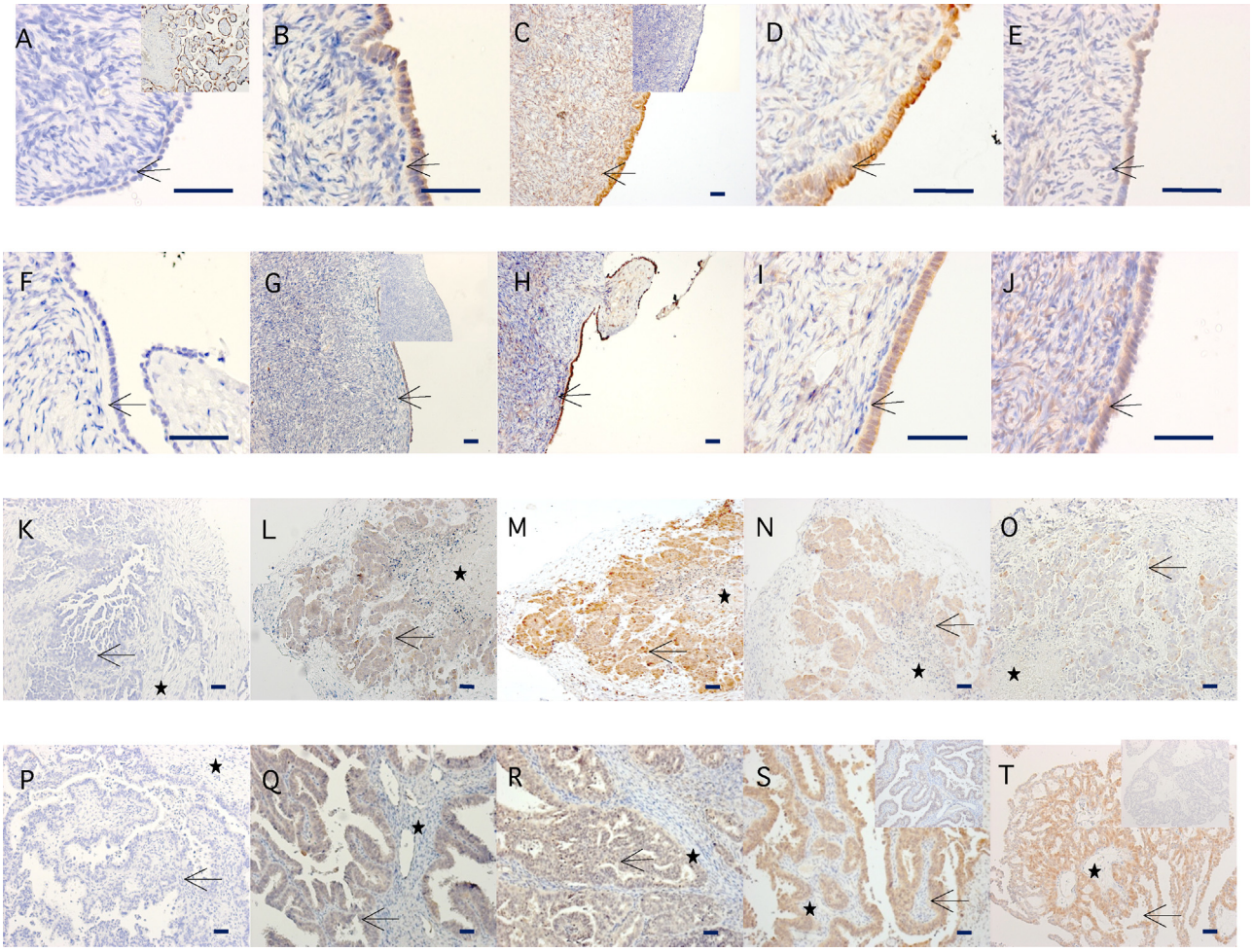

Fig. 1. Immunolocalization of Aromatase (A, F, K, P), STS (B, G, L, Q), EST (C, H, M, R), 17BHSD2 (D, I, N, S) and 17BHSD5 (E, J, O, T) proteins in pre-menopausal (A–E), post-menopausal (F–J) ovaries and epithelial ovarian cancer (K–T). Expression was localized using specific antibodies raised against Aromatase, STS, EST, 17BHSD2 and 17BHSD5, as described in Section 2. Examples shown are representative of 3 pre-menopausal, 6 post-menopausal and 7 EOC patient samples. The clinicopathalogical profiles of the samples are given in Supplementary Tables 1 and 3. (K–O) Aromatase, STS, EST, 17BHSD2 and 17BHSD5 in high grade serous carcinoma, (P) Aromatase in endometrioid carcinoma grade 3, (Q) STS in a mixed high grade serous and endometrioid carcinoma, (R) EST in endometrioid carcinoma grade 3, (S) 17BHSD2 in endometrioid carcinoma grade 3, (T) 17BHSD5 in mixed high grade serous and endometrioid carcinoma. Arrow indicates OSE cells (A–J) or epithelial-like cells (K–T). Asterisk marks stromal tissue. Inserts show positive control-placenta (A) or non-immune serum controls (C, G, S, T). Scale bars 40 μm.