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ESR1 methylation in primary tumors and paired circulating tumor DNA of patients with high-grade serous ovarian cancer

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HIGHLIGHTS

- *ESR1* methylation is detected in plasma ctDNA of high-grade serous ovarian cancer patients.
- The agreement between *ESR1* methylation in primary tumors and paired ctDNA is statistically significant.
- The presence of *ESR1* methylation indicates a better clinical outcome in high-grade serous ovarian cancer.

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ABSTRACT

Objective. Estrogen receptor, coded by the *ESR1* gene, is highly expressed in epithelial ovarian cancer. *ESR1* gene is frequently methylated in many types of gynecological malignancies. However, only a few studies attempted to investigate the role of *ESR1* methylation and its clinical significance in ovarian cancer so far. The aim of our study was to examine *ESR1* methylation status in primary tumors and corresponding circulating tumor DNA of patients with high-grade serous ovarian cancer (HGSC).

Methods. ESR1 methylation was detected by a highly specific and sensitive real-time methylation-specific PCR assay. Two groups of HGSC samples were analyzed: group A (n = 66 primary tumors) and group B (n = 53 primary tumors and 50 corresponding plasma samples).

Results. ESR1 was found methylated in both groups of primary tumors: in 32/66 (48.5%) of group A and in 15/53 (28.3%) of group B. 19/50 (38.0%) corresponding plasma samples of group B were also methylated for *ESR1*. A significant agreement for *ESR1* methylation was observed between primary tumors and paired plasma ctDNA samples (P = 0.004). Interestingly, the presence of *ESR1* methylation in primary tumor samples of group B was significantly correlated with a better overall survival (P = 0.027) and progression-free survival (P = 0.041).

Conclusions. We report for the first time the presence of *ESR1* methylation in plasma ctDNA of patients with HGSC. The agreement between *ESR1* methylation in primary tumors and paired ctDNA is statistically significant. Our results indicate a correlation between the presence of *ESR1* methylation and a better clinical outcome in HGSC patients.

1. Introduction

Ovarian cancer is one of the most frequent gynecological malignancies and the fifth cause of cancer-associated death in women [1]. The main type, epithelial ovarian cancer, is characterized by histological and molecular heterogeneity. High-grade serous ovarian cancer (HGSC) is the most common subtype, often diagnosed at an advanced stage [2]. The established treatment for the disease is a combination of debulking surgery followed by platinum and taxane-based chemotherapy [3]. Innovative targeted therapies have been additionally implemented into standard treatment of ovarian cancer, such as anti-angiogenetic therapy with Bevacizumab or PARP-inhibitors, which are FDA approved for platinum-sensitive recurrent ovarian cancer with a pathogenic mutation in the *BRCA1* or *BRCA2* gene [4,5]. Despite improved primary radical surgery response and despite the implementation of these therapies, >50% of patients suffer from recurrent disease,

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resulting in poor overall prognosis [6]. These facts suggest the need to develop different therapeutic options, either as a single agent or in combination with standard chemotherapy [7].

Epigenetic alterations hold an important role in cancer initiation and progression. DNA hypermethylation at specific sites of a gene, mainly at CpG islands, is a common event in human cancer and has a direct effect on gene expression [8,9]. Changes in DNA methylation patterns are frequently observed in epithelial ovarian cancer [10] and it is also argued that each histological subtype is characterized by different methylation motifs [11]. Aberrant methylation is also detected in the circulating tumor DNA (ctDNA) of patients with neoplasia [12]. Circulating tumor DNA is a tiny subgroup of cell-free DNA (cfDNA) that is shed in circulation by cancer cells and therefore carries all the genetic and epigenetic modifications identifying the primary tumors. ctDNA is a very promising liquid biopsy tool for the early diagnosis [13], prognosis and response to treatment, since it is considered an easily acceptable source of tumor DNA [14,15]. Our group has already reported *RASSFIA* methylation in ctDNA of ovarian cancer patients [16] and *CST6* [17], *SOX17* [18] and *BRMS1* [19] methylation in ctDNA of breast cancer patients, as well as *SOX17* methylation in NSCLC patients [20].

Estrogen receptors are members of a nuclear receptor superfamily of steroids receptors and act as ligand-inducible transcription factors. The two ER isoforms, namely ER-alpha (ERα) and ER-beta (ERβ) are encoded by *ESR1* and *ESR2* genes, respectively. They have slightly different functions and it is observed that the extended exposure to estrogen and the subsequent activation of ERα hold an important role in the development and progression of certain gynecological malignancies, such as breast and endometrial cancers [21,22]. ERα is the main subtype in the normal mammary gland and uterus; on the contrary, ERβ is the predominant subtype in the normal ovary. In serous ovarian cancer, the ERα subtype is highly expressed, while the ERβ expression is diminished and this decrease is significantly correlated with *ESR2* promoter methylation [21]. The expression of ERα is associated with endocrine sensitivity and estrogen's activity, and represents a target for endocrine therapy in selected ovarian cancer patients with recurrent, estrogen-sensitive tumors [23]. Phase II and phase III clinical trials of the anti-estrogen inhibitors tamoxifen and fulvestrant, as well as the aromatase inhibitors letrozole, anastrazole and exemestane, have been performed to evaluate the efficacy of endocrine therapy in ovarian cancer. Overall, endocrine agents show a moderate response rate of 10–15% in relapsed ovarian cancer. However, there are no phase III randomized controlled trials of endocrine therapy versus placebo in the relapsed setting nor for maintenance or adjuvant therapy. Its effect on progression free or overall survival is unknown [24].

In a very recent study, our group investigated *ESR1* methylation in primary tumors, circulating tumor cells (CTCs) and ctDNA of breast cancer patients [25]. A significant inverse correlation ($P < 0.001$) was observed between estrogen receptor (ER) expression and *ESR1* methylation status. Above all, the results of this study indicated that *ESR1* methylation in CTCs is strongly associated with lack of response to everolimus/exemestane treatment and has the potential to serve as a liquid biopsy-based biomarker for endocrine treatment efficacy [25].

The aim of the present study was to examine whether *ESR1* methylation is present in primary tumors and corresponding plasma samples of patients with HGSC and evaluate its prognostic significance. To detect *ESR1* methylation, we applied our recently developed highly specific and sensitive real-time MSP assay for *ESR1* methylation [25]. We also performed a direct comparison of the *ESR1* methylation status between primary tumors and matched ctDNA of HGSC patients. To the best of our knowledge, this is the first study on the examination of *ESR1* methylation status in ctDNA of ovarian cancer patients, and the first comparison study of *ESR1* methylation status in primary ovarian tumors and paired plasma ctDNA. Our results indicate that there is a concordance between primary tumors and paired ctDNA in respect to *ESR1* methylation status. Moreover, the presence of *ESR1* methylation in primary tumors is correlated with a potential benefit in the clinical outcome of HGSC patients.

2. Materials and methods

2.1. Clinical samples

Our study material consisted of two main groups of samples from patients with primary HGSC; a) Group A that consisted of 66 primary ovarian formalins fixed paraffin-embedded tissues (FFPEs) and b) group B that consisted of 53 primary tumor FFPEs and 50 available corresponding plasma samples (2 mL). Group A consists of mainly early stage tumor samples, whereas group B consists of mainly advanced stage tumor samples. For the plasma sampling, 2 × 5 mL ethylene-diamine-tetra-acetic acid (EDTA) blood samples were collected at time point of diagnosis, before tumor surgery and before the application of therapeutic substances with an S-Monovette (Sarstedt AG & Co.). Blood was centrifuged at 1500*g* for 10 min and the plasma supernatant was stored at −80 °C until further usage. The available clinicopathological features for both groups are shown in Table 1. In group B, all patients received at least six cycles of carboplatinum AUC 5 and paclitaxel 175 mg/m^2. Tumors were clinically defined as platinum-resistant if they recurred within six months after the completion of platinum-based chemotherapy. The tumor rest is not always indicated. In most cases, it is below 1 cm. In group B, all patients with distant metastasis (FIGO stage IV) were excluded from the methylation study, as per se have a poor prognosis. Two groups of normal samples were also recruited: a) a small group of 16 normal fallopian tube FFPEs that were obtained from women of the reproductive age group and b) a larger group of 51 plasma samples obtained from healthy women (2 mL). All group A and normal fallopian tube samples were obtained from the Pathology Department of IASO women's hospital, Athens, Greece. According to the protocol used in the pathology department, the whole fimbria is

Table 1
Clinicopathological characteristics of the patients.

Clinicopathological characteristics	Group A (total n = 66) n%	Group B (total n = 63) n%
Histology		
Serous	66 (100)	63 (100)
Tumor grade (G)		
G2	–	27 (42.9)
G3	66 (100)	36 (57.1)
FIGO stage		
I	13 (19.7)	4 (6.4)
II	37 (56.1)	3 (4.8)
III	14 (21.2)	48 (76.2)
IV	–	8 (12.7)
Unknown	2 (3.0)	–
Age	Median age = 55	Median age = 64
≥Median age	33 (50.0)	31(49.2)
<Median age	33 (50.0)	32 (50.8)
Regional lymph nodes (pN)		
N0		18 (28.6)
N1		29 (46.05)
NX		4 (6.3)
Not removed[a]		12 (19.05)
Tumor (pT)		
T1		6 (9.5)
T2		6 (9.5)
T3		51 (81.0)
Distant metastasis (M)		
M0		55 (87.3)
M1		8 (12.7)
Platinum resistance		
Positive		8 (12.7)
Negative		45 (71.4)
Unknown		10 (15.9)
Tumor rest		
Positive		24 (38.1)
Negative		38 (60.3)
Unknown		1 (1.6)

[a] Not removed because of residual tumor loads.

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