Estrogen receptor α and β expression in a case matched series of serous and endometrioid adenocarcinomas of the ovary

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Summary

Objective: The purpose of this study was to analyze estrogen receptor α and β (ERα, ERβ) expression in a stage and grade matched cohort of patients with serous and endometrioid adenocarcinoma of the ovary. *Methods:* Forty-two patients from 1991 to the present were found to have the diagnosis of endometrioid adenocarcinoma of the ovary and have tissue available for analysis. Of these 42, ten were selected for analysis. These were stage and grade matched with ten patients having serous adenocarcinoma of the ovary during the same time period. ERα and ERβ mRNA was detected by a multiplex RT-PCR and amplification of random hexamer generated cDNA using a housekeeping gene (G3PD) as a control for mRNA quality and quantity. Methylation specific PCR (MS-PCR) was used to correlate methylation of the ERα and ERβ CpG islands with mRNA expression status. *Results:* ERα expression was present in ten of ten endometrioid adenocarcinomas but in only five of ten serous carcinomas (χ^2, p = 0.01). ERβ expression was present in six of ten endometrioid adenocarcinomas and in four of ten serous caricinomas (χ^2, p = 0.65). Methylation of the ERα and ERβ CpG islands was found in tumors without mRNA expression but not in the tumors with mRNA expression (p = 0.005). *Conclusions:* ERα expression, but not ERβ expression, is significantly more common in endometrioid than serous adenocarcinomas of the ovary when controlled for stage and grade. The role of methylation in ER silencing may lead to potential therapeutic interventions.

Key words: Methylation; Estrogen receptor; Ovarian cancer; Serous; Endometrioid.

Introduction

In the United States, ovarian cancer is the most deadly of all gynecologic malignancies accounting for only 4% of newly diagnosed cancers annually, but 5% of deaths from cancer in women. In 2003 alone, an estimated 25,400 women were diagnosed with ovarian cancer and 14,300 would die from their disease [1]. More and more of the basic biology of these deadly cancers is beginning to be understood.

Histologically, multiple subtypes of epithelial ovarian cancer exist. Although it is somewhat controversial as to whether certain subtypes have different prognoses, it is believed that different histologies have different genetic alterations [2, 3, 4]. Buller demonstrated that clear cell carcinomas have a higher rate of BRCA2 dysfunction than is present in other ovarian cancers [4]. Others have shown that significant differences in p53 overexpression occur among serous, endometrioid, and clear cell carcinomas [5, 6]. In fact, some of these differences have led to the classification of clear cell carcinomas as "high-risk" [7].

The objective of this study was to look at the expression of both estrogen receptors (ERα and ERβ) in the tumors of women with endometrioid adenocarcinoma of the ovary and compare the results to a matched cohort of patients with serous carcinomas. A further aspect of the study was to try and determine if lack of expression was related to CpG promoter methylation.

Materials and Methods

This study was performed in accordance with the standards of our institutional committee for the Protection of Human Subjects. Patient selection was solely on the basis of the availability of snap frozen tissue for RT-PCR/cDNA amplification. Most of these tumors were 100% tumor with none being less than 90% tumor.

mRNA expression by RT-PCR

The techniques for RNA isolation and cDNA synthesis by RT-PCR were as previously described starting from snap frozen tumor samples stored at –140°C [8]. Failure to amplify a product in the cDNA reactions (despite appropriate amplification of a housekeeping gene sequence such as glycerol 3-phosphate dehydrogenase [G3PD]) provided candidate tumors where epigenetic phenomenon including promoter silencing may be operational. ERα and ERβ were amplified in a multiplex reaction [9]. A buffer with increased magnesium concentration (6.7 mM $MgCl_2$) [10] was used with 20 pMoles of the joint ERα/β forward primer and 10 pMoles of each reverse primer, 5 units, of Taq polymerase and 3 μl cDNA. G3PD expression was measured in a separate, concurrent reaction [11].

Methylation specific PCR

After EcoR1 restriction, methylation specific PCR (MS-PCR) was performed on $NaHSO_3$ converted DNA. The $NaHSO_3$ reaction has been previously described by Clark and others [10-14].

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Succintly, DNA (0.5-5 μg) was incubated first with 0.3 M NaOH at 37°C. The alkalinized mixture was exposed to 3.6 M $NaHSO_3$ and 1 mM hydroquinone at 55°C for 14 hours before recovering the products and desalting with Promega® Wizard Prep (Promega®, Madison, WI). Desalting was performed per manufacturer's recommendation except for the last elution in which 75°C deionized H_2O was incubated on the column at room temperature for 5 min before the final centrifuging. The solution was then incubated with 0.3 M NaOH at 37°C again before the addition of 3 M ammonium acetate and 95% ethanol. The mixture was next incubated at –20°C for 20 min and then centrifuged at 18.620 x g (4°C) for 30 min. The supernatant was removed, the DNA lyophilized, and finally re-suspended in 100 μl ddH_2O.

MS-PCR for ERα and ERβ was carried out on the converted DNA using the primers and conditions described previously [15, 16]. Both the ERαA and ERαβ regions were examined. The same buffer used for the multiplex PCR was used for all MS-PCR reactions [10]. CpGenome™ - Universal Methylated DNA (Intergen Company, Gaithersburg, MD) was used as the methylated control after $NaHSO_3$ conversion. Non-neoplastic ovarian epithelium and human placental tissue after $NaHSO_3$ conversion were used as unmethylated controls. A null control with all reagents except DNA template was also carried out.

Table 1. — *Clinical characteristics of matched patients*.

	Endometrioid	Serous	p value
FIGO Stage			
I	3	3	
II	2	2	NS
III	5	5	
IV	0	0	
Histologic grade			
1	1	1	
2	5	5	NS
3	4	4	

NS = not significant

Results

Forty-two patients with endometriod adenocarcinomas of the ovary were found to have banked frozen tissue. Ten of these samples were randomly chosen and then matched by stage and grade with the tumors from ten patients with ovarian serous carcinoma (Table 1). No peritoneal or fallopian tube carcinomas were used.

ERα expression was present in ten of ten endometrioid adenocarcinomas but in only five of ten serious carcinomas (χ^2, p = 0.01). Using MS-PCR, methylation of the A and B promoter regions was demonstrated in all samples not expressing ERα.

ERβ expression was present in six of ten endometrioid adenocarcinomas and in four of ten serous carcinomas (χ^2, p = 0.65). Methylation of the ERβ CpG island was found in tumors without mRNA expression but not in the tumors with mRNA expression (p = 0.005).

No correlation between stage, and ERα or ERβ expression could be demonstrated however the series was small. When looking at both histologies, loss of ERα or EPβ expression did correlate with methylation of the corresponding CpG island.

Discussion

Down-regulation of ER expression has been shown to be correlated with methylation in the promoter site [17, 18]. In ovarian cancer cell lines, methulation of the ERα promoter was only shown in tumors not expressing ER [19]. Methylation has been shown to be a common cause of gene down-regulation or lack of expression in ovarian malignancies [20-22].

Li and colleagues demonstrated that multiple alterations in steroid receptors are present in ovarian cancer cell lines [23]. The differences were more apparent in estrogen receptor subtypes than in progesterone receptor subtypes.

Although stage-matched endometrioid and serous carcinoma patients have equivalent five-year survivals, studies have shown different rates of gene expression and/or gene defects [2, 24]. Geisler *et al*. has shown that in optimally cytoreduced Stage IIIc serous ovarian carcinoma patients, decreased ER status was correlated with increased survival [25].

In this series, stage-matched controls demonstrated different expression of both ERα and β. ERα and β expression was much more common in endometrioid carcinomas than in serous carcinomas of the ovary. This correlates with what has been previously shown by immunohistochemical staining [25]. CpG promoter island methylation occurred in the promoter region in genes that were not expressed but not in the promoters of genes that were expressed.

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