The value of epithelial membrane antigen overexpression in hyperplastic and malignant endometrium and its relationship with steroid hormone receptor expression

G. Kir[1], H. Cetiner[1], A. Gurbuz[2], A. Karateke[2]

[1]Department of Pathology and [2]Obstetrics and Gynecology, Zeynep Kamil Maternity Hospital, Istanbul (Turkey)

Summary

The aim of this study was to evaluate the value of epithelial membrane antigen overexpression (EMA OE) in benign, hyperplastic and neoplastic endometrium and to analyze its association with estrogen and progesterone receptors (ER, PR) immunohistochemistry, tumor grade and myometrial invasion in patients with endometrial carcinoma (EC). The OE of EMA was analysed immunohistochemically in nine patients with benign endometrium (BE), in 18 patients with atypical complex endometrial hyperplasia (ACH) and in 29 patients with EC. EMA OE was present in 13 of 29 patients (44.8%) with EC, in two of 18 patients (11.1%) with ACH, and in none of nine patients with BE ($p < 0.05$). EMA OE of endometrial carcinoma was statistically correlated with the International Federation of Gynecology and Obstetrics (FIGO) grade (G1vs G2 and G3, $p < 0.05$) and depth of myometrial invasion (< 1/2 vs > 1/2, $p < 0.05$). EMA OE was significantly associated with PR negativity ($p < 0.001$). However it did not show any association with ER immunohistochemistry ($p = 0.14$). PR immunohistochemistry had significant correlations with FIGO grade ($p < 0.001$) and depth of myometrial invasion ($p < 0.05$) but ER loss showed a nearly significant association only with advanced FIGO grade ($p = 0.054$). In conclusion, EMA shows increased expression as the lesion progresses to malignancy and can also aid discrimination between hyperplastic and neoplastic states. The correlation of imunohistochemical findings with tumor grade and myometrial invasion could help in predicting behavior of the tumor and planning treatment in patients with endometrial carcinoma.

Key words: Endometrial cancer; Epithelial membrane antigen; Estrogen receptors; Progesterone receptors.

Introduction

Endometrial carcinoma (EC) is generally well known to be an endocrine-related neoplasm. Many studies have shown that the progesterone and estrogen receptor (PR and ER) content of EC is an important prognosticator for survival and a variety of clinicopathological parameters [1-14]. Epithelial membrane antigen (EMA) is a high-molecular weight transmembrane glycoprotein that is the antigen of an antiserum against human milk fat globule membranes [15]. Several human adenocarcinomas (breast, lung, colon, stomach, pancreas, ovary, kidney, prostate, endocrine glands and endometrium) exhibit positive immunostaining for EMA [16-18]. The prognostic value of EMA immunostaining was not provided in those studies. EMA expression has been shown to be useful in distinguishing between hyperplastic and neoplastic endometrium [15, 19, 20]. However there is only one study analysing the prognostic value of EMA in endometrial adenocarcinoma [15].

The aim of this study was to analyze the presence of EMA overexpression (OE) in neoplastic and benign endometrium and evaluating the its correlation with tumor grade, myometrial invasion and PR/ER immunohistochemistry.

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Materials and Methods

Endometrial paraffin-embedded specimens from 29 patients with EC (26 endometrioid, 3 adenosquamous), 18 patients with atypical complex hyperplasia (ACH) and nine patients with benign endometrium (BE) (3 proliferative, 3 secretory and 3 inactive) were analysed for EMA immunostaining. We also evaluated the steroid hormonal status of endometrial carcinomas.

Five-micron-thick representative tissue sections were immunohistochemically stained for EMA using the avidin-biotin complex peroxidase method and for ER/PR using a combination of the avidin-biotin complex peroxidase method with microwave antigen retrival (15min-0.001 N citrate buffer, pH 6). The tissue sections were incubated with monoclonal mouse antibodies (All Neo Markers-Fremont, CA; ready to use). The sections were then incubated with biotinylated goat anti-polyvalent and then with peroxidase conjugated streptavidin. They were stained with diaminobenzidine and counterstained with hematoxylin. Appropriate positive controls were performed at the the same time while tissue sections in which the primary antibody was omitted were used as negative controls.

EMA immunostaining was observed in the apical membrane cytoplasm and luminal content. Only the apical membrane stain was used. Staining cell numbers were scored as none, weak (+, < 33%), moderate (++, 33-66%), and intense (+++, > 66%). Overexpression (OE) was defined as intense staining [15].

The evaluation of ER and PR was performed according to the method described by Carcangiu *et al.* [21], based on the percentage of stained cells and the intensity of nuclear stain. The percentage of positive cells was graded as follows: 1) 0 to 25% of the nuclei stained; 2) 26 to 75% of the nuclei stained; 3) more than 75% of the nuclei stained. The staining intensity was

scored as follows: 1) absent or weak; 2) strong, and 3) very strong. The sum of both parameters provided the immunohistochemical score. Tumors were divided into three categories depending on the immunohistochemical score. Category I correspond to a score of 2, category II to a score of 3 or 4, and category III to a score of 5 or 6. Category I tumors were considered as immunonegative, whereas category II and III tumors were considered as immunopositive.

The relationships between the different variables were measured by Fisher's exact test and χ-square analysis, where appropriate. A p value of less than 0.05 was considered statistically significant. The analyses were performed using the Microsta statistical program for Windows.

Results

The average age of the patients with EC, ACH and BE was 54.2 ± 14.4 (range: 44-72), 46.7 ± 12.5 (range: 34-60), and 43.3 ± 9.8 (range 31-49), respectively. EMA immunostaining was confined to the endometrial glands. No stain was evidenced in the stroma or muscle. EMA OE was present in 13 of 29 patients (44.8%) with EC, in two of 18 patients (11.1%) with ACH, but none of nine patients with BE (Figure 1). A weak to moderate degree of EMA expression was observed in the glandular epithelium in secretory phases. The distribution was significantly different ($p < 0.05$) (Table 1).

Table 1. — *EMA OE in BE, ACH and EC.*

	EMA overexpression n (%)		p value
Benign endometrium	0/9	(0)	
Atypical complex hyperplasia	2/18	(11.1)	$p < 0.05$
Endometrial carcinoma	13/29	(44.8)	

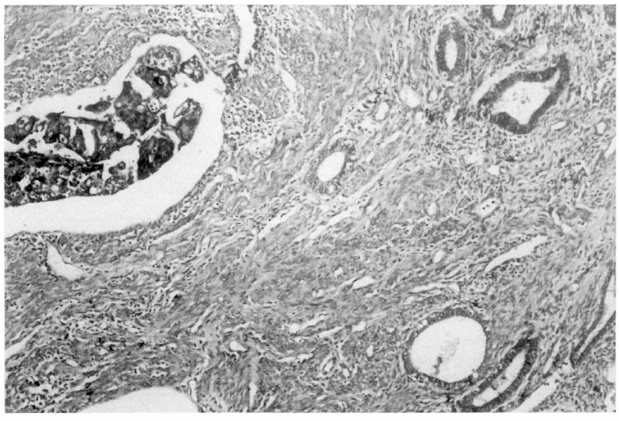

Figure 1. — EMA expression in neoplastic glands (left). No stain in normal glands (right) (hematoxylin-eosin x 100).

The association between ER/PR expression and tumor grade and myometrial invasion is shown in Table 2 and the relationship between EMA OE and tumor grade, and myometrial invasion and ER/PR immunohistochemistry is shown in Table 3.

Table 3. — *Relationship between EMA OE and some traditional prognostic factors.*

	EMA overexpression n (%)		p value
ER positive	1/5	(20.0)	0.14
ER negative	12/24	(50.0)	
PR positive	4/19	(21.0)	< 0.001
PR negative	9/10	(90.0)	
Grade 1	4/18	(22.2)	< 0.05
Grade 2, 3	9/11	(81.8)	
≤ 50% MI	5/18	(27.7)	< 0.05
> 50% MI	8/11	(72.7)	

EMA OE of endometrial carcinomas was statistically correlated with FIGO grade (G1vs G2 and G3, $p < 0.05$) and depth of myometrial invasion (< 1/2 vs > 1/2, $p < 0.05$). Furthermore EMA OE was significantly associated with PR negativity ($p < 0.001$). However it did not show any association with ER immunohistochemistry ($p = 0.14$).

PR immunohistochemistry had significant correlations with FIGO grade ($p < 0.001$) and depth of myometrial invasion ($p < 0.05$) but ER loss showed a nearly signifigant association only with advanced FIGO grade ($p = 0.054$).

Discussion

EC is a hormone-dependent tumor [22, 23] and therefore the detection of ER and PR in endometrial glandular tumor cells is not unexpected. The reported incidence of ER and PR in EC varies in several series from 35 to 70% [1, 6, 7, 24]. Our results are approximately at the lower end of this range for ER expression but at the highest level for PR expression.

In most series the lack of ER and PR expression by endometrial tumor cells has been a marker of aggressive tumor behavior and indeed many investigators connected the loss of hormone dependence with poorly differentiated endometrial tumors and advanced stage of disease [1, 4-8].

In this study decreased expression of PR was significantly associated with advanced tumor grade and deep myometrial invasion. However ER loss showed a nearly significant association only with advanced FIGO grade.

Table 2. — *Relationship between ER/PR positivity and some traditional prognostic factors.*

	ER positivity n (%)		p value	PR positivity n (%)		p value
Grade 1	5/18	(27.7)	0.054	16/18	(88.8)	$p < 0.001$
Grade 2, 3	0/11	(0.0)		3/11	(27.2)	
≤ 50% myometrial invasion	4/18	(22.2)	0.36	14/18	(77.7)	$p < 0.05$
> 50% myometrial invasion	1/11	(9.0)		4/11	(36.3)	

EMA is a high-molecular weight transmembrane glycoprotein. The practical value of EMA immunostaining resides in establishing the epithelial nature of neoplastic cells [25]. However, its biological role in tumors remains unknown. Previous studies have demonstrated that EMA expression is higher in malignant compared to normal or hyperplastic endometrium [19, 20]. Nokopoulou *et al.* [19] detected EMA expression in 26 % of adenomatous hyperplasia, 67% of atypical adenomatous hyperplasia and 95% of endometrial adenocarcinomas. In Coronado *et al.'s* study [15], EMA overexpression was signifigantly higher in malignant (60%) compared to hyperplastic (15%) or benign endometrium (9.1%). These findings are suggestive of EMA OE as a marker of proliferative endometrial leisons.

In the present study, EMA OE was detected more frequently in adenocarcinomas than in hyperplasias and no OE was detected in BEs. Well differentiated tumors showed less frequent EMA OE while EMA OE demonstrated a statistically signifigant increase as tumors became more poorly differentiated. A particularly important finding of the present study was the correlation of EMA OE with tumor grade, myometrial invasion and decreased PR expression. It did not show a correlation with ER immunohistochemistry. To our knowledge this is the first report correlating EMA OE with ER/PR status.

We suggest that EMA OE in EC is downregulated in a hormone-dependent manner, a function which it has apparently been endowed with from normal endometrium. Indeed EMA when expressed in normal endometrial tissue, appears to be under hormonal control, with the highest level occurring during the secretory phase of the menstrual cycle [26]; at this phase, the lowest ER and PR levels are presumed to occur [27].

In conclusion EMA OE has shown promising results in distinguishing between hyperplastic and neoplastic states and EMA OE seems to be of help for more precise tumor grading. It may be possible to consider the presence of intense EMA reactivity in hyperplastic endometrium as an additional risk factor for a malignant process.

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Address reprint requests to:
G. KIR, M.D.
Soyak Gokyuzu Konutlari
B-Blok D: 46
81150 Kosuyolu-Istanbul (Turkey)