

## Original Article

# Folate receptor $\alpha$ expression and significance in endometrioid endometrium carcinoma and endometrial hyperplasia

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**Abstract:** Endometrioid-type endometrial carcinoma (EEC) developing on the ground of endometrial hyperplasia (EH) is amongst the most commonly observed type of cancer in the world. Folate receptor  $\alpha$  (FR $\alpha$ ) is a vitamin molecule that has a role in cell proliferation. The fact that FR $\alpha$ , which is known to be needed extremely by the cells of malignancies that proliferate rapidly, is present in limited amounts in normal tissues while it is overexpressed in malignant cells of the same tissues makes folate a candidate for target molecular therapy. In our study, FR $\alpha$  expression in 214 cases, with 95 diagnosed within EEC and 119 with EH, was studied immunohistochemically. FR $\alpha$  expression in EEC was found significantly high compared to EH and normal endometrium ( $P<0.01$ ). Similarly, FR $\alpha$  expression in EH cases with complex atypia were significantly high compared to other hyperplasia subgroups ( $P<0.01$ ). The findings of our results make us think that FR $\alpha$  overexpression may play a role in the EEC carcinogenesis and carcinoma progression from EH. Furthermore, we suggest that it can be helpful in the treatment of EEC and/or transition from hyperplasia stage to EEC as a molecular therapy targeting receptors labeled with antibody-based prods containing FR $\alpha$ . Finally, we suggest that FR $\alpha$  may be used, based on the expression intensity, as a supplemental option to determine the patients that shall be directed to radical therapy amongst patients with complex atypical EH.

**Keywords:** Endometrial carcinoma, endometrial hyperplasia, folate receptor  $\alpha$ , target therapy

## Introduction

Endometrial carcinomas are the most common malignancy in the female genital system. The majority of these adenocarcinomas consist of endometrioid-type (type 1) endometrium carcinoma (EEC) that occurs as a result of unmet excess estrogen and develops on the ground of endometrial hyperplasia (EH) [1].

Having a role in cellular methylation in structures such as lipids, proteins, and DNA as well as being a basic cofactor in the synthesis of purine and pyrimidine, folate, also known as vitamin B9, is a molecule highly needed by rapidly proliferating cells [2, 3]. Folate receptor (FR), whose main task is to transfer folate vitamin through the cell membrane, also play a role

in cellular proliferation [3]. Folate receptor  $\alpha$  (FR $\alpha$ ) is the most important subunit of FR and the alpha isoform has been shown to be selectively overexpressed in cancer types like breast and ovarian cancer compared to normal breast and ovarian epithelial cells [4, 5]. It was determined that FR $\alpha$  exhibits a limited expression on the apical surfaces of the epithelial cells of normal lung, breast, thyroid, parathyroid, and kidney tissues [6-8]. For their uptake of folate, normal cells rely almost exclusively on the reduced folate carrier, whereas many carcinomas and myeloid leukemia cells overexpress a high-affinity FR on their surfaces, perhaps reflecting their increased need for folate to support rapid cell division [6, 9]. In recent years, studies were made reporting that FR $\alpha$  may play a role in cancer development and may be used in potential

anti-cancer therapies [10-17]. While importance and the details of the relationship between FR $\alpha$  overexpression and the deteriorated cellular proliferation have not been sufficiently cleared yet, high FR $\alpha$  expressions at various rates have been shown in previous studies in some carcinomas such as ovarian, non-small cell lung, breast, and colon [6, 7, 16-21]. Moreover, there are also studies suggesting that FR $\alpha$  expression is related to the survey and tumor stages and may be a prognostic indicator [6, 13, 21, 22].

Although FR $\alpha$  status has been considered from some aspects [15, 18, 23, 24], data on FR $\alpha$  expression in endometrium cancer is limited. In this study, we investigated the FR levels in EC and its precursor EH and the potential difference between these two entities, we aimed to present the effect of FR $\alpha$  on tumorigenesis in endometrium carcinoma and its potential to become a target molecule for this cancer.

### Material method

#### *Clinicopathologic data*

The study consisted of 95 patients diagnosed with EEC, 58 patients diagnosed with EH without simple atypia, 21 patients diagnosed with EH without complex atypia, 18 patients diagnosed with EH with simple atypia, and 22 patients diagnosed with EH with complex atypia in Medeniyet University Goztepe Training and Research Hospital, Istanbul, Turkey between the dates January 2007 and June 2014. Thirty normal endometrium tissues at secretory and proliferative phase were used as control.

Age and menopausal status of the patients together with nuclear grade taken as per the FIGO grading system in carcinoma cases, estrogen (ER) and progesterone (PR) hormone receptor status were used as study parameters. The study protocol was approved by the local ethical committee.

#### *Tissue microarray construction (TMA)*

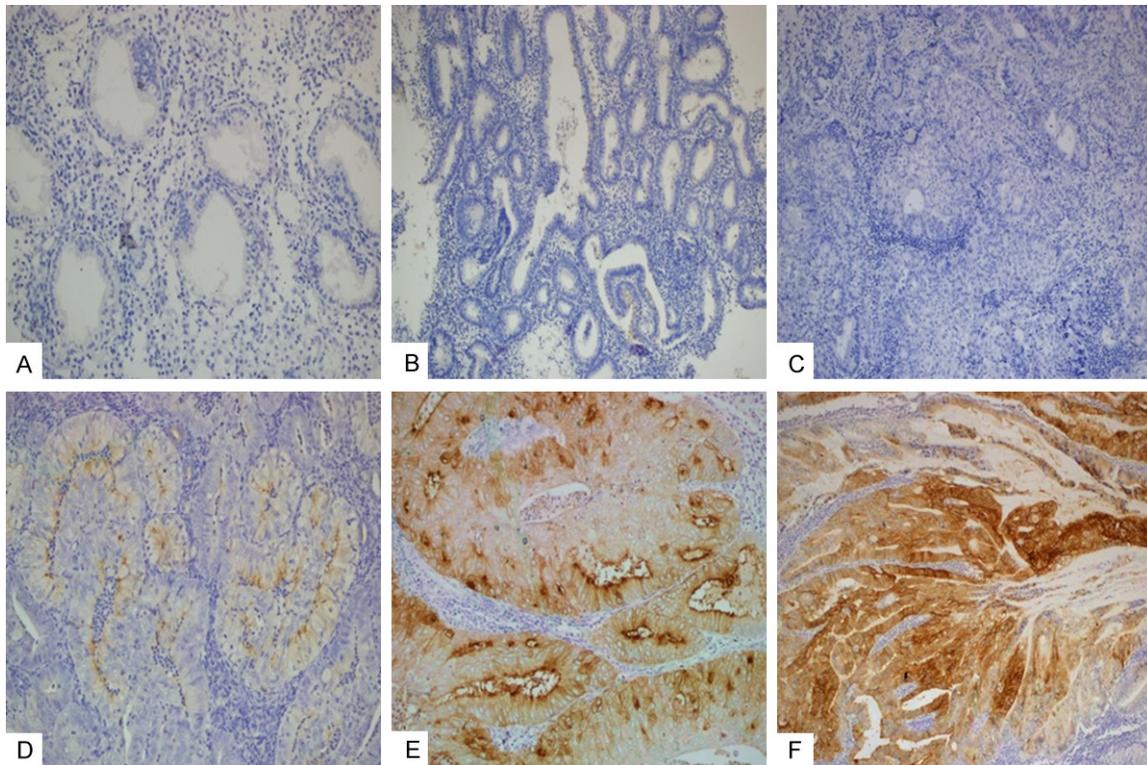
Cylindrical samples of 4-mm diameter were taken by comparing tissues in paraffin blocks and hemotoxylin-eosin cross-sections. This process was carried out using manual tissue microarrayer (Quick Ray; Unitma Co. Ltd., Seoul, Korea). The obtained tumor tissue samples

were mapped and re-blocked and made ready for immunohistochemical study.

#### *Immunohistochemistry and scoring*

Sections of 4  $\mu$ m thickness were cut from 224 THK tissues. Using specific primary antibodies, Dewaxed and rehydrated tissue sections were immunostained by the streptavidin-biotin peroxidase complex (SAB) method. Immunohistochemical staining for FR $\alpha$ , ER and PR receptor ER and PR receptor ER and PR receptor was done on step sections of TMA blocks. The slides were deparaffinized by 2 xylene rinses, followed by 2 rinses with 100% ethanol. Antigen retrieval was performed by heating the slides in a pressure cooker filled with either 7.5 mM sodium citrate with a pH of 6.0. After 5 minutes of casein blocking for nonspecific binding, the tissue sections were incubated for 25 minutes with primary antibodies for the detection of anti- FR $\alpha$  antibody (Leica Biosystems, NE, UK; BN 3.2, mouse monoclonal antibody, 1:100), ER receptor antibody (DAKO, ID5, mouse monoclonal antibody, ready to use) and PR receptor antibody (DAKO, PgR 636, mouse monoclonal antibody, ready to use). This step was followed by detection using the Bond Polymer Refine kit for 25 minutes on the Bond Max Autostainer (Leica Biosystems), visualization with diaminobenzidine chromogen, and counterstaining with hematoxylin. Breast and endometrium studies made previously were taken as basis in (FR) scoring [15, 25]. In short, regarding staining intensity, observation of no staining was evaluated as 0; membranous staining limited only to gland lumen and observed at  $\times$ 200 magnification as +1; staining observed at  $\times$ 100 magnification as +2; and membranous staining easily observed at even  $\times$ 40 magnification as +3. For staining rate, glandular cell staining over 25% was evaluated as positive. In statistical evaluation score 0 and 1 were classified as "low" expression and score 2 and 3 were classified as "high" expression. ER and PR immunoreactivity was scored as mild =1, medium =2 and severe =3 in intensity; and with regards to the ratio as follows: 0% as 0 points, 1-10% as 1 point, 11-33% as 2 points, 34-66% as 3 points, and 67-100% as 4 points. Ones with the ratio and intensity multiplication points of 0-1 were evaluated as negative (score 2), ones between 2-5 were low positive, and ones between 6-12 as high positive.

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**Figure 1.** FR $\alpha$  expression status in endometrial tissues. Negativity in secretory endometrium (A) and simple EH (B). Negative (C), +1/weak (D), +2/moderate (E) and +3/strong FR $\alpha$  immunoreactivity in EEC.

**Table 1.** Evaluation of FR $\alpha$  expression in EEC and EH main groups

		EEC (n=95)	EH (n=119)	Secretuar/Proliferative endometrial tissue (n=30)	P
		n (%)	n (%)	n (%)	
FR $\alpha$	Negative	18 (18.9%)	84 (70.6%)	30 (100.0%)	0.001**
	+	29 (30.5%)	27 (22.7%)	0 (0.0%)	
	++	27 (28.4%)	8 (6.7%)	0 (0.0%)	
	+++	21 (22.1%)	0 (0.0%)	0 (0.0%)	
FR $\alpha$	Low	47 (49.5%)	111 (93.3%)	30 (100.0%)	0.001**
	High	48 (50.5%)	8 (6.7%)	0 (0.0%)	

Fisher Freeman Halton Test, \*p<0.05, \*\*p<0.01.

### Statistical analysis

Pearson Chi-Square test was used for the comparison of the data from Yates Continuity Correction and Fisher Freeman Halton (Monte Carlo) tests. For consistency assessments between immunohistochemical variables, Spearman's correlation analysis was used. Significant was evaluated at the level of  $P < 0.05$ . Statistical analyses were carried out using the program NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and

Sample Size) 2008 Statistical Software (NCSS LLC, Kaysville, Utah, USA).

### Results

FR $\alpha$  expression was present in 81.1% of the EEC cases and was determined to have high expression in 50.5%. In endometrium hyperplasia, these ratios were 29.4% and 6.7%, respectively. All of the control tissues and 70.6% of EHs were negative (**Figure 1A, 1B**). FR $\alpha$  level in EEC was significantly high in comparison to EH

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**Table 2.** Evaluation of folate receptor  $\alpha$  (FR $\alpha$ ) expression in EEC, atypical-complex EH and simple without atypical EH

n (%)		EEC	Atypical-Complex EH	Simple w/o atypical	Normal endometrium	P
		(n=95)	(n=61)	EH (n=58)	(n=30)	
		n (%)	n (%)	n (%)	n (%)	
FR $\alpha$	Negative	18 (18.9%)	37 (60.7%)	47 (81.0%)	30 (100.0%)	0.001**
	+	29 (30.5%)	17 (27.9%)	10 (17.2%)	0 (0.0)	
	++	27 (28.4%)	7 (11.5%)	1 (1.7%)	0 (0.0%)	
	+++	21 (22.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
FR $\alpha$	Low	47 (49.5%)	54 (88.5%)	57 (98.3%)	30 (100.0%)	0.001**
	High	48 (50.5%)	7 (11.5%)	1 (1.7%)	0 (0.0%)	

Fisher Freeman Halton Test, \* $p < 0.05$ , \*\* $p < 0.01$ .

and normal endometrium ( $P < 0.01$ ). Similarly, FR $\alpha$  expression in EH cases with complex atypia were significantly higher when compared to other hyperplasia subgroups ( $P < 0.01$ ). When examined in two main groups as EEC and EH, observation of moderate (++) (Figure 1E) and severe (+++) (Figure 1F) FR $\alpha$  expression in EECs was determined to be significantly higher than the EHs and normal endometrial tissues ( $P < 0.001$ ). A statistical significance was also determined ( $P < 0.01$ ) when the FR $\alpha$  expression is assessed as low and high. Observation of high FR $\alpha$  expression in EECs was determined to be significantly higher than the EHs and normal endometrial tissues (Table 1).

When the EH subgroups are individually compared, observation of severe (+++) FR $\alpha$  expression in EECs was determined to be significantly higher than all the EH subgroups and normal endometrial tissues ( $P < 0.001$ ). Observation of moderate (++) FR $\alpha$  expression in EECs and EHs with complex atypia was determined to be significantly higher than the other EHs subtypes and normal endometrial tissues ( $P < 0.001$ ). A statistical significance was also determined for observation of FR $\alpha$  expression based on low and high positivity ( $P < 0.01$ ). Observation of high FR $\alpha$  in EHs with complex atypia was determined to be higher than EHs without complex atypia, EHs without simple atypia and normal endometrium group. The findings are summarized in Table 2.

While ER receptor expression did not exhibit a statistically significant relationship with age, PR expression increased with age ( $P < 0.05$ ). The proportion that the cases with high FR $\alpha$  expression were in patients 50 years old or more was significantly high ( $P < 0.05$ ). While ER and PR

expressions did not exhibit a statistically significant difference based on menopause ( $P > 0.05$ ), observation of high FR $\alpha$  expression in menopausal patients was significantly higher than non-menopausal patients ( $P < 0.05$ ). Although no correlation between the EEC grades and the FR $\alpha$ , ER and PR expressions were observed, the EEC cases with high FR $\alpha$  expression tended to have high grades. In EEC, the negativity and low expression of ER and PR were significantly higher than the EHs and normal endometrial tissues and high expressions were significantly low. Similarly, in EHs with complex atypia, the negativity and low expression of ER and PR were significantly higher than the EHs without simple atypia and normal endometrial tissues and high expressions were significantly low ( $P < 0.05$ ). When their consistency and correlations were compared, no statistical significance was observed between the FR $\alpha$ , ER and PR expressions ( $P < 0.05$ ). The results are shown in Table 3.

### Discussion

Current study is based on the evaluation of the FR $\alpha$  level immunohistochemically in EEC and its precursor EH using normal endometrium controls and thus presenting the possible difference of the FR $\alpha$  expression level between normal endometrial tissue, hyperplasia and carcinoma. We aimed to exhibit the possible contribution of FR $\alpha$  to the diagnosis and treatment of endometrial lesions by presenting FR $\alpha$  staining patterns between different endometrium tissues and lesions. The literature about FR $\alpha$  expression in endometrial malignancies and comparative analyses between the EHs and EECs in wide series is lacking.

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**Table 3.** Comparison between clinic-diagnostic parameters and FR $\alpha$ , ER-PR expressions

	Folate receptor $\alpha$			ER				PR			
	Low	High	<i>p</i> value	Negative	Low	High	<i>p</i> value	Negative	Low	High	<i>p</i> value
<b>Age</b>											
< 50	83 (86.5)	13 (13.5)	0.001**	11 (11.5)	9 (9.4)	76 (79.2)	0.016*	16 (16.7)	10 (10.4)	70 (72.9)	0.048*
$\geq$ 50	75 (63.6)	43 (36.4)		19 (16.1)	26 (22.0)	73 (61.9)		30 (25.4)	21 (17.8)	67 (56.7)	
<b>Menopause</b>											
-	78 (83.0)	16 (17.0)	0.011*	11 (11.7)	14 (14.9)	69 (73.4)	0.548	14 (14.9)	18 (19.1)	62 (66.0)	0.047*
+	80 (66.7)	40 (33.3)		19 (15.8)	21 (17.5)	80 (66.7)		32 (26.7)	13 (10.8)	75 (62.5)	
<b>Grade in EECs (n=101)</b>											
1	24 (61.5)	15 (38.5)	0.051	8 (20.5)	10 (25.6)	21 (53.8)	0.943	17 (43.6)	5 (12.8)	17 (43.6)	0.484
2	17 (48.6)	18 (51.4)		8 (22.9)	9 (25.7)	18 (51.4)		10 (28.6)	8 (22.9)	17 (48.6)	
3	6 (28.6)	15 (71.4)		5 (23.8)	7 (33.3)	9 (42.9)		7 (33.3)	6 (28.6)	8 (38.1)	
EEC	47 (49.5)	48 (50.0)	0.001**	21 (22.1)	26 (27.4)	48 (50.5)	0.001**	34 (35.8)	19 (20.0)	42 (44.2)	0.001**
EH	111 (93.3)	8 (6.7)		9 (7.6)	9 (7.6)	101 (84.9)		12 (10.1)	12 (10.1)	95 (79.8)	
EEC	47 (49.5)	48 (50.5)	0.001**	21 (22.1)	26 (27.4)	48 (50.5)	0.001**	34 (35.8)	19 (20.0)	42 (44.2)	0.001**
Atypical&Complex EH	54 (88.5)	7 (11.5)	0.004**	7 (11.5)	4 (6.6)	50 (82.0)	0.026*	9 (14.8)	7 (11.5)	45 (73.8)	0.243
Simple w/o atypical EH	57 (98.3)	1 (1.7)	0.001**	2 (3.4)	5 (8.6)	51 (87.9)	0.001**	3 (5.2)	5 (8.6)	50 (86.2)	0.001**

Fisher Freeman Halton Test, \* $p < 0.05$ , \*\* $p < 0.01$ .

It was shown that the FR $\alpha$  was expressed in normal progenitor cells and had a regulating effect on malign transformation [18]. Observation of high FR $\alpha$  expression with varying rates in some tumors in spite of its limited expression in normal and precancerous cells brings to mind that FR $\alpha$  may play a key role in carcinogenesis and might become a possible target in the treatment [6, 7, 18, 24]. It has been reported that FR $\alpha$  is a part of the malign transformation in breast carcinoma and increases proliferation in carcinoma cells [5, 25]. However, its expression in normal breast tissue epithelia was limited or non-existent [5]. Wu et al. showed that FR $\alpha$  expression did not disappear in serous ovarian and endometrioid malignancies although malign transformation from cervix glandular epithelia and the transformation from ovarian germinal epithelia to serous or mucinous benign neoplasia were related to FR $\alpha$  down-regulation [18]. Similarly, Shia et al. reported that FR $\alpha$  expression was significantly high in collateral carcinomas and metastasis compared to normal mucosa and adenomas and that they were related to poor survivals in univariate analyses. [16]. Christoph et al. reported that FR $\alpha$  was frequently expressed in advanced non-small cell lung carcinomas [26]. Cagle et al. determined high FR $\alpha$  expression in lung adenocarcinoma and squamous-cell carcinomas and suggested that FR-target therapy might be a good indicator especially in lung carcinomas unresponsive to therapy [27]. Toffoli et al. and Kalli et al. found that FR $\alpha$  expression was related to bad prognostic factors and aggressive progress in ovarian carcinoma and that FR $\alpha$  overexpression was significantly high in high-grade ovarian cancers compared to low-grade ones [13, 28]. Similarly, strong FR $\alpha$  expression in breast carcinoma was found to be correlated with poor clinical progress [2, 22]. Regarding the studies on endometrial carcinomas, Wu et al. reported that malign transformation was related to de novo expression of FR $\alpha$  in endometrial glandular cells [18]. Brown Jones et al. reported that high FR $\alpha$  has a significant relationship with poor prognostic factors such as late-stage and high-grade in endometrium carcinomas [15] and Allard et al. reported high FR $\alpha$  immunorexpression at a rate of 12% in EEC, 33% in the serous type, and 25% in the clear-cell type and no correlation was observed in the same study, in which radical resection materials were used,

between FR $\alpha$  and age, grade, stage, deep myometrial invasion and lymph node metastasis [23]. In a study carried out recently, O'Shannessy et al. showed that FR $\alpha$  was expressed negatively or weak apical positively in some of the normal endometrium tissues and strongly expressed in some of the complex EHs and EECs with atypia [24].

Although it is still unclear whether FR $\alpha$  overexpression encountered in some cancers is a passive condition caused by rapid proliferation of tumor cells or arises since it's a direct factor on pathogenesis [4, 6, 12, 18, 20], we suggest that FR $\alpha$  overexpression may play a role in EEC carcinogenesis and carcinoma progress from EH as we have found FR $\alpha$  expression is increased significantly in EECs in comparison to EHs and complex and atypia EHs when compared to simple hyperplasia. Furthermore, as established in some similar studies [6, 11], the non-existence of FR $\alpha$  expression in normal tissues brings to mind that folate might be used as a potential molecule in endometrium cancer therapy and receptor-targeted molecular therapies. Importantly, folate receptor-targeted therapies suppress metastasis and inhibit primary tumor growth in animal tumor models. And the recent data show that FR $\alpha$  is a target of immune system in breast and ovarian cancer patients [12, 31]. Moreover, for cancers with strong overexpression of folate receptor, targeting this receptor therapeutically shown to be beneficial [11, 12, 32, 33]. Recent studies reported promising results with experimental folate receptor labeled combination chemotherapies in ovarian and lung cancers with high FR $\alpha$  expression [34-36].

Moreover, there are applications as a serum marker and imaging in tumors such as lung, ovary and endometrium where FR $\alpha$  is overexpressed [37, 38] and studies suggesting a correlation between FR $\alpha$  expression and monitoring and follow-up of the disease and the response to therapy [29, 39, 40].

Although down-regulation or up-regulation has been reported between sex steroids in some studies and it has been shown that FR $\alpha$  regulation may be achieved through anti-estrogen therapy such as tamoxifen and ICI 162780 [2, 29, 30] we did not observe any correlation between the sex steroids, ER-PR receptor expression and FR $\alpha$  expression in both EHs

and EECs which is consistent with the results of the study on breast carcinomas by Hartman et al [22].

As a result, although hormonal therapy and various chemotherapeutics are used in the treatment of endometrioid-type carcinomas, target treatment is the treatment of choice in all tumor types. Significantly higher FR $\alpha$  expression encountered in endometrioid-type endometrium carcinomas in comparison to hyperplasia and normal endometrium brings to mind that FR $\alpha$  might have a role in the development of carcinoma along with other etiologic factors and folate may be used in the treatment of EEC and/or in the prevention of transformation to EEC from hyperplasia stage. Furthermore, the non-existence of FR $\alpha$  in normal endometrium glands, but its high expression in ECC may be interpreted as target therapies like paxlitaxel and anti-EGFR labeled with probes based on antibodies containing folate may be useful in the treatment of EEC. FR $\alpha$  might be used as an indicator in the wide spectrum of normal cyclic endometrium-simple hyperplasia-malign endometrial carcinoma transition and might become a supplemental marker to determine the patients that shall be directed to radical therapy amongst patients with complex atypical EH.

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### Disclosure of conflict of interest

None.

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