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Overexpression of folate binding protein is associated with shortened progression-free survival in uterine adenocarcinomas☆

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Abstract

Objectives. Oligonucleotide array and tissue microarray analysis (TMA) by our group has revealed that folate binding protein (FOLR1) is overexpressed in some types of uterine cancer, particularly tumors with serous histology. Since FOLR1 overexpression is a frequent event in some types of endometrial carcinoma, we examined the relationship between FOLR1 overexpression and clinical and pathologic features to determine its prognostic relevance.

Methods. A tissue microarray (TMA) comprised of primary tumor specimens from 485 patients diagnosed with endometrial adenocarcinoma was used to identify cases characterized by FOLR1 overexpression. A proportional hazards model was used to evaluate the association of FOLR1 overexpression with progression-free survival while accounting for confounding influences.

Results. Overexpression of FOLR1 was observed in 50/292 (17%) cases and was seen more often in poorly differentiated cancers (22/90 [24%], $p=0.051$) and tumors with serous histology (16/32 [50%], $p<0.001$). A shorter progression-free survival was noted in patients with FOLR1 overexpression (log-rank $p=0.016$) that persisted when the data were limited to patients with stage III/IV disease (log-rank $p=0.021$) or serous tumors (log-rank $p=0.020$). Multivariate Cox regression analysis revealed that patients with FOLR1 overexpression had a shorter progression-free survival (H.R. 2.14; 95% CI 1.07–4.28) even when controlling for stage, grade, myometrial invasion and adjuvant chemotherapy.

Conclusions. Our data show that FOLR1 overexpression is not only a biomarker associated with endometrial cancer, but it also appears to be a prognostic factor associated with adverse outcome. These findings suggest that FOLR1 may be an appealing target for biological therapies in some types of endometrial carcinomas.

Keywords: Folate binding protein; Papillary serous; Gynecologic tumors

Introduction

According to the American Cancer Society, an estimated 41,200 new cases of uterine cancer will be diagnosed in 2006 with approximately 7350 deaths that will be attributable to this disease [1]. Although most of these cases present at an early stage of disease associated with a good prognosis, many cases present at a later stage and tend to be more virulent. In addition, recurrence of disease occurs in approximately 7–12% of patients with early stage disease compared with 50–54% of patients with

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stages III–IV disease [2–4]. Additionally, recurrence is associated with a median survival of only 7 months for advanced stage disease [2–5]. Non-endometrioid histology, higher grade, advanced stage disease and increased depth of myometrial invasion are pathologic factors that are associated with a higher risk of recurrence and a decreased survival [6]. Alterations in the PTEN and p53 tumor suppressor genes, amplification and overexpression of the Her-2 oncogene and microsatellite instability are prognostic biomarkers associated with endometrial cancers [7–10]. Identification of patients with tumors having adverse clinical and molecular prognostic characteristics may facilitate adjuvant therapies aimed at improving outcome [11–14].

Our group has used microarray analysis to identify distinct gene expression profiles among various types of endometrial cancer in an effort to identify molecular alterations characteristic of more aggressive tumors [15–17]. Recently, our group has shown that FOLR1 is overexpressed in a significant proportion of endometrial adenocarcinomas, particularly tumors with serous histology [18]. The objective of this study was to correlate expression of FOLR1 using tissue microarray (TMA) with clinical and pathologic features in patients with endometrial carcinoma to confirm whether FOLR1 overexpression is associated with a shortened progression-free survival.

Materials and methods

An endometrial cancer tissue microarray (TMA) constructed from 485 primary tumor specimens that represented various histologic types and stages of endometrial cancer was used for immunohistochemical staining of FOLR1 [18]. For the purposes of this study, advanced stage disease is defined as FIGO (International Federation of Gynecology and Obstetrics) stages III–IV. Paraffin tissue samples used for the tissue microarray were collected from patients diagnosed with and treated for uterine malignancy between January 1st 1980 and July 31st 2003 at the Arthur James Cancer Hospital of the Ohio State University (OSU). Over 80% of patients underwent surgical staging including total abdominal hysterectomy, bilateral salpingo-oophorectomy with pelvic and para-aortic lymph node dissection. Patients without gross intraperitoneal disease and lymphadenectomy were staged according to the extent of uterine disease involvement. Platinum-based chemotherapy (with doxorubicin or paclitaxel) or pelvic radiation (with or without para-aortic coverage) was given at the discretion of the attending physician. The creation of the uterine cancer TMA and its use for the purposes of this study was performed following protocol approval by the OSU Institutional Review Board. The presence of tumor tissue for each of the arrayed cases was verified on a hematoxylin–eosin-stained section and each case was represented in quadruplicate on the TMA. Specimens selected for controls consisted of 50 specimens of normal endometrium (negative control) and 10 ovarian cancer specimens (positive control). Details regarding the immunohistochemical staining have been previously described [18].

Immunohistochemical staining of the endometrial cancer TMA for FOLR1 was performed using the Pu-17 antibody (Endocyte Inc.). FOLR1 staining was graded using the following criteria: 0=no staining; 1=weak cytoplasmic staining, no membranous immunoreactivity; 2=moderate cytoplasmic staining with weaker membranous immunoreactivity; 3=strong cytoplasmic and membranous immunoreactivity. The results were recorded as positive (consistent with overexpression) if at least 10% of the neoplastic cells exhibited a staining grade of 2–3. A case was considered as negative on TMA only if all four cores, representative of the case had 0–1 staining. The overall intensity of staining of the case was recorded for the core with the strongest intensity when there was variation between cores for a given case. All immunohistochemical staining was reviewed by a single board certified pathologist.

Only endometrial cancer cases with clinical follow-up (to include adjuvant chemotherapy or radiation therapy) were selected from the endometrial cancer TMA for an analysis of FOLR1 overexpression and survival. Using these selection criteria, 292/485 patients were available for this study. Demographic variables were evaluated according to FOLR1 overexpression status using Student's *t*-test and Fisher's exact test. Progression-free survival (PFS) was plotted according to the Kaplan–Meier method and differences in the curves evaluated via the log-rank test. Deaths due to a cause other than endometrial cancer were listed as censored events and not as endpoints for PFS. To analyze the independent contribution of various factors, survival comparisons were carried out utilizing the Cox proportional hazards model in univariate and multivariate analyses [19].

Results

The relationship between FOLR1 overexpression and the clinical and pathologic features of the 292 endometrial carcinoma specimens is shown in Table 1. Forty (40) patients were diagnosed with recurrent disease using either imaging and/or biopsy. Overexpression of FOLR1 was found more often in serous tumors ($p<0.001$) and in disease that was advanced stage ($p=0.054$) and poorly differentiated ($p=0.051$). There was no association between FOLR1 overexpression and age, race, depth of myometrial invasion, node metastasis and the administration of either chemotherapy or adjuvant radiation therapy.

Table 2 represents the univariate analysis of clinical and pathologic factors for PFS. Cox regression analysis revealed a

Table 1
Distribution of FOLR1 overexpression[a] by clinical and pathological factors ($n=292$)

	FOLR1− ($n=242$)	FOLR1+ ($n=50$)	p-value[b]
Age, mean (SD)	61.9 (13.5)	65.6 (12.0)	0.073
Race, *n* (%)			
Caucasian	228 (94.2)	45 (90.0)	
Other	14 (5.8)	5 (10.0)	0.339
FIGO stage, *n* (%)			
I	166 (68.6)	29 (58.0)	
II	22 (9.1)	4 (8.0)	
III	49 (20.3)	12 (24.0)	
IV	5 (2.1)	5 (10.0)	0.054
FIGO grade, *n* (%)			
1	108 (44.6)	14 (28.0)	
2	66 (27.3)	14 (28.0)	
3	68 (28.1)	22 (44.0)	0.051
Histologic type, *n* (%)			
Endometrioid	209 (86.4)	28 (56.0)	
Mixed epithelial	15 (6.2)	5 (10.0)	
Serous	16 (6.6)	16 (32.0)	
Clear cell	2 (0.8)	1 (2.0)	<0.001
Myometrial invasion, *n* (%)			
<50%	159 (65.7)	32 (64.0)	
≥50%	83 (34.3)	18 (36.0)	0.871
Nodes, %			
Positive	36 (14.9)	9 (18.0)	
Negative	206 (85.1)	41 (82.0)	0.667
Chemotherapy, *n* (%)			
Yes	93 (38.4)	23 (46.0)	
No	149 (61.6)	27 (54.0)	0.343
Radiation therapy, *n* (%)			
Yes	37 (15.3)	8 (16.0)	
No	205 (84.7)	42 (84.0)	0.833

[a] Overexpression of FOLR1 is defined as immunohistochemical staining=2–3.
[b] p-value for the difference in age was determined by the Student's *t*-test. All other p-values were determined by Fisher's exact test.

Table 2
Univariate analysis for progression-free survival

Variable	Hazard ratio	95% Confidence interval	p-value[a]
Age (10-year increase)[b]	1.46	1.12–1.91	0.005
Race			
Caucasian	Referent		
Other	2.47	0.97–6.31	0.059
FIGO Stage			
I–II	Referent		
III–IV	5.00	2.66–9.42	<0.001
Histologic grade			
Well-moderately differentiated	Referent		
Poorly differentiated	9.19	4.37–19.31	<0.001
Histologic type			
Endometrioid	Referent		
Other	4.68	2.52–8.71	<0.001
Myometrial invasion			
<50%	Referent		
≥50%	4.34	2.20–8.54	<0.001
Lymph node status			
Negative	Referent		
Positive	4.99	2.67–9.30	<0.001
Radiation			
No	Referent		
Yes	1.94	0.95–3.96	0.071
Chemotherapy			
No	Referent		
Yes	2.05	1.09–3.83	0.025
FOLR1			
Underexpression	Referent		
Overexpression	2.25	1.14–4.42	0.019

[a] Wald p-values determined via a univariate Cox model with the indicated factor.
[b] Univariate analysis of age was performed in a linear fashion; therefore, there is no referent variable.

shorter PFS was associated with older patients ($p=0.005$). PFS also was decreased among patients with poorly differentiated histology ($p<0.001$) and stage III/IV disease ($p<0.001$). Disease progression was noted to be increased among patients with tumors that had FOLR1 overexpression (HR 2.25, 95% CI 1.14–4.42). A shorter PFS was associated with non-endometrioid histology, deep myometrial invasion and lymph node metastasis. Patients who required adjuvant chemotherapy or radiation treatment had an increased risk of disease progression but the majority of these patients had advanced stage endometrial cancer.

An assessment of PFS according to FOLR1 expression depicted by Kaplan–Meier curves was performed. Endometrial cancer patients with carcinomas overexpressing FOLR1 had worse PFS when compared to patients with tumors demonstrating minimal to no detected FOLR1 expression (log rank, $p=0.016$) (Fig. 1). Estimates of five-year PFS were 82% (95% CI 74–87%) for patients with minimal FOLR1 expression and 68% (95% CI 49–81%) for those exhibiting overexpression. Analysis of FOLR1 in subgroups revealed that overexpression among serous tumors (Fig. 2) and stage III/IV disease (Fig. 3) was associated with a shorter PFS (log-rank, $p=0.020$ and $p=0.021$, respectively). FOLR1 overexpression in stage I/II

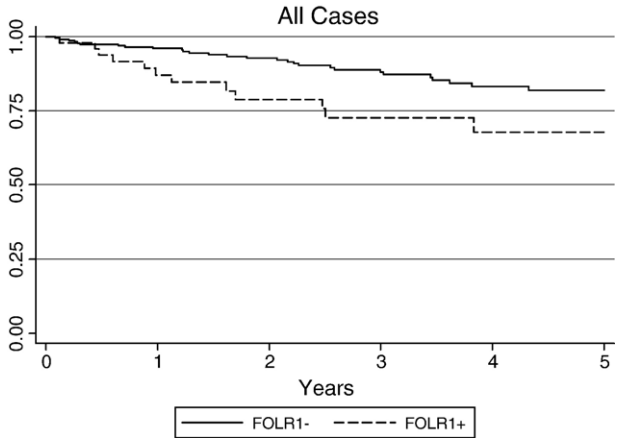

Fig. 1. Progression-free survival by FOLR1 expression for all cases (log-rank p-value=0.016). The solid line represents those patients who had weak staining (underexpression) of FOLR1. The broken line represents those patients who strong staining (overexpression) of FOLR1.

disease or endometrioid tumors did not appear to influence outcome.

Due to the limited number of events (40), backwards elimination was used to achieve a stable and parsimonious multivariate Cox model. All variables found to be significant univariately (at the 0.05 level) were considered in the initial model. Multivariate analysis revealed that overexpression of FOLR1 was associated with shorter PFS (H.R. 2.14; 95% CI 1.07–4.28) even when controlling for stage, grade, myometrial invasion and the administration of adjuvant chemotherapy (Table 3).

Discussion

Folate serves as a source of one-carbon units that are necessary for methylation of other molecules important in the regulation of multiple cellular processes including cell division,

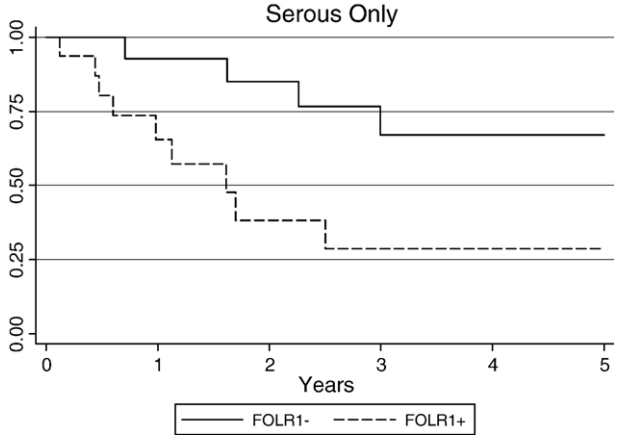

Fig. 2. Progression-free survival by FOLR1 expression for serous histology only (log-rank p-value=0.020). The solid line represents those patients who had weak staining (low expression) of FOLR1. The broken line represents those patients who strong staining (overexpression) of FOLR1.

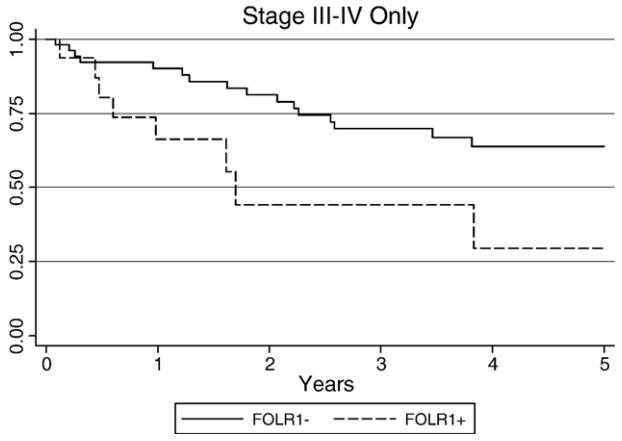

Fig. 3. Progression-free survival by FOLR1 expression for stages III–IV disease only (log-rank p-value = 0.021). The solid line represents those patients who had weak staining (underexpression) of FOLR1. The broken line represents those patients who strong staining (overexpression) of FOLR1.

growth and survival [20–24]. Methylation of DNA is frequently dysregulated in cancer, often leading to aberrant methylation in genes known to be implicated in the development of cancer [25]. More recently, folate has also been implicated as having a role in carcinogenesis via methylation of RNA, phospholipids and protein [21,23].

Folate binding protein is a cell-surface membrane bound protein linked to a glycosylphosphatidylinositol anchor that internalizes folate by receptor-mediated endocytosis. It exists in three isoforms (FRα, FRβ and FRγ) that are differentially expressed among various tissue types. The FRα isoform, also referred to as FOLR1, is the most widely studied isoform and is highly expressed in various non-mucinous tumors of epithelial origin [24,26,27]. Studies have revealed a growth advantage associated with cells transfected with FOLR1, suggesting that folate binding protein may be involved in the control and maintenance of cell proliferation [28,29].

Our study is the first to correlate overexpression of FOLR1 with clinical outcome in patients with endometrial adenocarcinoma. Although the patients with FOLR1 overexpression were slightly older than those with minimal or no FOLR1 expression, we measured progression-free survival as our outcome endpoint which should not have been influenced by age. In addition, Cox regression analysis did not show any significant association with age in the prediction of PFS in a multivariate model that included FOLR1 expression, stage, grade and myometrial invasion. In this model, FOLR1 overexpression was shown to be predictive of a shorter PFS (H.R. 2.14; 95% CI 1.07–4.28), even among patients with stage III/IV disease or serous histology. Although patients with serous histology have been shown to have higher rates of recurrence, there was not a significant effect of histology on PFS when the multivariate model included FOLR1, suggesting that this FOLR1 may play a significant role in the poor outcome observed among patients with serous tumors. In addition, we confirmed that adjuvant chemotherapy and radiation treatment was not administered differentially to serous cases with FOLR1 overexpression versus those with minimal to no expression (data not shown).

FOLR1 overexpression previously has been reported in association with poor prognostic features in endometrial and ovarian cancer. However, there is limited evidence demonstrating an association between FOLR1 overexpression and patient outcome. In an analysis of 99 patients with epithelial ovarian cancer, Toffoli et al. [28] found that FOLR1 overexpression correlated with a poor response to chemotherapy in patients with residual disease following cytoreduction. Although overexpression of FOLR1 was not found to significantly correlate with overall survival, the small sample size limited the power of confirming a trend associated poor outcome [28]. In the current investigation, the cause for shortened PFS among endometrial cancer patients with overexpression of FOLR1 is not clear. Further research is needed to confirm these findings and further clarify the biological role that FOLR1 plays in endometrial carcinogenesis.

Overexpression of FOLR1 has been reported in number of solid epithelial tumors, most notably ovarian and brain cancer where overexpression can be found in approximately 75–90% of tumors [30]. Therapeutics that target FOLR1 overexpression have appealing applications in treatment of cancer because FOLR1 has limited distribution in normal tissues. In addition, FOLR1 is located on the luminal surfaces of the normal epithelial cells minimizing exposure to systemically circulating therapeutics that target the folate receptor [31]. In contrast, the density of FOLR1 in the cellular membrane and the frequency of overexpression among cancer cells appear to be directly proportional to progressive stage of disease and poor differentiation making poor prognosis tumors ideal for FOLR1-mediated treatment [30]. A variety of therapeutic agents against cancers that overexpress FOLR1 recently have been developed [32,33]. Investigators have used the sequence of FOLR1 to predict potential CTL (cytotoxic T-lymphocyte) epitopes of

Table 3
Multivariate analysis for progression-free survival

Variable	Hazard ratio	95% Confidence interval	p-value
FIGO stage			
I–II	Referent		
III–IV	3.61	1.60–8.11	0.002
FIGO grade			
Well-moderately differentiated	Referent		
Poorly differentiated	5.99	2.72–13.22	<0.001
Myometrial invasion			
<50%	Referent		
≥50%	2.54	1.20–5.37	0.015
Chemotherapy			
No	Referent		
Yes	0.39	0.17–0.91	0.029
FOLR1			
Underexpression	Referent		
Overexpression	2.14	1.07–4.28	0.031

A multivariate regression model was designed utilizing a backwards elimination procedure. Although histology was a significant variable on univariate analysis, it was not significant in the multivariate model that included stage, grade, depth of myometrial invasion, chemotherapy and FOLR1 status.

HLA (human leukocyte antigen) binding peptides. Administration of these immunogenic peptides serves to direct activated T cells to cancer cells overexpressing FOLR1 in vitro. Additionally, folic acid has been linked to antigenic molecules (haptens) that in turn bind to FOLR1 on the surfaces of cancer cells making the tumor more immunogenic [32]. Finally, chemotherapeutics and other toxic conjugates have been linked to folate and subsequently used to target tumor cells overexpressing FOLR1 for direct cytotoxic effect [34].

Approximately 50% of the recurrences and deaths in patients with endometrial carcinomas are due to serous carcinoma [35]. Our data indicate that overexpression of folate binding protein in uterine carcinomas that are serous in histology are associated with shortened progression-free survival. Given these findings and the poor efficacy of current treatment modalities, it seems that a more aggressive treatment strategy using contemporary therapeutic options should be considered in the treatment of tumors that overexpress FOLR1. In addition, FOLR1 may provide a unique target for novel biologic approaches to therapy in subsets of endometrial cancer patients demonstrating overexpression of FOLR1.

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