Gynecologic Oncology 130 (2013) 192–199

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Folate receptor alpha (FRA) expression remains unchanged in epithelial ovarian and endometrial cancer after chemotherapy

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HIGHLIGHTS

- Both epithelial ovarian and endometrial cancer demonstrate high expression of FRA compared to normal ovarian and endometrial tissue.
- FRA expression is not altered by chemotherapy exposure at interval debulking surgery or at recurrence.
- Immunohistochemical FRA staining at diagnosis can guide the decision whether to use FRA targeted therapy upon recurrence.

ARTICLE INFO

Article history:
Received 21 December 2012
Accepted 23 March 2013
Available online 2 April 2013

Keywords:
Ovarian cancer
endometrial cancer
folate receptor
FRA
immunohistochemistry
chemotherapy

ABSTRACT

Objective. Based on its expression profile, folate receptor alpha (FRA) is an attractive candidate for targeted diagnostics and therapeutics. However, applicability of these agents in residual or recurrent disease could be influenced by chemotherapy. We evaluated whether chemotherapy modified FRA expression in non-mucinous epithelial ovarian (EOC) and endometrial carcinoma (EC).

Methods. FRA staining was evaluated by immunohistochemistry, using MAb 26B3, in 81 patients (41 EOCs and 40 ECs) and 17 control tissues (5 benign ovarian cysts, 5 normal ovarian, and 7 normal endometrial tissues). Chemotherapy effect was evaluated in 42 patients (30 paired samples at primary and interval debulking surgery and 12 from primary and recurrent disease). FRA expression was assessed using a semi-quantitative staining algorithm, the *M*-score (range 0–50).

Results. Median difference in *M*-score between tumor and control samples was 27.5 for EOC (95% CI 10.0 to 45.0) and 6.7 for EC (95% CI −6.7 to 21.7). Paired samples from both primary and interval debulking surgery did not differ in FRA expression in EOC (median difference of *M*-score between paired samples of 0.0 [95% CI −2.6 to 2.6]). Recurrent EOC tumors reflected FRA status at diagnosis (median difference of *M*-score between paired samples of 3.3 [95% CI −7.0 to 13.6]).

Conclusions. This study shows no significant difference in FRA expression after chemotherapy, strengthening the rationale for FRA targeted diagnostics and therapeutics in FRA expressing tumors, whether newly diagnosed or at recurrence.

Introduction

Folate receptor alpha (FRA) is a glycosylphosphatidyl-inositol-linked cell surface glycoprotein with high affinity for binding and transporting physiological levels of folate into cells [1]. Cellular uptake of folate, a basic component of cell metabolism and DNA synthesis and repair, occurs under physiological conditions by an ATP-dependent reduced folate carrier (RFC), which is ubiquitously expressed in normal cells, binds folate with low affinity and represents the sole folate uptake for most normal cells. In contrast, the expression of FRA in normal tissues is highly restricted to the apical surfaces of polarized epithelial cells [2], which are not exposed to circulating folate. FRA enhances folate uptake through receptor-mediated endocytosis, allowing internalization of folate but also folate-conjugates. FRA expression increases or decreases in response to folate repletion or depletion and is distinct in normal and malignant tissue. High expression patterns have been described in a range of epithelial cancers, including non-mucinous ovarian, endometrial, non-small cell lung carcinomas and to a lesser extent in clear cell renal, colorectal and breast cancers [3–10]. Moreover, in ovarian cancer the level of FRA expression has been correlated with tumor grade, stage, aggressiveness and response to chemotherapy [5,6,11–13].

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http://dx.doi.org/10.1016/j.ygyno.2013.03.024

Based on its highly tumor restricted expression profile, FRA represents an attractive candidate for targeted diagnostics and therapeutics. FRA expression has been observed in nearly 90% of the non-mucinous ovarian cancers [2,5,14] with enhancement of growth of tumorigenic cancer cells *in vitro* and *in vivo* [15], providing a rationale for targeting the folate receptor in non-mucinous EOC. In the widely accepted dualistic model of endometrial carcinogenesis, 80% of the tumors are early stage, estrogen-dependent endometrial cancers with a low grade endometrioid morphology (Type I) [16]. In contrast, high grade non-endometrioid endometrial carcinomas encompassing mainly serous and clear cell carcinomas, characterize the endometrial cancers associated with adverse outcome and are classified as "Type II". The association of FRA expression with high risk endometrial cancer features, as well as worse survival [17], suggests that FRA may also be a novel target for endometrial cancer therapy.

Preclinical studies have demonstrated that Farletuzumab (MORAb-003), a humanized, high-affinity monoclonal antibody against FRA, mediates robust antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity *in vitro*, and inhibits tumor growth in xenograft models [18]. In a phase I study (NCT00428766), Farletuzumab administered as an intravenous infusion did not demonstrate dose-limiting toxicities or severe adverse events in heavily pretreated patients with EOC [19]. A phase II study (NCT00318370) of Farletuzumab with carboplatin and taxane in patients with platinum-sensitive EOC in first relapse, has shown an improved response rate and time to progression compared with historical data. Also the duration of second remission was increased compared to first remission [20]. Currently, a randomized, double-blind, placebo-controlled phase III study with Farletuzumab plus chemotherapy has been recently closed and presentation of these results is awaited.

In vitro studies demonstrated that EC-145 (vintafolide), a conjugate of folic acid and the vinca alkaloid desacetylvinblastine hydrazide, also selectively binds to cells that express FRA, causing dose-dependent cytotoxicity [21]. Initial results from a phase II randomized study of EC145 in combination with pegylated liposomal doxorubicin (PRECEDENT study; NCT00722592) indicate a significant improvement in progression-free survival in patients with platinum-resistant ovarian cancer compared with pegylated liposomal doxorubicin alone [22].

Although promising, identification of patients who may benefit from FRA-targeted therapy depends on the presence of FRA expression. While chemotherapy remains one of the keystones in the treatment of EOC and EC, there is a paucity of data regarding the effect of chemotherapy on FRA expression. Chemotherapy could influence FRA expression, reflecting the increased need for folate to support rapid repair of DNA damage caused by platinum. The goal of the present study was to evaluate the expression of FRA in epithelial ovarian and endometrial cancer specimens and to explore whether FRA expression is altered by chemotherapy.

Materials and methods

Patient and tissue characteristics

In this retrospective cohort study, 41 patients with ovarian carcinoma, 40 patients with endometrial cancer and 17 control patients were selected from the historical tumor bank of the Department of Obstetrics and Gynaecology of the University Hospitals Leuven for participation in this study. Tumor samples were taken at primary diagnosis, at interval debulking surgery after 3 courses of chemotherapy and/or at recurrent disease, snap frozen in liquid nitrogen after prelevation and stored at −80 °C until further processing. All patients with at least 2 sequential representative frozen tumor tissues were selected for the analysis. These patients were diagnosed and underwent surgery between May, 1996 and November, 2010. Tissues were accepted independent of its origin, for example, surgical specimens from primary ovarian or endometrial tumor, lymph nodes or distant metastases. Clinical and pathologic data were abstracted from the surgical, pathologic and medical records. Histological diagnosis followed the classification of the World Health Organization [23] and the grading as well as staging were based on FIGO 2009 guidelines [24]. The collection of tissue samples was approved by the Institutional Review Board of the University Hospitals Leuven and written informed consent was obtained from all patients enrolled (ML2524).

Immunohistochemistry

FRA membrane staining was evaluated by IHC, using the highly specific mouse monoclonal anti-FRA antibody MAb 26B3 in formalin-fixed, paraffin-embedded (FFPE) tissue sections, as previously described [25]. Signals were detected using a MACH4 Universal HRP-Polymer Detection Kit (Biocare Medical, Concord, CA).

Immunohistochemical analysis

Scoring for staining was performed by an expert gynecologic pathologist (Dr. Y-S Fu, Laboratory Corporation of America, Los Angeles, CA), using conventional scoring for intensity and the percent of the tumor stained at each intensity. FRA IHC membrane and cytoplasmic staining intensities were scored using the following criteria: 0 = no staining, 1+ = weak staining, 2+ = moderate staining and 3+ = strong staining, as previously described [12]. Also the percentage of cells stained by MAb 26B3 was determined. Estimates to the nearest 5% were used.

If the percentage of the tumor area positive for membranous staining was greater than or equal to 5% at any intensity, FRA expression was considered positive. A sample was rejected for further analysis if it was composed of necrotic tissue or was deemed to represent normal tissue.

Table 1
Patient and tumor characteristics.

	Epithelial ovarian cancer $n = 41$	Endometrial cancer $n = 40$
Tumor grade, *n* (%)		
Grade 1	1 (2.4)	12 (30.0)
Grade 2	9 (22.0)	9 (22.5)
Grade 3	31 (75.6)	19 (47.5)
Tumor histology, *n* (%)		
Serous	36 (87.8)	10 (25.0)
Endometrioid	1 (2.4)	23 (57.5)
Clear cell	2 (4.9)	2 (5.0)
Mucinous	–	1 (2.5)
Mixed	2 (4.9)	3 (7.5)
Carcinosarcoma	–	1 (2.5)
FIGO[a] stage, *n* (%)		
I	2 (4.9)	18 (45.0)
II	–	2 (5.0)
III	30 (73.1)	6 (15.0)
IV	9 (22.0)	14 (35.0)
Debulking status, *n* (%)		
No residual disease	34 (82.9)	35 (87.5)
Residual disease > 1 mm	5 (12.2)	3 (7.5)
Inoperable	2 (4.9)	2 (5.0)
Follow–up, *median* (95% CI)		
Time to recurrence (months)	9.0 (6.3–15.3)	16.8 (2.9–Inf)
Time to death (months)	39.8 (26.3–50.0)	34.0 (7.1–Inf)
Vital status, *n* (%)		
Alive	4 (9.8)	29 (72.5)
Deceased	36 (87.8)	11 (27.5)
Missing	1 (2.4)	–

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