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Folate Receptor Alpha Expression in Lung and Ovarian Cancers by IHC Using a High Affinity Folate Receptor Alpha Antibody [26B3.F2]

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Context

Folate receptor alpha (FRA) is a cell-surface glycoprotein responsible for the transport of folate across cell membranes. FRA has demonstrated restricted expression in polarized epithelial cells in a subset of normal tissues, as well as certain cancers of epithelial origin. FRA expression has been identified in the majority of lung adenocarcinomas (LADC) and serous (non-mucinous) ovarian and endometrial carcinomas, as well as a limited subset of breast cancers.

FRA as a prognostic factor & therapeutic target in LADC

Analysis of mRNA levels in LADC has shown that higher expression levels of FRA is a positive prognostic factor for patient survival and disease-free survival, following surgical resection. Conversely, increased FRA expression has been found to be a predictor of negative outcomes in ovarian, endometrial, and breast cancers.

Currently, therapeutic strategies exploiting FRA are in late-stage clinical development. In particular, an anti-FRA monoclonal antibody, farletuzumab, is currently being evaluated as a therapeutic treatment in both non-small cell lung adenocarcinoma and ovarian cancer.

Immunohistochemistry of FRA

In order to consistently and reliably evaluate FRA expression for clinical and research purposes, a routine method for IHC of FRA in formalin-fixed paraffin-embedded tissues was necessary. Toward this goal, a new, highly specific mouse monoclonal anti-FRA antibody [26B3.F2] was developed and thoroughly characterized.[1]

IHC of FFPE using the anti-FRA [26B3] antibody demonstrated expression of FRA in a variety of normal tissues, including pancreas, thyroid, hypophysis, breast, lung, salivary gland, kidney, and cervix. Notable malignant tissues that exhibited FRA expression included serous ovarian carcinoma, endometrial carcinoma and the majority of cases of LADC.

A semi-quantitative scoring algorithm, the M-score, has been reported for evaluating FRA staining by intensity and proportion (see Design for details). In LADC patients treated by surgical resection, higher FRA expression, as measured by the M-score, was found to be a positive prognostic factor for overall survival.[2]

These results validate both the significance of FRA expression in LADC, as well as the utility of the M-score; thus, encouraging ongoing efforts to use IHC of FRA as a routine and informative tool.

Towards this end, an integrated kit of reagents for IHC of FRA has been assembled and validated, using the M-score, in order to provide a standardized toolkit for further studies and facilitate consistent data between laboratories.

Design

Formalin-fixed, paraffin-embedded whole tissues and tissue microarrays were analyzed by immunohistochemistry. The IHC protocol was optimized by evaluating various antigen retrieval protocols and antibody diluents and titers. The optimized protocol included heat-induced antigen retrieval using Diva Decloaker in a Decloaking Chamber (Biocare Medical), antibody incubation for 30 minutes, and detection using MACH 4 HRP Polymer (Biocare Medical) and visualization with DAB.

FRA staining was evaluated on a variety of normal and neoplastic tissues, including numerous cases of lung adenocarcinoma, lung squamous cell carcinoma, and ovarian cancer. A case was considered positive for FRA expression if 10% of the tumor cells were stained at any intensity.

In a further study, 26 cases of lung adenocarcinoma were evaluated using a semi-quantitative determination of FRA expression, the M-score, based on the proportion of cells staining at each intensity level. The M-score is calculated as follows:

$$M\text{-}score = (3x + 2y + z) / 6$$

x is the percent of tumor stained with intensity 3+, y is the percent of tumor stained with intensity 2+, and z is the percent of tumor stained with intensity 1+. The 26 cases of LADC, stained for FRA, were evaluated by three independent pathologists, at three separate sites, in order to assess the inter-observer reproducibility of the M-score as a method for evaluating FRA expression.

Results

Anti-FRA [26B3] exhibits membrane staining and is a sensitive marker of FRA expression. FRA staining was identified in 39/54 cases (72.2%) of LADC, with varying intensity (Figure 1). FRA is also strongly expressed in normal lung (Figure 2).

Notably, among non-small cell lung cancers (NSCLC), [26B3] stains the majority of lung adenocarcinomas, 39/54 (72.2%), whereas, a much smaller number of lung squamous cell carcinomas (SqCC) are positive for FRA, 4/37 (10.8%) (Table 1).

In ovarian cancers, [26B3] demonstrates high sensitivity for cases of serous cystadenocarcinoma (17/18, 94.4%) (Figure 3), while also staining a majority of endometriod adenocarcinoma cases (15/23, 65.2%). All mucinous cystadenocarcinoma cases were negative for FRalpha. (Table 1)

In order to investigate the inter-observer reproducibility of the integrated kit of reagents for IHC of FRA, 26 cases of LADC were stained with [26B3] and the M-score for each was determined by three independent pathologists. In 22/26 cases, the M-scores determined by each pathologist differed by ≤8 units (out of 50) between pathologists. Table 2 shows representative examples of 3 cases where at least one pathologist allocated the staining proportion and intensity significantly differently; however, in each case, the weighted nature of the semi-quantitative algorithm produced an M-score that was consistent between pathologists, a desirable result when different observers are reviewing the same stained slides.

Conclusions

IHC using anti-FRA [26B3] is a reliable method for the detection of FRA expression in LADC and ovarian cancers. [26B3] identified FRA in the majority of cases of LADC, while staining only a small subset of cases of lung SqCC. Among ovarian cancers, [26B3] was highly sensitive for serous cystadenocarcinoma, as well as endometrioid adenocarcinoma cases, but did not stain mucinous cystadenocarcinoma.

The inter-observer reproducibility of FRA staining interpretation and M-score determination encourages the continued development of [26B3] as the gold standard marker for FRA expression. Most importantly, as FRA associated therapies are currently in clinical development, determining the predictive and prognostic significance of FRA expression, as assessed by the M-score, is of the utmost importance.

Table 1

Sensitivity of anti-FRA [26B3] in non-small cell lung cancer and ovarian carcinoma.

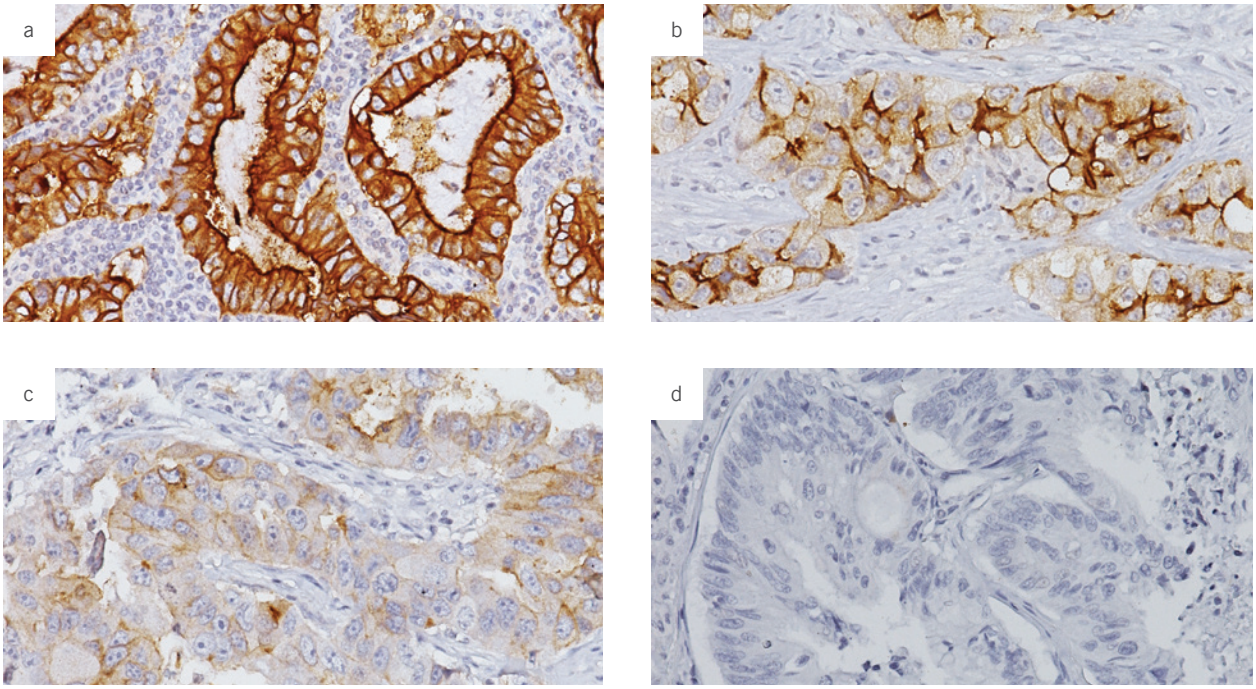
Anti-FRalpha [26B3.F2]	Lung ADC	Lung SqCC	Ovarian*
Positive Cases / Total Cases	39/54	4/37	32/41
Sensitivity	72.2%	10.8%	78.8%

Table 2

Sensitivity of anti-FRA [26B3] in non-small cell lung cancer and ovarian carcinoma.

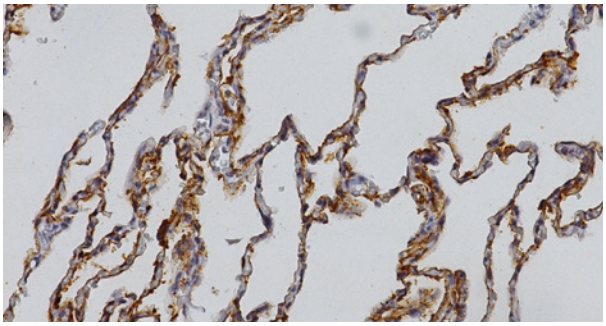
IHC of LADC with anti-FRA [26B3]		% cells staining at each intensity				M-score
		3+	2+	1+	0	
Case 1	Pathologist A	20	40	10	30	25
	Pathologist B	20	40	10	30	25
	Pathologist C	0	30	70	0	22
Case 2	Pathologist A	70	20	10	0	43
	Pathologist B	80	10	10	0	45
	Pathologist C	95	0	0	5	48
Case 3	Pathologist A	50	20	15	15	34
	Pathologist B	50	20	20	10	35
	Pathologist C	0	60	40	0	27

Figure 1



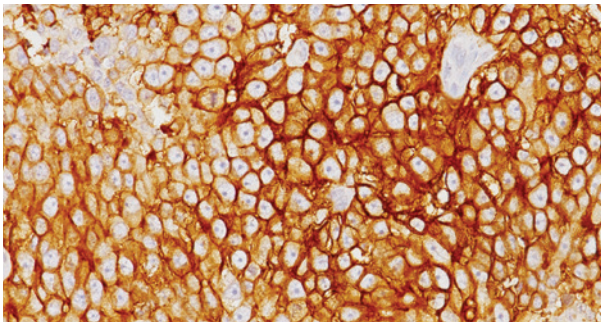
Anti-FRA [26B3] staining lung adenocarcinoma with intensity 3+ (A), 2+ (B), 1+ (C), and LADC negative for FRA (D).

Figure 2



Anti-FRA [26B3] staining normal lung.

Figure 3



Anti-FRA [26B3] staining ovarian serous cystadenocarcinoma.

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2. O'Shannessy DJ, Yu G, Smale R, Fu YS, Singhal S, Thiel RP, Somers EB, Vachani A. Folate Receptor Alpha Expression in Lung Cancer: Diagnostic and Prognostic Significance. Oncotarget. 2012 Apr;3(4):414-425.

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