High Expression of Folate Receptor Alpha in Lung Cancer Correlates with Adenocarcinoma Histology and Mutation

Maria Ines Nunez, MD,* Carmen Behrens, MD,† Denise M. Woods, BS,* Heather Lin, MD,‡ Milind Suraokar, PhD,† Humam Kadara, PhD,† Wayne Hofstetter, MD,§ Neda Kalhor, MD,* J. Jack Lee, PhD,‡ Wilbur Franklin, MD,‖ David J. Stewart, MD,† and Ignacio I. Wistuba, MD*‡

Introduction: Folate receptor alpha (FRα) and reduced folate carrier-1 (RFC1) regulate uptake of folate molecules inside the cell. FRα is a potential biomarker of tumors response to antifolate chemotherapy, and a target for therapies using humanized monoclonal antibody. Information on the protein expression of these receptors in non–small-cell lung carcinoma (NSCLC) is limited.

Material and Methods: Expressions of FRα and RFC1 were examined by immunohistochemistry (IHC) in 320 surgically resected NSCLC (202 adenocarcinomas and 118 squamous cell carcinomas) tissue specimens and correlated with patients’ clinicopathologic characteristics. Folate receptor α gene (FOLR1) mRNA expression was examined using publicly available microarray datasets. FRα expression was correlated with thymidylate synthase and p53 expression in NSCLCs, and with epidermal growth factor receptor (EGFR) and V-Ki-ras2 Kirsten rat sarcoma viral (KRAS) gene mutations in adenocarcinomas.

Results: NSCLC overexpressed FRα and RFC1. In a multivariate analysis, lung adenocarcinomas were more likely to express FRα in the cytoplasm (OR = 4.39; p < 0.0001) and membrane (OR = 5.34; p < 0.0001) of malignant cells than squamous cell carcinomas. Tumors from never-smokers were more likely to express cytoplasmic (OR = 3.35; p = 0.03) and membrane (OR = 3.60; p = 0.0005) FRα than those from smokers. In adenocarcinoma, EGFR mutations correlated with higher expression of membrane FRα and FOLR1 gene expressions. High levels of FRα expression was detected in 42 NSCLC advanced metastatic tumor tissues.

Conclusions: FRα and RFC1 proteins are overexpressed in NSCLC tumor tissues. The high levels of FRα in lung adenocarcinomas may be associated to these tumors’ better responses to antifolate chemotherapy and represents a potential novel target for this tumor type.

Key Words: Non–small-cell lung carcinoma, Epidermal growth factor receptor, membrane transporter, Folate receptor alpha, Reduced folate carrier-1.

Lung cancer represents the first cause of death for cancer worldwide.1 Most patients with lung cancer are diagnosed at advanced metastatic stage (IV), requiring systemic treatment.1 Two types of non–small-cell carcinoma (NSCLC), adenocarcinoma and squamous cell carcinoma (SCC), are the most frequent (~80%) histological types of lung cancer.2 Despite intensive research on molecular targeted therapy, chemotherapy still represents the main treatment option for patients with advanced NSCLC.3 In addition, over recent years chemotherapy after surgical resection has become the standard of care for treatment of selected patients with early-stage (i.e., stage IB, II, or IIIA) NSCLC.4 However, a subset of tumors does not respond to chemotherapy, and most tumors develop drug resistance, leading to chemotherapy failure.5 The factors associated with chemotherapy resistance are not well understood, but some phenomena have been associated with this resistance, including, among others, decreases or alterations in the membrane transporters involved in drug-uptake systems or increase in drug-efflux pumps.5 Folates are required in the synthesis of nucleotide bases, amino acids, and other methylated compounds, and consequently, they are required in larger quantities by proliferating cells.5 FRα is a glycoprotein that is anchored to the apical cell membrane of normal epithelial cells,5 and binds folate at a high affinity to mediate transport into the cytoplasm of cells.5 RFC1 is more ubiquitously expressed in normal cells, binds folate at low affinity, and represents the sole folate-uptake pathway for most normal cells.7

FRα expression is upregulated in a range of human tumors, including ovarian, mesothelioma, lung, and colorectal cancer.5–13 However, the level of expression of RFC1 in
tumors is less known. FRα has emerged as a potential marker for response to treatment of human carcinomas with the drug pemetrexed,\(^1\) a potent inhibitor of thymidylate synthase (TS) and other folate-dependent enzymes.\(^5\)–\(^7\) Interestingly, FRα has been also investigated as a potential novel molecular target for human tumors.\(^8\)–\(^\)\(^1\)\(^0\) Recently, a humanized monoclonal antibody against FRα has been tested in a phase I clinical trial in patients with advanced chemorefractory ovarian carcinomas.\(^1\)\(^9\)

In this study, we aimed to characterize the expression of FRα and RFC1 proteins in a large series (n = 320) of surgically resected NSCLC tissue specimens with annotated clinico-pathologic features. In addition, we investigated the expression of FRα in a small series (n = 42) of advanced metastatic NSCLC tumor tissues. In surgically resected tumors we correlated the expression of FRα with the expression of TS. Our findings of higher expression of FRα expression in lung tumors with adenocarcinoma histology and tumors obtained from never-smokers prompted us to correlate the expression of FRα with tumors’ epidermal growth factor receptor (EGFR) and KRAS mutation status in adenocarcinomas, and with tumors’ p53 protein expression in all NSCLCs.

**METHODS**

**Case Selection and Tissue Microarray Construction**

We obtained archived formalin-fixed and paraffin-embedded (FFPE) NSCLC tissues from the Lung Cancer Tissue Bank at The University of Texas M. D. Anderson Cancer Center (Houston, TX). We selected lung cancer tissue specimens from surgically resected NSCLCs with curative intent between 1997 and 2001, and constructed TMAs using three 1-mm diameter cores. Detailed clinico-pathologic information was available for most cases (Table 1). In addition, we selected FFPE NSCLC tumor tissues from diagnostic tissue specimens from 42 advanced metastatic NSCLCs. The tissue specimens were histologically classified according to the 2004 World Health Organization classification.\(^2\) The institutional review board at M. D. Anderson Cancer Center approved our study.

**Immunohistochemical Staining and Evaluation**

To test the expression of the membrane transporters, we used a monoclonal homemade antibody against FRα (clone Mb343, immunoglobulin G), dilution 1:500,\(^1\) and a polyclonal antibody against RFC1 (Abcam, Cambridge, MA), dilution 1:100. To assess the expression of TS, we used a monoclonal antibody (Zymed Carlsbad, CA), dilution 1:100. For p53 analysis, we used mouse monoclonal anti-human p53, clone DO7 (Dako, Carpinteria, CA), dilution 1:400.

For FRα we used a previously published IHC protocol.\(^1\) For RFC1 and TS, immunohistochemical staining was performed as follows: 5-µM FFPE tissue sections were deparaffinized and hydrated, and underwent heat-induced epitope retrieval in a DAKO antigen retrieval bath (Dako) at 121°C for 30 seconds and 90°C for 10 seconds in a decloaking chamber (Biocare, Concord, CA), followed by a 30-minute cool down. Before antibody immunostaining, endogenous peroxidase activity was blocked with 3% H\(_2\)O\(_2\) in methanol for 30 minutes. To block nonspecific antibody binding, tissue sections were incubated with 10% fetal bovine serum in Tris-buffered saline solution with Tween 20 for 30 minutes. The slides were incubated with primary antibody at ambient temperature for 60 minutes for all antibodies. This was followed by incubation with biotin-labeled secondary antibody (Envision Dual Link +, DAKO) for 30 minutes.

| TABLE 1. Summary of Clinico-Pathologic Features of Patients with NSCLC Examined for Membrane Transporter and Thymidylate Synthase Expression |
|-----------------|-----------------|-----------------|
| **Feature**     | **Squamous Cell Carcinoma (n = 118)** | **Adenocarcinoma (n = 202)** | **Total (n = 320)** |
| Mean age, years (SD), (range) | 68.4 (9.20), (43–90) | 64.9 (11.5), (33–88) | 66.2 (10.85), (33–90) |
| Sex             |                             |                             |                     |
| Male            | 73                          | 77                          | 150                 |
| Female          | 45                          | 125                         | 170                 |
| Smoking status* |                             |                             |                     |
| Never           | 4                           | 52                          | 56                  |
| Ever            | 113                         | 150                         | 263                 |
| TNM stage       |                             |                             |                     |
| I               | 62                          | 134                         | 196                 |
| II              | 36                          | 25                          | 61                  |
| III             | 18                          | 36                          | 54                  |
| IV              | 2                           | 7                           | 9                   |

*Smoking status and history were not available for one patient with squamous cell carcinoma.

NSCLC, non–small-cell lung carcinoma; TNM, Tumor, Node, Metastasis.
Staining was developed with 0.05% 3',3-diaminobenzidine tetrahydrochloride, which had been freshly prepared in 0.05 mol/L Tris buffer at pH 7.6 containing 0.024% H2O2, and then the slides were counterstained with hematoxylin, dehydrated, and mounted.

Two observers (M.N. and I.W.) jointly quantified the immunohistochemical expression of the membrane transporters (magnification ×20) in normal bronchial epithelium and lung tumor malignant epithelial cells. For each membrane transporter and TS, we defined three categories of intensity of immunostaining (0−3+). Next, an expression score (range, 0–300) was obtained by multiplying the intensity of staining by the percent of cells (0%–100%) staining. p53 expression was categorized by percentage of tumor cells expressing nuclear p53 as positive (greater than or equal to 5%) or negative (0%–5%).

**EGFR and KRAS Mutation Analysis**

Exons 18 through 21 of *EGFR* and exon 1 of *KRAS* were amplified by polymerase chain reaction (PCR) using intron-based primers as previously described.21,22

**Assessment of Membrane Transporter Expression in Microarray Data Sets**

The cancer microarray database and integrated data-mining platform Oncomine was utilized to analyze the expression of *FOLR1* (FRɑ) and *SLC19A1* (RFC1), and in microarray databases of NSCLC available online.24–27 The statistical significances of differences in expression of the genes were provided by Oncomine and confirmed by a two-tailed t test with random variance. Gene expression data of lung adenocarcinomas with annotated mutation data of *EGFR* and *KRAS* were obtained from the Ladanyi and Gerald laboratories at the Memorial Sloan-Kettering Cancer Center (http://cbio.mskcc.org/Public/lung_array_data/).28 Available Affymetrix raw data files of the transcriptomes of 190 adenocarcinomas (set I, n = 88; set II, n = 102) were analyzed using the BRB-ArrayTools version 3.7.0 software developed by using the BRB-ArrayTools v.3.7.0 developed by Dr. Richard Simon and BRB-ArrayTools Development Team.29 Robust multiarray analysis was used for normalization of gene expression data using the R language environment.30 *FOLR1* mRNA expression levels in both Memorial Sloan-Kettering Cancer Center data sets were median-centered by the Cluster v.2.11 software. Differences in normalized median-centered *FOLR1* expression levels were assessed for statistical significance by the two-tailed t test and p < 0.05 was considered statistically significant.

**Statistical Methods**

Associations between biomarker expression scores and patient clinico-pathologic data were assessed using the Wilcoxon ranked sum test or Kruskal-Wallis test, as appropriate, for continuous variables and the Chi-square test for categorical variables. The immunohistochemical expression of the markers was dichotomized in negative (score = 0) and positive (score > 0) expressions based on the graphical distribution of the scores. For recurrence free survival (RFS) and overall survival (OS) analyses, we tested binary cutoff points of biomarkers using the median expression score for each marker. Univariate and multivariate Cox proportional hazards regression models were used to assess the effects of covariates on survival. All statistical tests were two-sided, and p values <0.05 were considered statistically significant.

**RESULTS**

Expression of FRɑ and RFC1 in Surgically Resected Tumors

Both adenocarcinoma and SCC expressed relatively high levels of FRɑ and RFC1 in the malignant cells (Fig. 1

<table>
<thead>
<tr>
<th>Marker</th>
<th>Adenocarcinoma (Score &gt; 0)</th>
<th>SCC (Score &gt; 0)</th>
<th>p Valueb</th>
<th>Adenocarcinoma Average Score</th>
<th>SCC Average Score</th>
<th>p Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRɑ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Positive/Total (%)</td>
<td>Positive/Total (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>152/174 (87)</td>
<td>63/110 (57)</td>
<td>&lt;0.001</td>
<td>91.6 (66.4)</td>
<td>35.9 (40.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RFC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>107/174 (61)</td>
<td>29/110 (26)</td>
<td>&lt;0.001</td>
<td>72.2 (89.0)</td>
<td>11.29 (28.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Membrane</td>
<td>181/182 (99)</td>
<td>110/112 (98)</td>
<td>0.56</td>
<td>162.7 (83.2)</td>
<td>153.2 (72.0)</td>
<td>0.34</td>
</tr>
<tr>
<td>TS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>164/182 (90)</td>
<td>103/112 (92)</td>
<td>0.68</td>
<td>128.1 (95.9)</td>
<td>119.2 (86.1)</td>
<td>0.59</td>
</tr>
<tr>
<td>Nuclear</td>
<td>130/165 (79)</td>
<td>82/102 (80)</td>
<td>0.75</td>
<td>52.2 (40.1)</td>
<td>55.6 (42.0)</td>
<td>0.565</td>
</tr>
<tr>
<td></td>
<td>58/165 (35)</td>
<td>59/102 (58)</td>
<td>0.0003</td>
<td>9.3 (21.1)</td>
<td>13.8 (27.7)</td>
<td>0.0043</td>
</tr>
</tbody>
</table>

aFisher’s exact test.

bWilcoxon ranked sum test.

SCC, squamous cell carcinoma; FRɑ, folate receptor alpha; RFC1, reduced folate carrier-1; TS, thymidylate synthase.

Copyright © 2012 by the International Association for the Study of Lung Cancer
and Table 2). For FRα, the average expression scores and frequency of any expression (score > 0) were significantly higher in adenocarcinomas than in SCCs at membrane (p < 0.001) and cytoplasmic (p < 0.001) localizations (Fig. 2). Both NSCLC histologies demonstrated similar levels of cytoplasmic and membrane RFC1 expression. For both markers the tumor cells exhibited stronger immunohistochemical expression than the 11 samples of normal bronchial epithelia adjacent to tumors (data not shown).

**FIGURE 1.** Photomicrographs showing immunohistochemical expression of folate receptor alpha (FRα), reduced folate carrier-1 (RFC1) and thymidylate synthase (TS) in non–small-cell lung carcinoma tissue specimens by histologic type. FRα: (A), strong cytoplasmic and membrane expression in tumor cells; (B and C), moderate expression in tumor cells; (D), lack of expression in malignant cells. RFC1: (E and G), strong cytoplasmic expression in malignant cells. TS: (F and H), negative and moderate cytoplasmic and nuclear expression in tumor cells, respectively. Original magnification, ×200. FRα, folate receptor alpha; TS, thymidylate synthase; RFC1, reduced folate carrier-1.

**Correlation of FRα and RFC1 Expression with Clinico-Pathologic Features in Surgically Resected Tumors**

The multivariate analysis of the immunohistochemical expression of the two membrane transporters as a dichotomized variable (positive, score >0, vs. negative, score =0), after adjustment for patient’s tumor histology, smoking history, sex, and disease stage, revealed that adenocarcinomas were more likely

**FIGURE 2.** FRα expression scores by tumor histology. In the box-plots, indicates median scores. FRα, folate receptor alpha.
than SCCs to express cytoplasmic (odds ratio [OR] = 4.39; \( p < 0.0001 \)) and membrane (OR = 5.34; \( p < 0.0001 \)) FR\( \alpha \). In addition, tumors from never-smokers were significantly more likely to express cytoplasmic (OR = 3.35; \( p < 0.03 \)) and membrane (OR = 3.60; \( p = 0.0005 \)) FR\( \alpha \) than those of smokers. In the multivariate analysis, the patient’s sex was not an independent significant factor influencing tumor expression of FR\( \alpha \). No correlation was found between expression of both membrane transporters and RFS or OS in 230 patients with stage I or II NSCLCs (median follow-up, 7.2 years).

**Correlation between FR\( \alpha \) Expression and Tumors’ p53 Expression and EGFR and KRAS Mutation Status**

Our findings of higher expression of FR\( \alpha \) expression in lung tumors with adenocarcinoma histology and tumors obtained from never-smokers prompted us to correlate the expression of FR\( \alpha \) with tumors’ EGFR and KRAS mutation status in adenocarcinomas, and with tumors’ p53 protein expression in all NSCLCs.

In lung adenocarcinomas, EGFR mutant tumors demonstrated significantly higher expression scores for membrane FR\( \alpha \) (mean scores: mutant 134.8 versus wild-type 67.1; \( p = 0.002 \)) than wild-type tumors (Fig. 3A). No correlation between FR\( \alpha \) expression and adenocarcinoma tumors’ KRAS mutation status was detected.

Of all NSCLCs tested, 38% (75 of 195) of adenocarcinomas and 69% (80 of 116) of SCCs had a positive p53 level (5% or more). Interestingly, we found that the scores for FR\( \alpha \) expression in both membrane (\( p = 0.001 \)) and cytoplasm (\( p < 0.001 \)) were significantly lower in malignant cells from NSCLC tumors with positive p53 expression (mean score: membrane 33.4, SD 59.9, and cytoplasm 58.3, SD 60.0) than...
in tumors with negative p53 expression (mean score: membrane 65.3, SD 90.6, and cytoplasm 83.55.3, SD 65.3).

**Expression of FRα in Advanced Metastatic Tumors**

To determine the levels of FRα expression in the entire spectrum of NSCLC, we examined FRα expression in 42 tumor tissues obtained from advanced NSCLCs (27 from lung/pleural tumors and 15 from metastatic sites). The tumor histologies corresponded to 23 adenocarcinomas, 5 SCCs, and 14 tumors classified as NSCLCs without features of specific histology (NSCLC—not otherwise specified). We found that advanced tumors demonstrated similar levels of FRα expression than surgically resected tumors by examining the average expression scores and frequency of any expression (score >0). Although small numbers, in the advanced tumors the FRα average expression scores were higher in adenocarcinomas than SCCs at membrane (mean score: adenocarcinoma 62.2, SD 81.2; SCC 20.0, SD 44.7; p = 0.042) and cytoplasmic (mean score: adenocarcinoma 104.1, SD 88.5; SCC 22.0, SD 39.0; p = 0.319) locations. NSCLC—not otherwise specified showed intermediate levels of FRα expression (mean score: membrane 20.7, SD 56.9, and cytoplasm 64.6, SD 96.5). In advanced tumors, any membrane expression (score > 0) of FRα was detected in 48% (11 of 23) of adenocarcinomas and 20% (1 of 5) of SCCs, whereas cytoplasmic expression was observed in 78% (18 of 23) and 40% (2 of 5) SCCs. No significant difference in the expression of FRα was detected when comparing lung/pleural tumors with metastatic sites (data not shown).

**FOLR1 mRNA Expression in Tumor Tissues**

Our findings that protein levels of FRα was greater in adenocarcinomas than in SCCs incited us to analyze expression levels of the mRNA of the *FOLR1* in published microarray data sets of surgically resected (stages I–III) NSCLC tumor specimens and compare them by histologic type. To confirm our findings on the increased FRα immunoreactivity in tumors obtained from *EGFR* mutant lung adenocarcinomas compared with wild-type tumors, we probed this association using the mRNA expression levels of *FOLR1* in publicly available microarray data sets with information on *EGFR* and *KRAS* mutation status. Notably, the analysis of the microarray data further revealed the statistically significant upregulation of *FOLR1* mRNA levels in *EGFR* mutant lung adenocarcinomas compared with wild-type tumors in both available data sets (p = 0.00016 and p = 0.003) (Fig. 3B and C). In addition, no statistically significant differences were found in *FOLR1* expression levels between *KRAS* mutant lung adenocarcinomas and wild-type tumors (data not shown). These findings confirm the close positive association between *FOLR1* expression and *EGFR* mutation status, which we had found at the protein level by assessment of FRα immunoreactivity.

**Correlation of Immunohistochemical Expression of TS and FRα**

TS was expressed frequently in the nucleus and cytoplasm of malignant NSCLC cells. However, the frequency of any TS expression (score > 0) was higher in the cytoplasm (212 of 267, 79%) than in the nucleus (117 of 267, 44%). Although cytoplasmic expression of TS was similar in both NSCLC histologic types (Table 2), nuclear expression was significantly higher (p = 0.003) in SCCs (mean score: 13.8, SD 27.7) than in adenocarcinomas (mean score: 9.3, SD 27.1). The level of TS expression did not correlate with clinico-pathologic characteristics, including RFS and OS. In all NSCLC, significantly (p = 0.02) higher expression of nuclear TS immunostaining was detected in tumors with positive p53 expression (67 of 114, 58%) than in those with negative p53 staining (65 of 147, 44%). In adenocarcinomas, there was no correlation between TS expression and *EGFR* or *KRAS* mutation status.

We correlated the expression of TS and FRα in NSCLC tissue specimens. The score for nuclear TS expression correlated negatively with the score for cytoplasmic FRα expression in SCCs (r = −0.20; p = 0.04), and showed marginally significant negative correlation with membrane FRα expression in adenocarcinomas (r = −0.16; p = 0.05). When we examined the correlation of any expression (score > 0) of both markers in tumors, we found that in SCCs expression of nuclear TS was significantly inversely correlated (p = 0.03) with membrane expression of FRα, and that most tumors positive for TS (62 of 79, 79%) lacked membrane FRα. This correlation was not detected in adenocarcinomas.

**DISCUSSION**

Membrane transporters FRα and RFC1 are considered potential biomarkers of tumor response to antifolate chemotherapy. In addition, FRα represents a novel target for therapy in human carcinomas utilizing monoclonal antibodies. Information on the protein expression of these receptors in NSCLC is limited. Here, we report for the first time that NSCLC frequently overexpresses both FRα and RFC1 proteins by studying a large series of cases with annotated clini-co-pathologic information. Importantly, we report that tumor cells from lung adenocarcinoma histology expressed significantly higher levels of cytoplasmic and membrane FRα than SCC, and tumors from never-smokers were significantly more likely to express cytoplasmic FRα than those from smokers. In lung adenocarcinomas, the presence of *EGFR* mutations correlated with higher expression of membrane FRα and *FOLR1* gene expression. NSCLC tissue specimens from advanced metastatic tumors showed similar levels of FRα expression than surgically resected tumors. We postulate that this information may be useful in selecting which patients with NSCLC may benefit from receiving treatment with antifolate inhibiting agents and monoclonal antibodies against FRα.

Our study showed that RFC1 is expressed frequently in the membrane and cytoplasm of malignant cells of NSCLC tumor tissues. Although RFC1 performs its important biological functions at the cell membrane, the cytoplasmic expression can be explained as part of synthesis of the protein. The only report available on the expression of RFC1 in human tumors showed relatively high
levels of mRNA gene expression in NSCLC, with similar expression in adenocarcinomas and SCCs. These data are consistent with our protein expression data showing that levels of expression of RFC1 were similar in the two histologic types.

Interestingly, in our study the expression of membrane and cytoplasmic FRα was significantly higher in surgically resected lung adenocarcinomas compared with SCCs. FRα is bound to the cell membrane which binds to folate and internalizes it in the cytoplasm through endocytosis. Similar trend was detected in a small set of advanced metastatic NSCLC tumor tissues. FRα has been shown by immunohistochemical studies to be overexpressed previously in small sets of NSCLC tissue specimens. However, to the best of our knowledge, there is no published report of FRα protein expression in NSCLC tumors and correlation with clinical and pathological features. Our protein expression findings agree with the significantly higher levels of expression of FOLR1 (FRα gene) mRNA in adenocarcinomas than in SCCs in all four public microarray data sets available. Similar findings have been reported in a quantitative PCR study of mRNA expression of 119 NSCLC tissue specimens.

The findings of higher levels of FRα protein and FOLR1 mRNA expression in adenocarcinomas than in SCCs of the lung may have important clinical implications. The higher level of FRα protein expression in adenocarcinomas may explain the better response of advanced NSCLC of nonsquamous histology when treated with the combination of cisplatin and the multitargeted antifolate agent pemetrexed. However, this needs to be further tested in NSCLC tumor tissue specimens obtained from patients treated with pemetrexed. In addition, FRα is currently considered an attractive target for biologic therapy in tumors in which it is overexpressed compared with corresponding normal epithelium such as ovarian cancer by using the humanized monoclonal antibody against FRα Farletuzumab. Our findings of high expression of FRα in NSCLC compared with normal bronchial epithelium suggest that this protein could be considered a novel potential target for NSCLC, particularly in lung adenocarcinomas.

Our finding that NSCLCs of never-smokers have a higher expression of FRα than those of smokers is of interest. Our data showing significantly higher cytoplasmic and membrane FRα expression in NSCLCs obtained from never-smokers are in agreement with the previous report of higher levels of mRNA FOLR1 by quantitative PCR in adenocarcinomas from nonsmokers and light smokers than in those from heavy smokers. These differences in the expression of FRα by smoking status are consistent with our findings of higher FRα expression in NSCLCs lacking p53 expression and in adenocarcinomas harboring EGFR mutation, two features associated with the pathogenesis of non–smoking-related lung cancer. Of interest, the analysis of the publicly available microarray data confirmed at mRNA gene expression level our observation that EGFR mutant adenocarcinoma tumors expressed higher levels of FRα protein. There are not data available on the response to antifolate chemotherapy agents in lung adenocarcinomas based on EGFR mutation status. However, it has been shown that advanced stage adenocarcinoma harbouring this mutation showed improved response to other type of (carboplatin and paclitaxel) chemotherapy.

Because of their roles in metabolism of the chemotherapy agent pemetrexed, we correlated the expressions of TS and FRα in NSCLC tissue specimens by histologic type. As previously reported, TS protein was expressed frequently in the nucleus (44%) and cytoplasm (79%) of malignant NSCLC cells. In our analysis, we determined that nuclear expression was significantly higher in SCCs than in adenocarcinomas. Ceppi et al. previously reported that immunohistochemical expression of TS mRNA and protein was significantly higher in SCCs of the lung than in adenocarcinomas. In this previously reported immunohistochemical analysis, however, expression of TS in the malignant cells was not distinguished as nuclear or cytoplasmic. It has been shown that low levels of TS mRNA expression significantly correlated with in vitro chemosensitivity of freshly explanted human tumor specimens to pemetrexed. It has been hypothesized that the higher mRNA and protein expressions of TS observed in SCCs explains the lower rate of response to pemetrexed in this NSCLC type. Recently, Sun et al. reported that low immunohistochemical TS protein expression in tumors correlated with worse progression-free survival in stage IIIIB and IV patients with nonsquamous cell lung carcinomas treated with pemetrexed.

When we correlated FRα and TS protein expression in NSCLC tumors, we found that in SCCs the expression of nuclear TS had a significant inverse correlation with expression of membrane FRα, and most TS-positive SCCs (79%) lacked membrane FRα immunostaining. Furthermore, we speculate that the more frequent occurrence of the FRα-membrane–nuclear expression pattern in lung SCCs than in adenocarcinomas could be associated with the lower response rate to pemetrexed in this tumor type. Although FRα is most biologically active at the cell membrane, there is strong evidence of the important role of TS as translational regulation in the nucleus of cells.

In summary, our findings indicate that membrane transporter FRα and RFC1 proteins are frequently overexpressed in NSCLC tissues. The higher level of FRα in adenocarcinomas than in SCCs may help explain differences in efficacy of antifolate chemotherapy between these tumor types. We postulate that this information may be useful in selecting which patients with NSCLC may benefit from and should receive treatment with antifolate inhibiting agents and with monoclonal antibodies against FRα.

ACKNOWLEDGMENTS

This study was supported in part by grants from the Department of Defense (W81XWH-07-1-0306 to David J. Stewart, MD and Ignacio I. Wistuba, MD), the Specialized Program of Research Excellence in Lung Cancer (P50CA70907 to Ignacio I. Wistuba), and the National Cancer Institute (Cancer Center Support Grant CA-16672).

REFERENCES


