Folate Receptor in Adenocarcinoma and Squamous Cell Carcinoma of the Lung
Potential Target for Folate-Linked Therapeutic Agents

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Context.—Lung cancer is the number one cause of cancer deaths in the United States and globally. The advent of targeted therapies has offered a new treatment paradigm for lung cancer, but currently validated and emerging drugs are effective in only a small minority of lung cancers, predominantly adenocarcinomas. Folate receptors can serve as targets for drugs attached to folate and are overexpressed in many cancers.

Objective.—To determine the frequency of folate receptor overexpression in lung cancers of different cell types as potential targets for folate-linked therapy.

Design.—High-density tissue microarrays were constructed from archival formalin-fixed, paraffin-embedded resection specimens from 188 primary stage I or stage II adenocarcinomas or squamous cell carcinomas of the lung with three 0.1-cm cores from each tumor. Tissue microarrays were immunostained for folate receptor with mAb343 and the results scored (0 to 1+ = weak expression, 2+ to 3+ = strong expression).

Results.—Eighty-four of 117 (72%) of the adenocarcinomas were strongly positive for the folate receptor, and 36 of 71 (51%) of the squamous cell carcinomas were strongly positive for the folate receptor.

Conclusions.—Our data indicate that a large percentage of lung cancers, including squamous cell carcinomas in addition to adenocarcinomas, strongly express folate receptor. This suggests that folate-linked targeted therapy can potentially be used to treat the majority of lung cancers, both adenocarcinomas and, particularly, squamous cell carcinomas, that do not respond to current targeted therapies.

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Lung cancer is the number one cause of cancer deaths in the United States and in the world, with little improvement in 5-year survival during the past half century. Five-year survival for male lung cancer patients ranges from 6% to 14% and for female patients ranges from 7% to 18%. In the United States, annual deaths from lung cancer surpass the combined total deaths from the next 3 leading causes of cancer deaths (colon, breast, and prostate cancers) combined. In 2012, there will be an estimated 160,340 deaths from lung cancer in the United States, representing about 29% of all cancer deaths among men and 26% of all cancer deaths among women. Worldwide, the number of estimated deaths from lung cancer in 2011 was 1,378,400.

In the past few years, a new paradigm in the treatment of lung cancer has emerged with the molecular targeted therapy of lung cancer, offering hope of improved prognosis to select groups of lung cancer patients. The most reliable basis for selecting lung cancer patients for these targeted therapies is identification of predictive biomarkers in the lung cancer tissue. To date, 2 predictive markers and their associated therapies are validated by clinical trials: activating mutations of the epidermal growth factor receptor (EGFR) gene are more prevalent in patients who respond to EGFR therapy (tyrosine kinase inhibitors: gefitinib and erlotinib, with afatinib on the horizon), and the presence of anaplastic lymphoma kinase (ALK) fusion genes predicts response to ALK therapy (crizotinib). Beginning in 2010, the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology initiated an expert panel, steering committee, and advisory board to write lung cancer biomarkers guidelines under the auspices of the College of American Pathologists Pathology and Laboratory Quality Center, with an initial focus on EGFR and ALK testing. Multiple other potential predictive biomarkers and corresponding targeted therapies are in various stages of investigation.
The frequency of EGFR mutations in lung cancers ranges from 32% in East Asians to 7% in whites to 2% in African Americans.\textsuperscript{21,22} The frequency of ALK rearrangements is generally estimated at 4% of adenocarcinomas, ranging from 1.6% in unselected advanced-stage non–small cell carcinomas in the United States to 3.7% in advanced-stage adenocarcinomas to 13.7% in advanced-stage adenocarcinomas in patients who have never smoked.\textsuperscript{23,24} EGFR and ALK rearrangements are reported overwhelmingly in adenocarcinomas, and it seems likely that squamous cell carcinomas do not harbor these mutations.\textsuperscript{25} Therefore, there is a great need to discover targeted therapies that can potentially be used to treat the majority of lung cancers, both adenocarcinomas and, particularly, squamous cell carcinomas, that do not respond to current targeted therapies.

Folate is a water-soluble vitamin required for nucleotide base biosynthesis in both normal and malignant cells. Cellular uptake of folates may be mediated by a reduced folate carrier, a proton-coupled folate transporter, or a high-affinity folate receptor (FR).\textsuperscript{26–29} Normal cells rely almost exclusively on the reduced folate carrier for their uptake of folate, 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.\textsuperscript{30} Folate has been recently exploited to deliver attached drugs selectively into cells that overexpress a cell-surface FR.\textsuperscript{28–33} Because FRs are expressed and accessible primarily on pathologic cells, folate conjugation allows selective delivery of nonspecific drugs into these pathologic cells. As a consequence, normal tissues lacking FR are spared the toxicity that commonly limits nontargeted therapies. Distribution and binding studies of the high-affinity FR have shown elevated expression in the majority of ovarian cancers, and lesser degrees of positivity in kidney, breast, uterine, colon, and lung cancers.\textsuperscript{34,35} Studies in ovarian,\textsuperscript{36} breast,\textsuperscript{37} and colon cancers\textsuperscript{38} have shown a negative correlation between FR overexpression and survival. In contrast, a similar study of lung cancer revealed a positive correlation with survival.\textsuperscript{39}

In a multi-institutional collaboration, we immunostained a lung cancer tissue microarray with mAb343,\textsuperscript{40} a murine monoclonal antibody directed against the $\alpha$ isoform of the human FR (FR-$\alpha$), to determine if this might be used as a target for folate-linked drugs.

**MATERIALS AND METHODS**

Institutional review board approval of research protocols for this project was obtained through The Methodist Hospital Research Institute. High-density tissue microarrays were constructed from archival formalin-fixed, paraffin-embedded resection specimens from 188 primary stage I or stage II adenocarcinomas or squamous cell carcinomas of the lung, resected between 1974 and 1991 at The Methodist Hospital, Houston, Texas. Histopathologic classification of all selected cases was performed, taking into consideration currently proposed revisions for classification.\textsuperscript{8} For each sample, areas rich in viable tumor cells were identified by light microscopic examination of hematoxylin-eosin–stained sections and selected for use in the tissue microarrays. Three cores measuring 0.1 cm in diameter were taken from the donor paraffin tissue blocks of each case and were arranged in a recipient paraffin tissue array block by using a manual tissue arrayer (MTA-1, Beecher Instruments Inc, Sun Prairie, Wisconsin). Retrospective chart review was performed to obtain clinical data, including patient age, sex, and smoking history.
Tissue microarrays were immunostained for FR-α with mAb343 using the following protocol. Formalin-fixed, paraffin-embedded samples were deparaffinized with 3 changes of xylene and then rehydrated in a series of ethanol/water solutions (100%, 95%, 70% ethanol) prior to rinsing in running distilled water. Slides were then incubated in a preheated (99°C) Dako Target Retrieval Buffer (Dako Cytomation, Carpinteria, California) for 40 minutes in an Isotemp 205 water bath (Fisher Scientific), then cooled in the same buffer for 20 minutes at room temperature prior to a 5-minute rinse in running distilled water. After epitope retrieval, slides were placed on a Dako autostainer for staining with mAb343 at room temperature. Slides were first incubated with peroxidase blocking reagent for 5 minutes to inactivate endogenous peroxidase, followed by 5 minutes incubation with a sniper block reagent. Sections were then incubated in a 1:500 dilution (Background Reducing Diluent, Dako Cytomation) of mAb343 (1.8 mg/mL) for 30 minutes prior to rinsing with Tris-buffered saline solution (TBST), containing Tween 20, pH 7.6 (Dako, Carpinteria, California). Staining was performed using a Mouse MACH3 detection kit (Biocare Medical, Walnut Creek, California) according to the manufacturer’s instructions. Stained sections were then rinsed with the TBST wash buffer and counterstained with modified Schmidt hematoxylin for 5 minutes, followed by a 3-minute rinse to blue sections. Samples were mounted with permanent mounting media on an automated coverslipper (Leica, North Central Instruments, Plymouth, Minnesota).

Positive immunostaining was membranous and cytoplasmic and scored in the same manner as previously reported for this antibody in breast cancer tissue microarrays. Immunopositivity in tumor cells was scored on a scale from 0 to 3 and then averaged for the 3 punches from the same tumor specimen. A score of 0 indicated absence of discernible staining; a score of 1 equaled weak, finely granular staining; a score of 2 represented a coarser granular staining; and a score of 3 reflected a strong, intense, coarsely granular staining. An average score of 0 or 1 was classified as weak expression, and an average score of 2 or 3 was classified as strong expression.

RESULTS

There were 117 primary adenocarcinomas and 71 squamous cell carcinomas. There were 54 women and 63 men among the patients with adenocarcinoma and 15 women and 56 men among the patients with squamous cell carcinoma. The average age of patients with adenocarcinoma was 67.5 years at diagnosis (range, 45–84 years). There were 5 never-smokers among the patients with adenocarcinoma and 15 never-smokers among the patients with squamous cell carcinomas. There were 54 women and 63 men among the patients with squamous cell carcinoma (a 75-year-old woman).

Of the adenocarcinomas, 84 (72%) were strongly positive for FR (Figure) and of the squamous cell carcinomas, 36 (51%) were strongly positive for FR.

COMMENT

Currently, a limited percentage of lung cancers are candidates for targeted therapies based on mutations such as EGFR mutations or ALK fusion genes. To date, the validated or investigational targets are largely limited to adenocarcinomas. By comparison, our data indicate that a much larger percentage of lung carcinomas are potential targets for FR-targeted therapies, including squamous cell carcinomas (51%) as well as adenocarcinomas (72%). In addition to their availability in a greater percentage of lung cancers, including squamous cell type, increased FR expression was not observed to be skewed toward special demographic groups, at least in terms of age, sex, or smoking status. This is in contrast to the current targeted therapies, which have tendencies to be more frequent in special demographic groups: female sex and never- or light-smokers for EGFR mutation and younger age and never- or light-smokers for ALK fusion gene (we did not investigate ethnicity in our series). All of these observations suggest that a greater percentage of lung cancer patients may benefit from FR-targeted therapies than from specific targeted therapy.

Folate and folate-linked drugs bind to FRs with nanomolar to subnanomolar affinities. Because cells lacking FR exhibit no affinity for folate-linked drugs, only cells that express accessible FR are observed to take up appreciable levels of folate-targeted drugs. Not surprisingly, lungs of healthy individuals display no uptake of folate-targeted radiolabeling agents, whereas lungs of many cancer patients display localized uptake at sites of malignant disease.

Based on the high affinity of folate conjugates for FR, 4 FR-targeted therapeutic agents and 1 FR-targeted imaging agent are currently undergoing human clinical trials. The folate-targeted therapeutic agents include a highly immunogenic hapten, a modified vinblastine, an epothilone, and a dual warhead folate conjugate containing both desacetylvinblastine and mitomycin C in the same molecule. The imaging agent is comprised of folate linked to a 99mTc chelating agent. The high level of FR expression in lung cancers argues that lung cancers could be a good indication for FR-targeted therapies.

References


