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ORIGINAL ARTICLE

Relationship of thyroid transcription factor 1 to *EGFR* status in non-small-cell lung cancer

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ABSTRACT

Background

Activating mutations of the epidermal growth factor receptor (*EGFR*) gene are known to drive a proportion of non-small-cell lung cancers. Identification of lung cancers harbouring such mutations can lead to effective treatment using one of the agents that targets and blocks EGFR-mediated signalling.

Methods

All specimens received at the BC Cancer Agency (Vancouver) for *EGFR* testing were prospectively identified and catalogued, together with clinical information and *EGFR* status, over a 14-month period.

Results

Specimens from 586 patients were received for *EGFR* testing, and *EGFR* status was reported for 509 patients. No relationship between specimen type or site of origin and *EGFR* test failure rate was identified. Concurrent immunohistochemical (IHC) status for thyroid transcription factor 1 (TTF1) was available for 309 patients. The negative predictive value of TTF1-negative status by IHC was 94.2% for predicting negative *EGFR* status.

Conclusions

In patients with limited tissue available for testing, a surrogate for *EGFR* status would aid in timely management. Immunohistochemistry for TTF1 is readily available and correlates highly with *EGFR* status. In conjunction with genetic assays, TTF1 could be used to optimize an *EGFR* testing strategy.

KEY WORDS

EGFR, TTF1, thyroid transcription factor 1, NSCLC, non-small-cell lung cancer, lung cancer, adenocarcinoma, biomarker testing

1. INTRODUCTION

Lung cancers are the leading cause of cancer-related mortality in the developed world. In recent decades, great strides have been made in treating this group of diseases, including improved surgical management, increased early detection, and newly available targeted therapies[1,2].

The epidermal growth factor receptor (EGFR) is the most well-characterized biologic target in lung cancer[3]. Activating mutations in this receptor tyrosine kinase cause constitutive activation of the mitogen-activated protein kinase pathway, driving increased cellular motility, invasiveness, and resistance to apoptosis. Activating *EGFR* mutations are known to underlie a significant number of adenocarcinomas[4], but can also be the drivers behind a smaller number of adenosquamous[5] or squamous cell carcinomas harbouring adenocarcinomatous components[6]. Established clinical risk factors for an EGFR-driven lung cancer include female sex and absence of a heavy tobacco-smoking history[7].

Monoclonal antibodies and small-molecule inhibitors have both been effective in blocking EGFR signalling and subsequently retarding tumour growth in lung cancer and other malignancies[8]. Optimal outcomes are achieved when targeted therapy is delivered selectively to patients with EGFR-driven lung cancers[1], thus establishing the need to accurately identify lung tumours driven by EGFR activation.

Recent guidelines on *EGFR* testing in lung cancers have advocated the use of polymerase chain reaction (PCR)–based testing for all lung tumours with adenocarcinoma-like components (and discretionary testing on additional patients based on the clinical risk factors mentioned earlier)[9].

Up to 75% of patients with lung cancer are diagnosed with advanced or metastatic disease and therefore do not undergo surgical procedures. The initial, often scanty, tissue samples are the only material available for biomarker testing. In patients with limited or no tumour tissue available for ancillary

studies, identification of surrogates for *EGFR* status can greatly contribute to timely management.

Thyroid transcription factor 1 (TTF1) is a tissue-specific transcription factor expressed in epithelial tissues of lung and thyroid. It is an important immunohistochemical marker for a diagnosis of pulmonary adenocarcinoma in routine pathology practice[10]. Furthermore, TTF1 likely plays a role in lung cancer biology, because amplifications of the *NKX2-1* locus (which codes for the TTF1 protein) occur frequently in the lung cancer genome[11].

Increased expression of TTF1 protein, detectable by immunohistochemistry (IHC), is well studied and has been associated with increased survival in lung adenocarcinoma patients[12]. Prior studies have shown significant correlations between TTF1 IHC and *EGFR* status[13,14]. It is clear from the medical literature that TTF1 is emerging, not just as a diagnostic tool, but also as a relevant biomarker in the treatment and study of lung adenocarcinoma.

2. METHODS

Institutional review board approval was obtained from the University of British Columbia and the BC Cancer Agency before initiation of the present research.

All cases referred to the BC Cancer agency for *EGFR* status assessment were prospectively collected over a 14-month period. Diagnostic material was obtained from formalin-fixed paraffin-embedded blocks for all cases. Each case was evaluated by a single pathologist (DNI) before genetic testing for cellularity and tumour content (expressed as the number of viable tumour nuclei divided by the total number of viable nuclei), and tumour-rich areas were marked for macrodissection.

Samples were tested by previously validated methods for in-frame deletions in exon 19 of *EGFR* by PCR and fragment length analysis. Additionally, samples were tested for the L858R point mutation in exon 21 by PCR and restriction fragment length polymorphism analysis. Both PCR assays were controlled for a minimum detection threshold of 2% mutant DNA[14,15].

Results of the *EGFR* status testing and clinicopathologic variables were compiled for statistical analysis. Among the included variables was the patient's TTF1 status as reported by the referring laboratory. The relationships between specimen type, anatomic site, TTF1 immunoreactivity, and *EGFR* status were examined.

3. RESULTS

Specimens from 586 patients were referred for *EGFR* testing. Table I shows the demographic data of the patients included in the study. On initial assessment, specimens from 38 patients were rejected because of insufficient tumour quantity or quality. *EGFR* testing failed to yield an interpretable result in an additional 39 cases.

TABLE I Demographic data of the patients tested for *EGFR* status

Variable	*Value*	
	(n)	*(%)*
Samples received	586	
Samples tested[a]	548	—
Test failure	39	7
Reportable result obtained	509	93
Referring institution		
Regional centre (*n*=9)	73	13
Community hospital (*n*=18)	475	87
Anatomic site		
Lung	321	59
Non-mediastinal lymph node	65	12
Pleura	60	11
Bone	35	6
Brain	25	5
Mediastinal lymph node	13	2
Other distant metastatic site[b]	29	5
Specimen type		
Resection	110	20
Biopsy	329	60
Cytology	109	20
Histology		
Adenocarcinoma	476	87
Non-small-cell carcinoma	72	13
EGFR status		
Exon 19 deletion	70	14
Exon 21 L858R	39	8
No mutation reported	398	78

[a] Of the samples received, 38 were rejected on initial screening and were not tested.

[b] Includes kidney, adrenal gland, omentum, and other abdominal viscera.

EGFR mutations were detected in 109 of the remaining 509 specimens (21.4%): 70 (13.7%) in exon 19, and 39 (7.7%) in exon 21. Of 323 samples for which TTF1 IHC results were available, 248 (76.8%) were TTF1-positive, and 75 (23.2%) were TTF1-negative. *EGFR* mutation status and TTF1 IHC results were both available for 306 specimens. In that subset, TTF1 expression was detected in 58 of 62 mutation-positive samples; however, in 244 TTF1-positive specimens, no *EGFR* mutation was detected in 178. Of the 4 TTF1-negative, *EGFR*-positive cases, 3 were reported negative by a laboratory using the TTF1 8G7G3/1 clone (Dako, Glostrup, Denmark); the 1 remaining case had been subjected to acid decalcification before IHC. For the 58 *EGFR*-positive, TTF1-positive patients, referring laboratories had used two primary antibodies: 24 specimens were positive by the TTF1 SPT24 clone

(Leica, Wetzlar, Germany), and 34, by the 8G7G3/1 clone. Those results demonstrate that TTF1 IHC is 93.5% sensitive and 27.1% specific for predicting the presence of *EGFR* activating mutations (Figure 1). As noted in the Methods section, all IHC was performed and interpreted at the referring laboratories.

Comparisons of the *EGFR* test failure rate with the origin site of the specimen failed to reveal any significant differences (data not shown). When interrogated for *EGFR* status, specimens originating from lung, lymph nodes (mediastinal and distant), pleura, bone, brain, and other sites showed similar test characteristics. A trend toward an elevated test failure rate (compared with the overall failure rate of 7.1%) was observed for specimens derived from mediastinal lymph nodes [23.1% (3 of 13)] and from bony metastases [22.8% (8 of 35)]. That observation was believed to be a result either of poor tumour content (in the mediastinal lymph node specimens) or poor DNA quality (extreme fragmentation of DNA in the bony metastasis specimens was probably attributable to the use of decalcification agents).

EGFR results were similarly compared with the specimen type—specifically, resection, biopsy, or cell block from cytology preparations. No significant differences in the test failure rate were identified by specimen type.

4. DISCUSSION

In the modern era of targeted therapeutics, laboratories are confronting the new challenge of biomarker testing. Many hospital laboratories are now successfully reporting *EGFR* status, a forerunner in terms of molecular targets. That work confirms that PCR determination of *EGFR* status is robust and highly effective in a wide array of tissues and specimen types. Nonetheless, current trends in cancer therapeutics are leading toward additional targeted therapies that require testing for additional biomarkers. Currently, *ALK* is the only other such biomarker routinely tested in lung cancers, but *ROS1, MET,* and others are on the horizon. Although PCR has been shown to be effective, it requires a significant quantity of tumour cells and is associated with both high cost and rapid turnaround time. New testing platforms, including next-generation sequencing, could eventually address those issues. It is of practical importance, however, to define additional tools that can help clinicians to decide on a treatment option when patients have a poor performance status, a high burden of comorbidity, or limited tissue samples, or when a long turnaround time for molecular testing seems likely.

In TTF1-negative non-small-cell lung cancer, the probability that a patient harbours non-mutated *EGFR* is 94.2%. That information can be used in the interpretation of equivocal *EGFR* mutation results or to allow for early initiation of chemotherapy when the wait for formal *EGFR* test results could

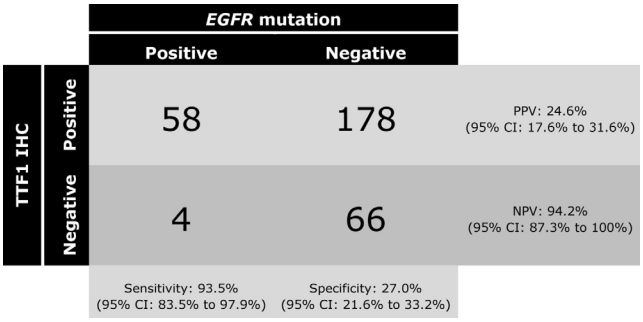

FIGURE 1 Test characteristics of thyroid transcription factor (TTF1) immunohistochemistry (IHC) as a predictor of epidermal growth factor receptor (*EGFR*) gene status in non-small-cell lung carcinoma. Note the high sensitivity (93.5%) and negative predictive value (94.2%). PPV = positive predictive value; CI = confidence interval; NPV = negative predictive value.

be detrimental. Negativity for TTF1 can also guide clinical testing toward biomarkers other than *EGFR* (specifically, *ALK* rearrangement), improving the testing algorithm in patients with limited tumour samples. In addition to improving patient care, such a change would maximize the economy of biomarker testing for laboratories operating under budgetary constraints. Other authors have suggested that clinicians use TTF1 status as a surrogate marker for *EGFR* during the lengthy turnaround time associated with genetic testing[13]. Perhaps revised guidelines could indicate *EGFR* testing for any lung tumours showing TTF1 immunoreactivity. Such a guideline might offer less ambiguity than the current indication of "IHC features of adenocarcinoma"[9]. The negative predictive value of the 8G7G3/1 clone compared with the SPT24 clone could also be further explored in future studies.

As more targeted therapies are added to the clinician's toolbox, more biomarker testing will be requested of the laboratory. It follows that continued refinement and reassessment of biomarker testing strategies should take place, optimizing the information extracted from tumours to maximize patient benefit. The data presented here and in the medical literature clearly indicate that TTF1 plays a significant role in the biology of lung adenocarcinoma. Consideration of the predictive and prognostic significance of TTF1 could further optimize patient outcomes.

5. CONCLUSIONS

EGFR testing by PCR is highly robust and reliable in a variety of sample and tissue types, but its incorporation into treatment strategies for patients with advanced lung cancer remains challenging because of the necessary turnaround time. Although TTF1 immunoreactivity is not specific for the presence of activating *EGFR* mutations, its absence can reliably predict an absence of activating *EGFR* mutations with an accuracy of 94.2%. Thus, IHC is not able to

replace mutational testing, but TTF1 status could be informative in the selection of patients for *EGFR* mutation testing. A marriage of IHC and genetic testing could provide optimal biomarker results when considered within the milieu of additional biomarkers and economic constraints.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST DISCLOSURES

The authors of this manuscript have no relevant conflicts of interest to disclose.

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