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

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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 therapies1,2.

The epidermal growth factor receptor (EGFR) is the most well-characterized biologic target in lung cancer3. 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 adenocarcinomas4, but can also be the drivers behind a smaller number of adenosquamous5 or squamous cell carcinomas harbouring adenocarcinomatous components6. Established clinical risk factors for an EGFR-driven lung cancer include female sex and absence of a heavy tobacco-smoking history7.

Monoclonal antibodies and small-molecule inhibitors have both been effective in blocking EGFR signalling and subsequently retarding tumour growth in lung cancer and other malignancies8. Optimal outcomes are achieved when targeted therapy is delivered selectively to patients with EGFR-driven lung cancers1, 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 \textit{EGFR} status can greatly contribute to timely management.

Thyroid transcription factor 1 (\texttt{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\textsuperscript{10}. Furthermore, \texttt{ttf1} likely plays a role in lung cancer biology, because amplifications of the \textit{NKK2-I} locus (which codes for the \texttt{ttf1} protein) occur frequently in the lung cancer genome\textsuperscript{11}.

Increased expression of \texttt{ttf1} protein, detectable by immunohistochemistry (\texttt{ihc}), is well studied and has been associated with increased survival in lung adenocarcinoma patients\textsuperscript{12}. Prior studies have shown significant correlations between \texttt{ttf1} \texttt{ihc} and \textit{EGFR} status\textsuperscript{13,14}. It is clear from the medical literature that \texttt{ttf1} is emerging, not just as a diagnostic tool, but also as a relevant biomarker in the treatment and study of lung adenocarcinoma.

\section{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 \textit{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 \textit{EGFR} by \texttt{pcr} and fragment length analysis. Additionally, samples were tested for the L858R point mutation in exon 21 by \texttt{pcr} and restriction fragment length polymorphism analysis. Both \texttt{pcr} assays were controlled for a minimum detection threshold of 2\% mutant \texttt{DNA}\textsuperscript{14,15}.

Results of the \textit{EGFR} status testing and clinicopathologic variables were compiled for statistical analysis. Among the included variables was the patient’s \texttt{ttf1} status as reported by the referring laboratory. The relationships between specimen type, anatomic site, \texttt{ttf1} immunoreactivity, and \textit{EGFR} status were examined.

\section{RESULTS}

Specimens from 586 patients were referred for \textit{EGFR} testing. Table 1 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. \textit{EGFR} testing failed to yield an interpretable result in an additional 39 cases.

\textit{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 \texttt{ttf1} \texttt{ihc} results were available, 248 (76.8\%) were \texttt{ttf1}-positive, and 75 (23.2\%) were \texttt{ttf1}-negative. \textit{EGFR} mutation status and \texttt{ttf1} \texttt{ihc} results were both available for 306 specimens. In that subset, \texttt{ttf1} expression was detected in 58 of 62 mutation-positive samples; however, in 244 \texttt{ttf1}-positive specimens, no \textit{EGFR} mutation was detected in 178. Of the 4 \texttt{ttf1}-negative, \textit{EGFR}-positive cases, 3 were reported negative by a laboratory using the \texttt{ttf1} 8G7G3/1 clone (Dako, Glostrup, Denmark); the 1 remaining case had been subjected to acid decalcification before \texttt{ihc}. For the 58 \textit{EGFR}-positive, \texttt{ttf1}-positive patients, referring laboratories had used two primary antibodies: 24 specimens were positive by the \texttt{ttf1} SPT24 clone.

\begin{table}[h]
\centering
\caption{Demographic data of the patients tested for \textit{EGFR} status}
\begin{tabular}{llll}
\hline
\textbf{Variable} & \textbf{Value} \\
\hline
\textit{n} & \textit{(\%)} \\
\textbf{Samples received} & 586  \\
\textbf{Samples tested}\textsuperscript{a} & 548  \\
\textbf{Test failure} & 39 (7)  \\
\textbf{Reportable result obtained} & 509 (93)  \\
\textbf{Referring institution} &  \\
\textit{Regional centre (n=9)} & 73 (13)  \\
\textit{Community hospital (n=18)} & 475 (87)  \\
\textbf{Anatomic site} &  \\
\textit{Lung} & 321 (59)  \\
\textit{Non-mediastinal lymph node} & 65 (12)  \\
\textit{Plera} & 60 (11)  \\
\textit{Bone} & 35 (6)  \\
\textit{Brain} & 25 (5)  \\
\textit{Mediastinal lymph node} & 13 (2)  \\
\textbf{Other distant metastatic site}\textsuperscript{b} & 29 (5)  \\
\textbf{Specimen type} &  \\
\textit{Resection} & 110 (20)  \\
\textit{Biopsy} & 329 (60)  \\
\textit{Cytology} & 109 (20)  \\
\textbf{Histology} &  \\
\textit{Adenocarcinoma} & 476 (87)  \\
\textit{Non-small-cell carcinoma} & 72 (13)  \\
\textbf{EGFR status} &  \\
\textit{Exon 19 deletion} & 70 (14)  \\
\textit{Exon 21 L858R} & 39 (8)  \\
\textit{No mutation reported} & 398 (78)  \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a} Of the samples received, 38 were rejected on initial screening and were not tested.

\textsuperscript{b} Includes kidney, adrenal gland, omentum, and other abdominal viscera.
TTF-1 IHC PREDICTS EGFR STATUS IN NSCLC

(Leica, Wetzlar, Germany), and 34, by the 8G7G3/1 clone. Those results demonstrate that TTF-1 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 TTF-1-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 be detrimental. Negativity for TTF-1 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 TTF-1 status as a surrogate marker for EGFR during the lengthy turnaround time associated with genetic testing. Perhaps revised guidelines could indicate EGFR testing for any lung tumours showing TTF-1 immunoreactivity. Such a guideline might offer less ambiguity than the current indication of “IHC features of adenocarcinoma”. 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 TTF-1 plays a significant role in the biology of lung adenocarcinoma. Consideration of the predictive and prognostic significance of TTF-1 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 TTF-1 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 \textit{ttf}1 status could be informative in the selection of patients for \textit{EGFR} mutation testing. A marriage of \textit{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.

8. REFERENCES


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