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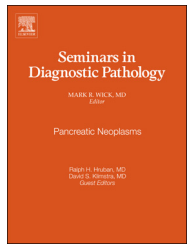

Protein correlates of molecular alterations in lung adenocarcinoma: Immunohistochemistry as a surrogate for molecular analysis

Lynette M. Sholl, MD

Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston, Massachusetts 02115

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ABSTRACT

Most clinically actionable alterations in lung adenocarcinomas are detected using molecular or cytogenetic techniques. However, many such alterations have a protein-level correlate that can be interrogated using immunohistochemistry. This review will summarize the therapeutic relevance of predictive biomarkers in lung adenocarcinoma including the oncogenes *EGFR*, *MET*, *ALK*, *RET*, and *ROS1* and tumor suppressors *PTEN* and *LKB1* with an emphasis on established and emerging protein immunohistochemistry reagents and their promise in clinical practice.

Introduction

The therapeutic course of patients with lung adenocarcinoma (ACA) is increasingly dictated by specific tumor genomic alterations. Identification of such "predictive" DNA-level changes has led to more rational application of specific oncogenic pathway inhibitors. The best described and most common targetable alterations are *EGFR* mutations and *ALK* rearrangements; testing for these alterations is recommended for all patients with advanced (stage IV) lung ACA (Fig. 1).[1] Other alterations with proven or potential actionability may occur in a variety of other targets, although at the time of this writing, universal testing recommendations for lung ACA were limited to *EGFR* and *ALK*.

Tumor genetic changes may take the form of single nucleotide variants (SNVs), small insertions–deletions (indels), and larger structural variations including chromosomal rearrangements. Comprehensive molecular profiling to identify these targetable alterations requires significant institutional resources in the form of clinical molecular diagnostics and and/or cytogenetics laboratories. The DNA requirements necessary to complete a larger panel of tests often exceeds that available from the small biopsies typically obtained for lung cancer diagnoses. The costs of multiple FISH and molecular assays may be prohibitive for patients and payers. The adoption of next-generation sequencing (NGS) may help to ameliorate these logistical hurdles; however, the capital expenditure necessary to implement this technology is not insignificant. Thus, NGS is unlikely to be readily available in all settings.

In contrast, immunohistochemistry (IHC) for protein expression is relatively inexpensive and already an established component of the surgical pathologist's diagnostic armamentarium. Indeed, protein expression as detected by IHC can reflect aberrant pathway activation or inactivation and generate a functional readout of underlying genetic alterations. It is, therefore, a powerful and complementary tool to genomic analyses, especially those that uncover alterations of unclear significance to tumorigenesis—this being one of the major challenges of interpreting NGS data.

In most cases, assays that detect gene-level alterations are considered the gold standard for analysis of predictive

E-mail address: lmsholl@partners.org

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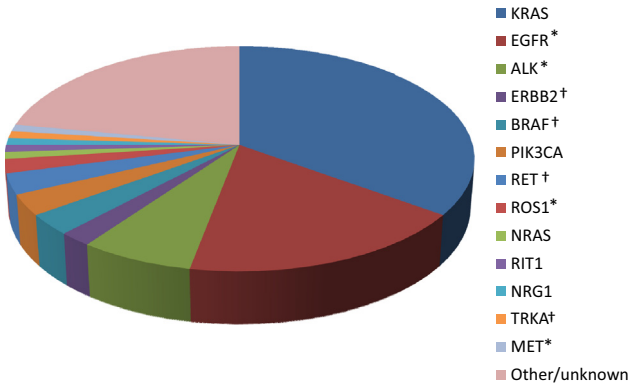

Fig. 1 – Genetic drivers of lung adenocarcinoma. The starred genes are known drivers of lung adenocarcinoma for which immunohistochemical reagents with demonstrated clinical utility exist to detect protein overexpression. The daggers mark genes for which immunohistochemical reagents exist to detect protein expression; however, their practical utility is limited or not yet established.

biomarkers in lung ACA. However, many IHC correlates have been demonstrated to have excellent clinical performance and in some cases outperform the equivalent molecular or cytogenetic assays. This review will discuss the advantages and pitfalls of some common and evolving predictive IHC assays for lung ACA.

EGFR

The epidermal growth factor receptor (*EGFR* or *ERBB1*) is a cell surface protein kinase and a member of the ErbB transmembrane growth factor receptor family. Ligand binding triggers homo- or heterodimerization with other ErbB family members, leading to kinase domain tyrosine autophosphorylation and activation of PI3K/AKT/mTOR and RAS/RAF/MAPK pathways. EGFR activation is a well-established driver of tumor cell proliferation, cell survival, and angiogenesis.[2] In the late 1980s, EGFR protein overexpression was described in lung squamous cell carcinomas and ACA and therefore proposed for use as a diagnostic marker to distinguish between small cell and non-small cell carcinomas or as a predictor for antibody therapies.[3] Monoclonal antibody-based therapies, however, have been largely ineffective in lung cancer.[4] In contrast, EGFR tyrosine kinase inhibitors (TKIs) designed to block the ATP-binding groove showed significant anti-tumor activity both *in vitro* and in early clinical trials.[5] Response to these drugs (including first-generation TKIs gefitinib and erlotinib) is associated with somatic gain-of-function mutations in the *EGFR* tyrosine kinase domain.[6,7] Phase III clinical trials have confirmed that somatic *EGFR* mutation predicts efficacy of EGFR TKIs and that patients whose tumors are *EGFR* wild type derive more benefit from traditional chemotherapy.[8] Knowledge of the tumor *EGFR* status is therefore of paramount importance.

EGFR-activating mutations are enriched in, but not exclusive to, lung ACA with papillary features arising in women, nonsmokers, and patients of East Asian descent.[9] Approximately 90% of EGFR TKI-sensitizing mutations occur either as variable in-frame indels around the LREA motif in exon 19 or as missense mutations in exon 21 (L858R). Overall, 10% of *EGFR* mutations occur outside of these hotspots in exons 18, 20, and elsewhere in 21.[2] Most are sensitizing; however, exon 20 insertion/duplication mutations are largely EGFR TKI resistant and tend not to respond to first-generation inhibitors.[10]

Total EGFR protein expression is neither sensitive nor specific as a predictor of response to EGFR TKIs.[11] As a result, current practice guidelines for EGFR testing in lung ACA recommend use of mutation analysis, but not EGFR protein expression for selection of patients for targeted therapy.[1] That said, mutation-specific IHC has offered a promising approach to protein-based detection for a variety of predictive targets, with EGFR-specific tools among the first to be developed and validated for limited uses. Introduced in 2009, multiple groups have since evaluated the clinical utility of the available mutation-specific antibody clones 43B2 for EGFR L858R and 6B6 for EGFR exon 19 deletion (ex19del) mutations (Fig. 2).[12–14] In general, these studies have demonstrated that both the L858R and ex19del mutation-specific antibodies are indeed highly specific but variably sensitive when compared to molecular analyses; the ex19del antibody in particular may have sensitivity as low as 50%, this driven by the fact that the antibody was raised against the most common L746_A750 deletion event that accounts for only about half of the ex19del mutations. The ex19del antibody has variable to poor sensitivity for other deletion events involving only a segment of or occurring adjacent to the canonical LREA site.[13] It is interesting to note that the majority of published studies also find the specificity of both antibodies to be slightly less than 100%. The reason for IHC expression despite wild-type mutational analysis is not entirely clear, but it may be driven in some cases by ambiguous molecular results[12] or tumoral heterogeneity[14]; however, this latter proposal is controversial given that *EGFR*-activating mutations are thought to be early drivers in lung ACA tumorigenesis and lead to oncogene dependency.[2] It is possible that these types of discrepancies are driven by technical artifacts, such as falsely negative molecular results in tumor specimens with a low ratio of

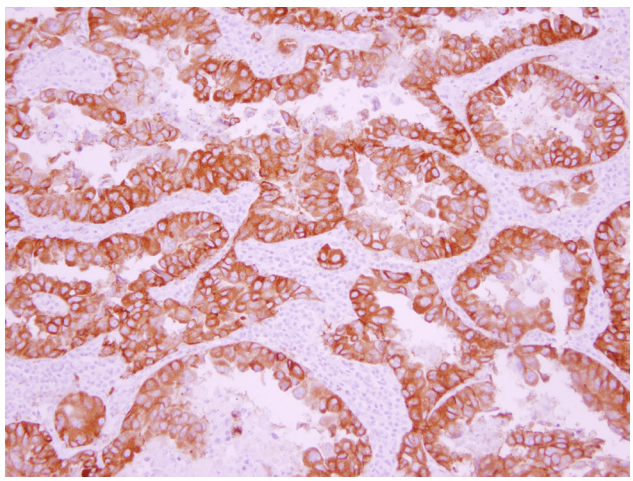

Fig. 2 – EGFR exon 19 deletion mutation-specific immunohistochemistry. Characteristic staining is cytoplasmic with occasional membranous accentuation.