NEXT GENERATION LEARNING

2016 ANNUAL MEETING

March 12-18 Seattle, Washington

USCAP

Creating a Better Pathologist
How has Molecular Diagnostics changed my practice?

*Pulmonary Pathology*

Lynette M. Sholl, M.D.
Associate Pathologist
Brigham and Women’s Hospital
Associate Director, Center for Advanced Molecular Diagnostics
Chief, Pulmonary Pathology Division
Conflicts of Interest

• Genentech Scientific Advisory Board (past)
Objectives

• Identify contexts in which molecular techniques may aid in diagnostic pulmonary pathology
  – Multiple primaries vs intrapulmonary metastases?
  – Primary lung tumor or metastasis?
    • Mutational signatures
    • Site-specific driver gene alterations

• Increase familiarity with molecular diagnostic tools
20% of screened population will have multiple lung tumors

...or T1a, multicentric?

Murphy et al. *J Clin Oncol* 2014
1975 Martini-Melamed Criteria

1990s Comparative genomic hybridization

2000s Genomic + histologic profiling

2010s Next gen sequencing

Weiss MM Am J Pathol 2003
Diagnosis of multiple primary lung tumors

Table 1—Criteria for Diagnosis of MPLC

Martini and Melamed, 1975
Metachronous tumors
I. Histologic type different
II. Histologic type the same, if:
   A. Free interval between cancers is at least 2 yr, or
   B. Origin from carcinoma in situ, or
   C. Second cancer in different lobe or lung, but:
      1. No carcinoma in lymphatics common to both
      2. No extrapulmonary metastases at time of diagnosis

Synchronous tumors
I. Tumors physically distinct and separate
II. Histologic type:
   A. Different
   B. Same, but in different segment, lobe, or lung, if:
      1. Origin from carcinoma in situ
      2. No carcinoma in lymphatics common to both
      3. No extrapulmonary metastases at time of diagnosis

Antalde et al., 1995
A. Different histologic condition
B. Same histologic condition with two or more of the following:
   1. Anatomically distinct
   2. Associated premalignant lesion
   3. No systemic metastases
   4. No mediastinal spread
   5. Different DNA ploidy
Comparative genomic hybridization

- Distinguish tumors based on copy number changes across the genome
- Requires significant amount of input DNA
- Technically difficult—especially from FFPE tissue
- Degraded specimens may deliver equivocal results

Girard N et al *Clin Cancer Res* 2009
Molecular testing--2000s

Single Gene Assays
Molecular testing--2000s

KRAS variants in lung adenocarcinoma

WT
KRAS
EGFR

Few Targets
Histology outperforms molecular analysis?

Classical criteria

aCGH + targeted genotyping

Comprehensive analysis of histologic subtypes and cytologic features

Definitive molecular methods?

- Mate-pair whole genome sequencing
  - Fusion-enriched sequencing data
- Presence of identical large-rearrangement breakpoints supports shared tumor lineage
- Breakpoints for common rearrangements (i.e. EML4-ALK) are highly variable between individuals

Murphy et al. J Clin Oncol 2014
TARGETED NGS PANELS: TODAY

- Routinely available
- Capture majority of known drivers in lung adenocarcinoma
- Permit testing of scant/highly contaminated specimens
74 year old woman, former smoker, incidental RUL mass

MET c.3028+2T>C (p.D1010_splice)

KRAS c.35G>A (p.G12D)
LUNG PRIMARY TUMOR VS METASTASIS?
Breast vs. Lung?

- 58 year old woman, h/o well differentiated T1aN0 invasive ductal carcinoma (ER+, PR+, HER2-) 5 years prior
- Now with lung left upper lobe mass and diaphragmatic implants

Lung tumor: CK7+, TTF1-, GATA3 weak, ER weak, mammoglobin -

“The carcinoma in the lung is poorly differentiated with high grade nuclei, abundant cytoplasm, mucin production, and necrosis. The carcinoma does not resemble the breast carcinoma in the excision from 2010. The possibility that the patient has a different breast primary carcinoma should be considered.”
Back of the envelope:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency in breast</th>
<th>Frequency in Lung</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS mutation</td>
<td>1%</td>
<td>25%</td>
<td>98% (for lung)</td>
</tr>
<tr>
<td>EGFR mutation</td>
<td>0</td>
<td>13%</td>
<td>100% (for lung)</td>
</tr>
<tr>
<td>ERBB2 amp</td>
<td>16%</td>
<td>0.5%</td>
<td>91% (for breast)</td>
</tr>
</tbody>
</table>
Breast vs. Lung?

KRAS c.34G>T (p.G12C), exon 1
STK11 c.206C>A (p.S69*), exon 1
TP53 c.499_501CAG>G (p.Q167fs), exon 5

KEAP1 c.1016T>C (p.L339P), exon 3
KEAP1 c.382A>T (p.I128F), exon 2
JAK2 c.3214C>T (p.Q1072*), exon 24
BRIP1 c.434C>G (p.S145C), exon 5
CDC73 c.26G>T (p.R9L), exon 1
CHEK2 c.349A>G (p.R117G), exon 3
DMD c.10567G>A (p.E3523K), exon 75
SMARCA4 c.2439_splice (p.S813_splice)
TCF3 c.136G>A (p.G46R), exon 3

Genomic evidence supports a lung primary.

More on mutational signatures

Alexandrov et al. *Nature* 2013
## Clinical correlates of targeted NGS mutation signatures

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tumor</th>
<th>Mutation #</th>
<th>Mutational signature</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Glioblastoma</td>
<td>169</td>
<td>Temozolamide + MMRd</td>
<td>PMS2 gene deletion; treatment history n/a</td>
</tr>
<tr>
<td>Brain</td>
<td>Oligodendroglioma</td>
<td>36</td>
<td>Temozolamide</td>
<td>Recurrent dz s/p temozolamide</td>
</tr>
<tr>
<td>Brain</td>
<td>Oligodendroglioma</td>
<td>204</td>
<td>Temozolamide</td>
<td>Recurrent dz s/p temozolamide</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Adenocarcinoma</td>
<td>104</td>
<td>POL ε</td>
<td>POL ε mutation</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Adenocarcinoma</td>
<td>153</td>
<td>POL ε + aging</td>
<td>POL ε mutation</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Adenocarcinoma</td>
<td>32</td>
<td>MMRD</td>
<td>MLH1 IHC defect</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Adenocarcinoma</td>
<td>197</td>
<td>POL ε</td>
<td>POL ε mutation</td>
</tr>
<tr>
<td>Lung</td>
<td>SCC</td>
<td>36</td>
<td>Smoking</td>
<td>40 p.y. TOB</td>
</tr>
<tr>
<td>Lung</td>
<td>Adenocarcinoma</td>
<td>32</td>
<td>Smoking</td>
<td>55 p.y. TOB</td>
</tr>
<tr>
<td>Lung</td>
<td>SCC</td>
<td>44</td>
<td>UV</td>
<td>History of cutaneous invasive SCC</td>
</tr>
<tr>
<td>Skin</td>
<td>SCC</td>
<td>47</td>
<td>UV</td>
<td>Scalp SCC</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Adenocarcinoma</td>
<td>45</td>
<td>MMRD</td>
<td>MSH2 IHC defect</td>
</tr>
</tbody>
</table>
Mutational signatures informing diagnosis I

- 56 year old nonsmoking man with incidental, peripheral 1.4 cm RUL lung nodule, diagnosed and staged as a primary lung squamous cell carcinoma.

- Patient developed multiple lung nodules.

- 300 gene panel sequencing identified 70 missense mutations with a UV mutational signature.

- On further review of past medical history: Cutaneous basosquamous carcinoma with regional lymph node metastases, one decade earlier.
Mutational signatures informing diagnosis II

- Lung, liver and soft tissue masses in a 50 year old man with a light smoking history
- Inconclusive immunohistochemistry profile
- Metastatic poorly differentiated carcinoma, lung origin, was favored
Clinical course:

- No targetable alterations identified.
- Patient progressed on standard chemotherapy for lung cancer.
- Excellent systemic response to immunotherapy, with brain-only progression.
Mutational profiling performed on the chest wall mass:

- RB1 Q637*
- TP53 R213*
- 46 additional mutations

Ultraviolet light mutational signature

Courtesy Frank Kuo, BWH
What is the value of a correct diagnosis?

<table>
<thead>
<tr>
<th>Test</th>
<th>Cost in US $ Medicare rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>136</td>
</tr>
<tr>
<td>Single gene assay</td>
<td>201</td>
</tr>
<tr>
<td>FISH</td>
<td>489</td>
</tr>
<tr>
<td>Panel testing</td>
<td>500-1000</td>
</tr>
<tr>
<td>Large NGS panel</td>
<td>5000*</td>
</tr>
</tbody>
</table>

* “Retail” cost

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cost in US $ per cycle Medicare rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-Pem</td>
<td>5721</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>937</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>3982</td>
</tr>
<tr>
<td>Crizotinib</td>
<td>8041</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>100,000†</td>
</tr>
</tbody>
</table>

† Estimated total cost of treatment for melanoma patient
Molecular in Pulmonary Pathology: Summary

• Advances in molecular technology permit for more routine genetic analysis of tumor specimens
  – Whole genome sequencing and high resolution aCGH probably not necessary in most cases
• Pathologists and oncologists increasingly aware of this resource and employing it for diagnosis
  – Synchronous/metachronous tumors vs. metastases
  – Primary lung vs. metastasis
  – Guidelines on validation/implementation in practice are lacking
• Sequencing panels can uncover mutational signatures that inform diagnosis
Major challenges exist in lung cancer diagnostics, despite advances in classification schema. Some of these challenges can be addressed by incorporating molecular techniques into routine diagnostics.

1) Distinguishing between synchronous/metachronous lung primary tumors versus metastatic disease

Computed tomography (CT) screening is recommended to permit early detection of lung cancers in high-risk populations. Based on available data from CT screening studies, 20% of subjects will have multiple lung tumors diagnosed simultaneously or at follow up. Pathologists may apply a variety of criteria in order to distinguish between independent synchronous/metachronous tumors and related, metastatic tumors. Martini-Melamed criteria, established over 40 years ago but still employed in practice, recommend considering histology (adenocarcinoma, squamous cell carcinoma or “bronchioloalveolar cell carcinoma”), location relative to lymphatic drainage, the presence of a carcinoma in situ component, the presence of lymphatic invasion and systemic metastases may reflect the relatedness of tumors. However, multiple studies have suggested that alternative approaches outperform the Martini-Melamed criteria when benchmarked against clinical
outcomes. Morphologic analyses that take into account the diversity of adenocarcinoma phenotypes, including histologic subtypes, mucin production, and unique cytologic features appear to effectively distinguish between distinct primary and metastatic tumors. Genomic methods, including (array) comparative genomic hybridization, targeted genotyping, whole genome sequencing for structural rearrangements, and targeted next generation sequencing (NGS), may also be employed. Practical limitations such as cost and tissue availability will dictate the feasibility of clinical use of these assays. Targeted NGS assays that query most of the common driver mutations in lung adenocarcinoma and that are low in cost may permit more routine use of molecular testing for this purpose.

2) Distinguishing between primary lung and metastatic tumors

Immunohistochemistry allows the pathologist to determine the primary site for the vast majority of metastatic carcinomas, however a subset of carcinomas may be difficult to classify, and clinical history may confound the diagnosis. For example, CK7 positive carcinomas that lack convincing expression of differentiation-specific transcription factors (such as TTF-1, GATA-3 and PAX8) may be difficult to classify with certainty, particularly in women where the differential commonly includes metastatic breast or Müllerian carcinoma. Molecular analysis may be informative in this context, as certain driver mutations such as KRAS and EGFR are significantly more likely to occur in lung adenocarcinoma whereas PIK3CA and PTEN mutations and ERBB2 amplification are more common in breast tumors. Although these mutations are not necessarily exclusive to a single tumor type, expanded mutational profiling may provide additional clues to the likely tumor origin and should be considered in the diagnostic algorithm.

NGS-based mutational profiling, including whole genome, whole exome, and targeted sequencing, can reveal unique mutational signatures that reflect an
underlying etiology, such as ultraviolet light exposure in cutaneous malignancies, tobacco-related mutagenesis in lung tumors, and mismatch repair defects (MMRd) in patients with germline or sporadic MMRd. In the lung, identification of a smoking signature can confirm its primary status. In contrast, identification of UV signatures in lung carcinomas or undifferentiated malignancies point to a skin origin such cutaneous squamous cell carcinoma or malignant melanoma.

Clinicians including pathologists and oncologists are increasingly interested in incorporating molecular testing into routine diagnostics for lung cancers. Pathology labs should consider practical approaches to mutational profiling for in situations where it is likely to clarify the diagnosis. The benefits of making the correct diagnosis early in a patient’s course are likely to far outweigh the costs incurred by these additional tests.

References


