The new role of cytology in the diagnosis and management of lung cancer

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Outline

• Role of cytology in the diagnosis of lung cancer
• Non-small lung cancer subtypes in cytology
• Role of cytology in molecular analysis
• Update on MSKCC work
Lung Cancer

• Remains the major cancer killer
• Most patients are not surgical candidates
• More than half of all patients will be diagnosed by cytology alone

• Targeted therapy changed the rules
Change in respiratory tract cytology past several decades due to
flexible bronchoscopy, better imaging, better FNA techniques

Primary neoplastic disease shifted from
central squamous carcinoma to
peripheral adenocarcinoma

New targeted therapies depend on
histologic/cytologic subtyping
genotyping mutation status

Clinicians’ expectations have changed–do more with less
• Conventional cytology  sputum, brushes, washes, lavages

• Transbronchial FNA  best for lesions below the bronchial epithelial surface,

• EBUS biopsies essentially a real time staging procedure

Eliminates need for surgery

Cost is 1/3 of a mediastinoscopy
Transthoracic FNA/EBUS

Cytotechnologist or Cytopathologist should be present during procedure

Assures adequacy of the specimen and allows for special studies to be done (flow, mutational analyses, microbiology, etc)
Accuracy

Transbronchial FNA
Very good at separating NSCLC from SCC
PPV 100%, NPV 70%

Conventional Cytology (BWL)
PPV 94% for each
NPV 30%

Bronchial biopsy comparable to cytology
Accuracy

Transthoracic

Most FN are sampling errors; reliability of a negative result is questionable

PPV 98%
NPV 70%

FP rate 0.85% huge impact on patient
FN rate 8%

SCLC v NSCLC 95%
Small Cell Carcinoma
Adenocarcinoma in bronchial brush
• Targeted therapies require separation of adenocarcinoma from squamous cell carcinoma: Tarceva and Avastin

• Classic morphologic criteria to separate adenocarcinoma from squamous

• Additionally, IHC can be applied to cytology material, to cell blocks, Thin Preps and direct smears

• Subtyping adenocarcinoma more challenging
Alcohol fixed paraffin embedded cell blocks

- Excellent source for IHC stains and other special stains such as mucin, AFB
- Can be utilized for molecular analysis such as EGFR mutations
- Problem exists with cell block artifact:
  Cell blocks can show squamoid features even in adenocarcinoma
Adenocarcinoma in cell block
Use of IHC in Pulmonary Cytology

- TTF-1 and PE10, napsin support pulmonary primary and adenocarcinoma
- 4A4, p63, 34βE12 support squamous carcinoma
- CK7 and CK20 useful for distinguishing lung tumors from GI metastasis
TTF-1 in cell block
Adenocarcinoma subtypes in cytology

• Separate mucinous from non-mucinous (IR), no *EGFR* mutations with mucin
• Papillary and micropapillary can be recognized
• Distinction between acinar and BAC difficult
• Is BAC worth mentioning? Does it play a role in management?
管理和管理

TKI therapy with EGFR mutations: best response is female non-smokers with adeno/BAC

BAC with GGO: wedge not lobe, fewer surgeries

Analyze cytology for mutations

1Pao W, Zakowski M et al. Proc Natl Acad Sci USA. 8/04; 101 (360:13306-11)
BAC and Cytology

- Cytology cannot unequivocally diagnose BAC (WHO definition)
- Patterns can be recognized that suggest in-situ proliferation of:
  - pure BAC
  - BAC with invasion
  - adeno with BAC features
BAC on FNA
BAC
Adenocarcinoma bronchial wash
Cytologic features of BAC

- Clean/mucinous background
- Monolayer sheets
- Orderly arrangement
- Slight nuclear enlargement
- Smooth to slightly irregular nuclear contours

- Nuclear grooves
- Intranuclear inclusions
- Finely granular chromatin
- Nucleoli single to few but not very conspicuous
- No psammoma bodies
- Histiocytes present
Differential Diagnosis

- Abundant reactive epithelium
- Clear cell tumors (glycogen, not mucin)
- Mesothelium
- Carcinoid
- Hamartoma
- Well differentiated adenocarcinoma
- Metastatic adenocarcinoma (pancreas, breast)
Reporting of BAC

- Positive. Adenocarcinoma with BAC features
- Positive. BAC. The possibility of an invasive component cannot be excluded
- Positive. Adenocarcinoma. The possibility of BAC cannot be excluded
Use of cytologic material for mutational analysis

- *EGFR/KRAS* most frequently studied
- FNA cell blocks, fluids, archival slides all used successfully

- Touch preps done to ascertain the adequacy of core biopsy material for studies, protocols, etc
Underutilization of FNA material for targeted therapy decisions

Impediments to FNA based predictive tests:

- Few studies compare cellularity of FNA with quantity/quality of desired analyte
- Pre analytical handling/processing impact on molecular tests
- Need for standardized/optimized FNA processing devices for molecular testing
- Cytopathologists must be engaged in clinical therapeutic aspects of cancer care to be knowledgeable partners
- Cytologists must become more scientific or others will get there first (circulating tumor cells, etc.)

Doug Clark Cancer Cytopathology Oct 2009
Molecular techniques and cytology

• Krishnamurthy: FNA for FISH, PCR, Southern blotting, gene microarrays
• Smouse et al: EGFR mutation detection in cytology (FNA, pleural fluid, bronchial washes and lavages) comparable to that in surgical pathology specimens
• Savic et al: FNAs, washes brushes, BAL, pleural fluids used for EGFR gene mutation and EGFR gene copy number by FISH
• Centeno et al: extracted RNA from bench prep FNAs from surgically resected tumors with good yield
• Symmans et al: total RNA yield and microarray gene expression from FNAs and cores of breast similar
MSKCC Trial 04-103: Rebiopsy of Patients with Acquired Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non-small Cell Lung Cancer
(P.I. Vincent Miller, M.D.)

• **Aims:**
  – Determine the feasibility of rebiopsy in this setting
  – Determine the frequency and spectrum of secondary EGFR mutations
  – To collect material to search for novel mechanisms of acquired resistance to EGFR TKIs
Total Samples Tested For *EGFR* Mutations

- 134 samples (111 patients) *

22 patients had multiple specimens studied (range 2-3)
7 patients not rebiopsied
Sample Adequacy

93% (94/101) surgical pathology and FNA samples were adequate for mutation analysis

- 73% (24/33) fluid cytology samples adequate for mutation analysis
- 9 fluid cytology samples insufficient or degenerated
- Bone samples challenging due to decalcification - poor DNA quality
- FNAs performed better than cores
“The Lung Adenocarcinoma Oncogenome”

Pie chart of mutually exclusive mutations (ca 2009)

- **Unknown (33%)**
- **KRAS (1987)**
- **EGFR (2004)**
- **NF1 (2008)**
- **MEK1 (2008)**
- **ALK fusions (2007)**
- **ERBB2 (2004)**
- **BRAF (2002)**

1. Sensitive to EGFR inhibitors
2. Resistant to EGFR inhibitors
3. Sensitive to MEK1 inhibitors
4. Sensitive to BRAF inhibitors
5. Sensitive to ALK inhibitors
6. Sensitive to MTOR inhibitors
EML4-ALK positive lung adenocarcinoma

H&E cell block lung adenocarcinoma

ALK Ab D5F3 (Cell Signaling)

Abbott-Vysis ALK FISH assay
Assessment of EGFR mutation status in needle biopsies and cytology specimens of lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR.

IHC with mutation specific monoclonal Abs against exon 21 L858R and exon 19 mutant (15bp) is sensitive and specific for mutation status.

Ang D, Ladanyi M, Zakowski M. USCAP 2009
94 lung adenocarcinomas with known *EGFR* status on resection material with corresponding needle biopsy or cytology: cell block or Thin prep

47 exon 19 deletion
35 exon 21 L858R mutation
12 wild type
Staining with Cell Signaling Technology Abs in cytology and tissue

0,1+ negative
2+,3+ positive

Sensitivity
ex 19 (15bp only)  79%    85%
ex 19 (all bp)      57%
ex 21               66%    76%

Specificity and PPV 100%

No wild type case reactive
### IHC for EGFR ex19 deletion mutant

<table>
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<tr>
<th>Mutation Status</th>
<th>n=47</th>
<th>Specimen type</th>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>Exon 19 (15bp del) n=34</td>
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<td>Biopsy (n=22)</td>
<td>18 (92%)</td>
<td>4 (8%)</td>
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<td></td>
<td>Cytology (n=12)</td>
<td>Thin Prep (n=5)</td>
<td>5 (100%)</td>
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<tr>
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<td></td>
<td></td>
<td>Cell block (n=7)</td>
<td>4 (57%)</td>
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<tr>
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<td>Biopsy (n=9)</td>
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<td>9 (100%)</td>
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<tr>
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<td>Cytology (n=4)</td>
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<td>4 (100%)</td>
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</table>

### IHC for EGFR L858R mutant

<table>
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<th>Mutation Status</th>
<th>n=35</th>
<th>Specimen type</th>
<th>Positive</th>
<th>Negative</th>
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<tr>
<td>EGFR L858R</td>
<td></td>
<td>Biopsy (n=12)</td>
<td>10 (83%)</td>
<td>2 (17%)</td>
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<tr>
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<td>Cytology (n=23)</td>
<td>Thin Prep (n=4)</td>
<td>3 (75%)</td>
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<td>Cell block (n=19)</td>
<td>10 (53%)</td>
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</table>
Tumor with EGFR del-15 (three different patients)

Biopsy

Cell block

Thin Prep

2085 Ab (EGFR ex19 Ab)
• Good sensitivity and high specificity
• No FP
• Eliminates need for additional biopsy for mutation studies
• Would better pre-analytical methods to improve results?
Lung adenocarcinoma samples

Possible flowchart for use of EGFR mutation-specific antibodies

EGFR exon 19 deletion mutant specific Ab

5-10%

Positive

EGFR molecular testing

EGFR mutant status reported

EGFR L858R mutant specific Ab

5-10%

Positive

Negative

Negative

January 2006 we instituted reflex testing for *EGFR/KRAS* mutations on all lung adenocarcinomas resected at MSKCC

**Reflex Testing:** No clinical order needed; a pathology finding automatically initiates another test (example: HPV testing in cytology)
Reflex testing background

Primary lung adeno is preferentially susceptible to the effects of TKIs

Identification of activating mutations in the *EGFR* TK domain is the best predictor of response to EGFR TKIs

*EGFR* mutations are never found in association with *KRAS* mutations

The presence of a *KRAS* mutation is a contraindication for *EGFR* TKI therapy
Methodology

- At surgical sign out of adenocarcinoma, 10-15 unstained slides are ordered through CoPath.
- H&E and unstained slides sent to Molecular lab.
- Non neoplastic material on slides is trimmed away using H&E as a guide.
- Results reported from Diagnostic Molecular Lab in 2 weeks with separate molecular report in CoPath.
294 cases studied for *EGFR* and *KRAS*
58 (20%) *EGFR* mutations found
85 (28%) *KRAS* mutations found

Most of the material used was frozen
Mutation data 2007

674 cases analyzed for EGFR/ KRAS mutations

reflex testing extended to submitted cases as well

Frozen tissue: 229
Paraffin: 119
Cytology cell blocks: 9 (1.3%)

357 in house cases (20% increase)

Plasma DNA: 1
Submitted slides: 316

317 submitted cases
Mutation Data 2008

847 cases studied for *EGFR/KRAS*

177 *EGFR* mutations  (20%)
216 *KRAS* mutations  (25%)

Samples included frozen, paraffin, submitted slides, cytology cell blocks
Mutation Data 2009

• 1400 cases studied for EGFR/KRAS
• 864 lung, remainder colon
• 70 (8%) cytology (FNA, pleural, BW)
• No reflex testing on cytology samples
The oncologists needs have changed and pathology practice is changing

Pathologists must distinguish small cell from NSCLC, consider subtyping adenocarcinoma, and secure tissue for mutational analysis using **cytology material**

Pathologists in an ideal position to influence appropriate testing and treatment in the era of targeted therapy
Molecular technique in cytology samples

- Cell block is cut into sections using H&E as a check of tumor content
- Slides scraped, DNA extracted with column based method (Qiagen kit)
- *EGFR* mutations assessed by 2 PCR based specific assays for exons 19, 21
- If *EGFR* is negative, sequencing for *KRAS* codon 12 and 13 mutations
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Has no conflicts of interest to report concerning the presentation
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