14 Cytologic Sub-Classification of Lung Cancer: A New Challenge for Practicing Pathologists

Parvin Ganjei-Azar MD
Merce Jorda MD, PhD

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AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
33 W. Monroe, Ste. 1600
Chicago, IL 60603
14 Cytologic Sub-Classification of Lung Cancer: A New Challenge for Practicing Pathologists

This session will present aspects of cytologic sub-classification of lung cancer, focusing on the introduction of target therapy and its associated significant therapeutic implications for patients with advanced stage lung cancer. Included in the presentation will be subclassification of non-small cell carcinomas into adenocarcinomas and squamous cell carcinomas, cytologic samples including fine needle aspiration (FNA), bronchoscopic brushing (BB) and washings (BW), and bronchoalveolar lavages (BAL) to establish the diagnosis of lung cancer and subclassification of these tumors. Molecular testing for lung cancer will also be discussed. Formulate a differential diagnosis based on routinely prepared cytologic slides, based on clinical, imaging and cytologic findings.

- Learn how to identify and mark diagnostic cells properly for further application of immunocytochemistry, select a limited panel of antibodies based on available clinical and cytomorphologic information and to interpret immunostain results, and become fam

- Differentiate lung primary carcinomas from those of metastatic origin, accurately diagnose small cell carcinomas of lung and subclassify non small cell carcinomas into those with squamous differentiation and distinguish between malignant mesothelioma and

FACULTY:

Parvin Ganjei-Azar MD
Merce Jorda MD, PhD

Practicing Pathologists
Cytopathology
Cytopathology (Non-Gynecologic)
2.0 CME/CMLE Credits

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Cytologic Sub-Classification of Lung Cancer
New Challenge for Practicing Pathologists

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Disclosure

I do not have, and have not had, any relevant financial relationship with any commercial interests within the past 12 months, as pertaining to this presentation.

Parvin Ganjei-Azar MD.
Merce Jorda MD, PhD

Lung cancer is the most frequent cause of major cancer incidence and mortality worldwide.

More people die of lung cancer than of colon, breast, and prostate cancers combined.
• Lung cancer is the second most common cancer in both men (after prostate cancer) and women (after breast cancer)

• In 2011…..
  • 221,130 newly diagnosed cases
  • 156,940 deaths from lung (27% of all cancer deaths)

• Black men are about 40% more likely to develop lung cancer than white men

• The rate is about the same in black women and in white women

• The rate of lung cancer has been dropping among men for many years

• The rate of lung cancer is fairly stable among women.
• Significant decline in incidence/mortality for African American and white males
• Increase in incidence/mortality for females of both races.

Source: North American Association of Central Cancer Registries (NAACCR), the National Cancer Institute (NCI), and the American Cancer Society. Journal of the National Cancer Institute, May 4, 2011.

Cytology of Lung Cancer
Sampling Methods
• Sputum
• Transthoracic (FNA)
• Transbronchial (FNA, BB, BL)
• Transesophageal (FNA)
• Body Cavity Fluids

Cytology of Lung Cancer: Goals
• Preventive
• Diagnostic
• Prognostic
• Predictive
Preventive

Spiral (helical) CT scans of the lungs
Chest X-rays
Sputum cytology

• They can find cancers earlier
• There is little evidence that they prevent death from lung cancer

High-risk patients

The International Early Lung Cancer Action Program (I-ELCAP):
– 48 institutions in 9 countries
– Helical CT scan with followed biopsy or cytology
– Curability of stage I lung cancers is 80-90%
– Costs of CT screening for lung cancer compare favorably with breast, cervical, and colon cancer screenings

The U.S. Preventive Services Task Force concludes that evidence is insufficient to recommend for or against screening asymptomatic persons for lung cancer with either low dose computerized tomography, chest x-ray, sputum cytology, or a combination of these tests.
Pulmonary Cytology: Goals

- Preventive
- Diagnostic
- Prognostic
- Predictive

Diagnostic Goals

- Malignant neoplasms
- Benign neoplasms
- Infectious/Inflammatory processes

Malignant Neoplasms of Lung

- Primary
- Secondary
Primary Lung Carcinoma

Primary Lung Neoplasms: Classification

Based on histomorphology

WHO 2004
Based on histomorphology, genetics and clinical information

Primary Lung Neoplasms: Classification

International Multidisciplinary Classification
IASLC/ATS/ERS, 2011

Identification of prognostic and predictive factors

- International Association for the study of lung cancer (IASLC)
- American Thoracic Society (ATS)
- European Respiratory Society (ERS)

J Thorac Oncol. 2011; 6: 244-285
International Multidisciplinary Classification, 2011

- Applies to
  - Resection specimens
  - Biopsies
  - Cytology samples

- Bronchioloalveolar carcinoma and mixed subtypes adenocarcinoma are **NOT used**

- Provides guidance for diagnosis in cytology samples (70% of lung cancers are diagnosed by cytology)

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**Primary Lung Carcinoma Cytomorphology**

<table>
<thead>
<tr>
<th>Type</th>
<th>Frequency</th>
<th>5-year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>80 %</td>
<td>17%</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td>15%</td>
</tr>
<tr>
<td>Squamous Cell</td>
<td></td>
<td>11%</td>
</tr>
<tr>
<td>Large Cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small Cell</td>
<td>20 %</td>
<td>5%</td>
</tr>
</tbody>
</table>
Adenocarcinoma

Mixed Type
Acinar
Papillary
Solid

Bronchioloalveolar (non-mucinous)
Bronchioloalveolar (mucinous)
Fetal
Mucinous (colloid)
Signet ring
Clear cell
No counterpart

Adenocarcinoma with lepidic pattern
Mucinous adenocarcinoma
Adenocarcinoma with fetal pattern
Adenocarcinoma with colloid pattern
Adenocarcinoma with signet ring cell features
Adenocarcinoma with clear cell features
NSCLC, favor Adenocarcinoma

WHO, 2004
Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

Adenocarcinoma

Cytomorphology

- Cohesive groups
- Acinar, papillary, micropapillary formations
- Cytoplasmic vacuoles
- Mucinous background

Small Biopsy/Cytology IASLC/ATS/ERS, 2011

Adenocarcinoma

<table>
<thead>
<tr>
<th>Gland Forming</th>
<th>Biopsy</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Micropapillary</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lepidic growth</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mucinous diff.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>“Colloid”</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Signet-ring</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clear cell</td>
<td>Yes</td>
<td>?</td>
</tr>
</tbody>
</table>
Squamous Cell Carcinoma Cytomorphology

• Cohesive groups
• Isolated pleomorphic cells
  • Hyperchromatic nuclei
  • Eosinophilic cytoplasm
• Necrotic background
Small cell carcinoma

WHO, 2004

Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

Incidence

Small cell Carcinoma  20%

Non-small cell carcinoma  80%

About 75% of lung cancers are NSCLC.
Small Cell Carcinoma
Cytomorphology

- Isolated small cells / small groups
- Crushing artifact
- Nuclear molding
- Cellular fragmentation
- Tumor necrosis

Large Cell Carcinoma
WHO, 2004

Large cell carcinoma
Large cell neuroendocrine carcinoma (LCNEC)
Large cell carcinoma with neuroendocrine morphology (LCNEM)

Non-small cell carcinoma, NOS
Non-small cell carcinoma, with neuroendocrine morphology (positive NE markers) possibly LCNEC
Non-small cell carcinoma, with neuroendocrine morphology (negative NE markers); LCNEC is suspected

J Thorac Oncol. 2011; 6: 244-285
Large Cell Carcinoma
Cytomorphology

- No squamous or glandular differentiation
- Isolated cells
- Pleomorphic nuclei
- Macronucleoli
- Binucleation

Adenosquamous Carcinoma

WHO, 2004
Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

- Adenosquamous Carcinoma
- Non-small cell carcinoma with squamous cell and adenocarcinoma patterns
- No counterpart
- Non-small cell carcinoma, NOS with IHC favoring both squamous and adenocarcinoma patterns
Primary Lung Carcinoma

Immunocytochemistry

Malignant Neoplasms in Lung

Immunocytochemistry

Applications

• Sub-classification of primary lung cancer
### Immunocytochemistry in Primary Lung Carcinomas

<table>
<thead>
<tr>
<th></th>
<th>Adenocarcinoma</th>
<th>Squamous Cell</th>
<th>Small Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF-1</td>
<td>Positive</td>
<td>Negative</td>
<td>TTF-1</td>
</tr>
<tr>
<td>CK 7</td>
<td>Positive</td>
<td>Negative</td>
<td>CK</td>
</tr>
<tr>
<td>p63</td>
<td>Negative</td>
<td>Positive (dot-like)</td>
<td>Positive</td>
</tr>
<tr>
<td>CK 20</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) positive in intestinal type

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### NSCLC

**Prognostic and Predictive Molecular Markers**

- A *prognostic biomarker* is a biomolecule that is indicative of patient survival independent of the treatment received.
- A *predictive biomarker* is a biomolecule that is indicative of therapeutic efficacy.

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NSCLC: Prognostic and Predictive Biomarkers

- EGFR mutation:
  - 15% of Caucasians
  - 30% of Asians
- EGFR high copy number
  - 40% Caucasians (FISH, IHC)
- KRAS mutation: 25%
- EML4-ALK translocation: 6%
- BRAF mutation: 3%

NSCLC: Target Therapy

- Patients with EGFR mutation respond to TKI (Erlotinib, Getifinib).
- Patients with KRAS or BRAF mutation do not respond to TKI, ALKI
- Patients with EML4-ALK fusion, respond to ALK inhibitor (Crizotinib)

Secondary Lung Carcinoma
Secondary Lung Carcinomas

- Metastatic carcinomas are the most common malignant neoplasms found in lung
- Adenocarcinomas are the most common secondary neoplasms
- Sites of origin: Breast, colorectal, endometrium, others
- Distinction between lung primary and secondary carcinomas can not be made, based on cytomorphology alone

Secondary Lung Carcinomas

- Final diagnosis should be based on:
  - Clinical
  - Imaging
  - Cytomorphology
  - Immunocytochemistry

Malignant Neoplasms in Lung Immunocytochemistry Applications

- Distinction between primary vs. secondary carcinomas
Primary vs. Secondary
Commonly Used Markers

- TTF-1
- Napsin
- TGB
- PSA
- S100, HMB45
- ER, PR
- CK 7 and CK20
- P63
- CDX-2
- HCA, RCC
- Endocrine markers
- Lymphoid markers

Primary vs. Secondary

- Adenocarcinomas
- Squamous cell carcinomas
- Small cell carcinomas
- Large cell carcinomas
Adenocarcinoma

<table>
<thead>
<tr>
<th>Male</th>
<th>Lung</th>
<th>Upper GI</th>
<th>Colorectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF-1</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CK7</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CK20</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Cancer Cytopathology 2002

Thyroid Transcription Factor 1: A Marker for Lung Adenocarcinomas in Body Cavity Fluids

Lung FNA; 76 M
History of Colon Ca.

Adenocarcinoma of Lung

Ganjeh & Nadji
Springer 2007
Lung FNA: Patient with history of colorectal carcinoma

CDX2

CK20

Adenocarcinoma

<table>
<thead>
<tr>
<th>Female</th>
<th>Lung</th>
<th>Breast</th>
<th>Colorectal</th>
<th>FGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF-1</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CK 7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CK 20</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CA 125</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- Breast
- Colorectal
- Female
- Genital tract
- Others
"Cellular evidence of adenocarcinoma. ICC for further subclassification to follow."

ER(+) "This immunophenotype is consistent with metastasis from the patient’s known primary breast carcinoma."

Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

Squamous cell carcinoma
Papillary
Clear cell
Small cell
Bazaloid

NSCLC, favor Squamous Cell Carcinoma

Secondary Squamous Cell Carcinomas

Squamous cell Carcinoma

<table>
<thead>
<tr>
<th>Male</th>
<th>Lung</th>
<th>Head &amp; Neck</th>
<th>Esophagus</th>
<th>Urothelial</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TP 2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>TP 3</td>
<td>- / +</td>
<td>- / +</td>
<td>- / +</td>
<td>- / +</td>
</tr>
</tbody>
</table>
P63 Differentiates Subtypes of Nonsmall Cell Carcinomas of Lung in Cytologic Samples

Implications in Treatment Selection

Horse Lords, M.D., M.S.; Permten, Fierce-Fernandez, M.D.; Vivas-Barcia, M.D.; Fatemeh Moosavi, M.D.; Calo Wolk, M.D.; Aili Rekai, M.D.; Gustavo Femandez-Castro, M.D.; and Fabio Gorga, M.D.

Cancer Cytopathology February 25, 2009

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Squamous cell Carcinoma

Female

• Lung
  • Head & Neck
  • Esophagus
  • Uterine Cervix
  • Urothelial
  • Others

---

<table>
<thead>
<tr>
<th>Female</th>
<th>Lung</th>
<th>Head &amp; Neck</th>
<th>Esophagus</th>
<th>Uterine Cervix</th>
<th>Urothelial</th>
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<tbody>
<tr>
<td>TTF-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P16</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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Squamous Cell Carcinoma

Secondary Small Cell Carcinomas

Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

Small cell carcinoma

Male
Female

Skin
Others

TTF-1
CK 20

Lung Skin ( Merkel)

+  -
-  +
Differential Diagnosis
Small Cell Carcinoma vs. Other Small Cell Neoplasms

<table>
<thead>
<tr>
<th></th>
<th>CK</th>
<th>CD45</th>
<th>DES</th>
<th>CD99</th>
<th>SYN</th>
<th>TTF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Cell Ca</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PPNET</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
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</tbody>
</table>

Malignant Neoplasms in Lung
Immunocytochemistry Applications

• Distinction between lung primary and malignant mesothelioma

Lung Primary vs. Malignant Mesothelioma

<table>
<thead>
<tr>
<th></th>
<th>CEA</th>
<th>TTF-1</th>
<th>Calretinin</th>
<th>p63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Adenocarcinoma</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malignant Mesothelioma</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Squamous Cell Carcinoma</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Calretinin is the most useful marker for mesothelial cells. Expression is both nuclear & cytoplasmic. Calretinin does not differentiate benign from malignant mesothelial cells.

Sarcomatoid mesotheliomas are negative for calretinin. Lung spindle cell carcinomas & sarcomatoid mesotheliomas express CK but are negative for TTF-1. Lung spindle cell carcinomas may express p63, whereas mesotheliomas are p63 negative.
Intended Learning Outcomes

• Formulate a differential diagnosis based on clinical, imaging and cytologic findings
• Differentiate lung primary carcinomas from those of metastatic origin, and sub-classify primary carcinomas
• Application of immunocytochemistry in cytologic material
• Applicability of molecular assays