Cytological Sub-classification of Lung Cancer: Morphologic and Molecular Characteristics

Una Patología para el siglo XXI
Patología molecular, imagen digital y gestión
Cádiz
22-24 mayo 2013

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

- **Decline** in mortality for African American and white males
- **Increase** in mortality for African American and white females

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

• Basis for diagnosis
  70% of lung cancers are diagnosed by cytology

• Equivalent results to biopsy for
  • Diagnostic
  • Prognostic
  • Predictive

Cytology of Lung Cancer
Sampling Methods

- Sputum
- Transthoracic (FNA)
- Transbronchial (FNA, BB, BW)
- Transesophageal (FNA)
- Effusion
Primary Lung Neoplasms: Classification

Based on histomorphology

WHO 2004
Based on histomorphology, genetics and clinical information
<table>
<thead>
<tr>
<th>Type</th>
<th>Frequency</th>
<th>5-year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>80 %</td>
<td>17%</td>
</tr>
<tr>
<td>Squamous Cell</td>
<td></td>
<td>15%</td>
</tr>
<tr>
<td>Large Cell</td>
<td></td>
<td>11%</td>
</tr>
<tr>
<td>Small Cell</td>
<td>20 %</td>
<td>5%</td>
</tr>
</tbody>
</table>
Primary Lung Neoplasms: Classification

International Multidisciplinary Classification
IASLC/ATS/ERS, 2011

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

Identification of
prognostic and predictive factors
Applies to resection specimens and
Small biopsies-Cytology samples

J Thorac Oncol. 2011; 6: 244-285
Current treatment of Lung cancer is focus in personalized medicine based on histologic cell type and molecular status.
NSCLC: Target Therapy

– tyrosine kinase inhibitors (TKI) first-line therapy in patients with advanced lung adenocarcinoma with **EGFR mutations**

– adenocarcinomas with **ALK rearrangements** are responsive to crizotinib (ALK inhibitor).

– Patients with **KRAS or BRAF mutation** do not respond to TKI, ALKI
NSCLC: Target Therapy

– patients with adenocarcinoma or NSCLC, not otherwise specified (NSCLC-NOS), are more responsive to pemetrexed than those squamous cell carcinoma
– squamous cell carcinoma is associated with life-threatening hemorrhage in patients treated with bevacizumab; therefore, it is contraindicated in lung cancer patients with this histology.

Arch Pathol Lab Med 2013, 137:668-684
International Multidisciplinary Classification, 2011

Based on histologic cell type and molecular status

Recommendation:

– **NSCLC be further classified** into a more specific type, such as adenocarcinoma or squamous cell carcinoma, whenever possible (strong recommendation).

– **NSCLC-NOS be used as little as possible**, and we recommend it be applied only when a more specific diagnosis is not possible by morphology and/or special stains.
**Adenocarcinoma**

**WHO, 2004**

- Mixed Type, Acinar, Papillary, Solid

**Bronchioloalveolar (non-mucinous)**

- Adenocarcinoma with lepidic pattern

**Bronchioloalveolar (mucinous)**

- Mucinous adenocarcinoma

**Fetal**

- Adenocarcinoma with fetal pattern

**Mucinous (colloid)**

- Adenocarcinoma with colloid pattern

**Signet ring**

- Adenocarcinoma signet ring cell features

**Clear cell**

- Adenocarcinoma with clear cell features

**No counterpart**

* Eliminated in IASLC/ATS/ERS, 2011

**Small Biopsy/Cytology**

**IASLC/ATS/ERS, 2011**

- **NSCLC, favor Adenocarcinoma**
  - Morphologic patterns not present
  - Positive TTF-1

**J Thorac Oncol. 2011; 6: 244-285**

**Arch Pathol Lab Med 2013, 137: 668-684**

**New in IASLC/ATS/ERS, 2011**
Adenocarcinoma: Cytomorphology

Cohesive groups:
- flat sheets or 3-dimensional cell balls
- pseudopapillary aggregates
- true papillae with central fibrovascular cores
- acinar structures
- “drunken honeycomb”

Individual tumor cells are:
- basophilic
- cytoplasm: granular, foamy, translucent, vacuoles

The nuclei are:
- eccentrically located
- chromatin: from granular to hyperchromatic macronucleoli
Small Biopsy/Cytology IASLC/ATS/ERS, 2011

Adenocarcinoma

- Gland Forming
- Micropapillary
- Lepidic growth
- Mucinous diff.
- “Colloid”
- Signet-ring
- Clear cell

WHO 2004
Bronchoalveolar Carcinoma, non-mucinous

IASLC/ATS/ERS, 2011
Adenocarcinoma with lepidic pattern
WHO 2004
Bronchoalveolar Carcinoma, non-mucinous

IASLC/ATS/ERS, 2011
Adenocarcinoma with lepidic pattern
WHO 2004
Bronchoalveolar Carcinoma, non-mucinous

IASLC/ATS/ERS, 2011
Adenocarcinoma with lepidic pattern
WHO 2004
Bronchoalveolar Carcinoma, mucinous

IASLC/ATS/ERS, 2011
Mucinous Adenocarcinoma
WHO 2004
Bronchoalveolar Carcinoma, mucinous

IASLC/ATS/ERS, 2011
Mucinous Adenocarcinoma
WHO 2004
Bronchoalveolar Carcinoma, mucinous

IASLC/ATS/ERS, 2011
Mucinous Adenocarcinoma
WHO 2004
Adenocarcinoma

IASLC/ATS/ERS, 2011
Adenocarcinoma, micropapillary type
Use of Immunohistochemistry

• Use in cases lacking characteristics of adenocarcinoma or squamous cell carcinoma

• IHC can help in classifying NSCLC, nos into:
  NSCLC favor adenocarcinoma
  NSCLC favor squamous cell carcinoma

• Use only 2 markers to preserve material for molecular tests
  **TTF-1:** adenocarcinoma (or Mucin)
  **P63 or p40:** squamous cell carcinoma
  **Cocktails** of nuclear and cytoplasmic markers
    (TTF-1/cytokeratin 5/6 or p63/napsin A)
WHO 2004
No counterpart

IASLC/ATS/ERS, 2011
NSCLC, favor Adenocarcinoma
WHO 2004
No counterpart

IASLC/ATS/ERS, 2011
NSCLC, favor Adenocarcinoma

TTF-1
Pleural Fluid: Adenocarcinoma
Pleural Fluid: Adenocarcinoma from lung origin

Cancer Cytopathol 96:289-293, 2002
Squamous cell carcinoma

- Papillary
- Clear cell
- Small cell
- Basaloid

No counterpart

Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

Squamous cell carcinoma

- Papillary
- Clear cell
- Small cell
- Basaloid

NSCLC, favor Squamous Cell Carcinoma
Morphologic patterns not present
Positive p40/p63

WHO, 2004

J Thorac Oncol. 2011; 6: 244-285
Arch Pathol Lab Med 2013, 137: 668-684
Squamous cell carcinoma: Cytomorphology

**Cohesive groups:**
- flat sheets, keratinizing changes, pearls, necrotic background

**Individual tumor cells:**
- cytoplasm opaque or dense
- have round to ovoid to elongated contours with sharply defined cell borders, "tadpole" configurations

**The nuclei are:**
- solitary, centrally situated, and hyperchromatic chromatin is very dense, homogeneous, pyknotic, nucleoli are not well developed.
WHO 2004
Squamous cell carcinoma

IASLC/ATS/ERS, 2011
Squamous cell carcinoma
WHO 2004
Squamous cell carcinoma

IASLC/ATS/ERS, 2011
Squamous cell carcinoma
Use of Immunohistochemistry

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WHO 2004
No counterpart

IASLC/ATS/ERS, 2011
NSCLC, favor Squamous Cell Carcinoma
WHO 2004
No counterpart

IASLC/ATS/ERS, 2011
NSCLC, favor Squamous Cell Carcinoma


p63
WHO 2004
No counterpart

IASLC/ATS/ERS, 2011
NSCLC, favor Squamous Cell Carcinoma
WHO 2004
No counterpart

IASLC/ATS/ERS, 2011
NSCLC, favor Squamous Cell Carcinoma
Small cell carcinoma

WHO, 2004

Small cell carcinoma

Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

NSCLC with NE morphology
(Positive NE markers)

Large cell Neuroendocrine

Large cell Neuroendocrine
with NE morphology

J Thorac Oncol. 2011; 6: 244-285
Arch Pathol Lab Med 2013, 137: 668-684

NSCLC with NE morphology
(negative NE markers)
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
WHO 2004
Small Cell Carcinoma

IASLC/ATS/ERS, 2011
Small Cell Carcinoma
PanKeratin
WHO 2004
Small Cell Carcinoma

IASLC/ATS/ERS, 2011
Small Cell Carcinoma

TTF-1
WHO 2004
Small Cell Carcinoma

IASLC/ATS/ERS, 2011
Small Cell Carcinoma
WHO 2004
Large cell NE Carcinoma

IASLC/ATS/ERS, 2011
NSCLC with NE morphology (Positive NE markers)

Chromogranin
Large Cell Carcinoma

WHO, 2004
Large cell carcinoma

IASLC/ATS/ERS, 2011
Non-small cell carcinoma, nos

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Arch Pathol Lab Med 2013, 137: 668-684
Large Cell Carcinoma: Cytomorphology

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

WHO, 2004

Adenosquamous Carcinoma

Non-counterpart

Small Biopsy/Cytology
IASLC/ATS/ERS, 2011

Non-small cell carcinoma with squamous cell and adenocarcinoma patterns

Non-small cell carcinoma, NOS with IHC favoring both squamous and adenocarcinoma patterns

J Thorac Oncol. 2011; 6: 244-285
Arch Pathol Lab Med 2013, 137: 668-684
Sarcomatoid Carcinoma

WHO, 2004

Small cell carcinoma
IASLC/ATS/ERS, 2011

Sarcomatoid Carcinoma

NSCLC with spindle and or giant cell morphology (with or without adeno or squamous component)
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
VEGF
C-met
P13k
HER2
ROS1
NSCLC: Target Therapy

– tyrosine kinase inhibitors (TKI) first-line therapy in patients with advanced lung adenocarcinoma with **EGFR mutations**

– adenocarcinomas with **ALK rearrangements** are responsive to crizotinib (ALK inhibitor).

– Patients with **KRAS** or **BRAF** mutation do not respond to TKI, ALKI
Analysis of Deletions in Exon 19 of EGFR

Assay Setup

Theoretical Histogram of Wild Type

Summary

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample ID</th>
<th>Result</th>
<th>Frequency</th>
<th>Amino Acid Substitution</th>
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<tbody>
<tr>
<td>A6</td>
<td>13-3134</td>
<td>Mutation (2236del15)</td>
<td>44.5 %</td>
<td>del E746-A750</td>
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<tr>
<td>A8</td>
<td>DNA CONT 30</td>
<td>Wildtype</td>
<td></td>
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</tr>
</tbody>
</table>
FISH Assay for ALK Rearrangement

ALK break-apart FISH assay
[Courtesy John Lafrate, Massachusetts General Hospital]

Assay is positive if rearrangements can be detected in ≥15% of cells
FISH = fluorescence in situ hybridization

Analysis of Mutations in Codon 12 and 13 of KRAS.

Assay Setup

Theoretical Histogram of Wild Type

Summary
NOTE: Only the mutation with the highest frequency is reported.

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample ID</th>
<th>Result</th>
<th>Frequency</th>
<th>Codon Change</th>
<th>Amino Acid Substitution</th>
<th>Info</th>
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<tbody>
<tr>
<td>B1</td>
<td>13-2997</td>
<td>Mutation (codon 12)</td>
<td>46.8 %</td>
<td>GGT&gt;GAT</td>
<td>G12D</td>
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<tr>
<td>B3</td>
<td>DNA CONT 28</td>
<td>Wildtype</td>
<td></td>
<td></td>
<td></td>
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SDC4–ROS1 fusion identified in a patient with NSCLC who responded to crizotinib treatment.
