Molecular Pathobiology of Lung Cancer

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Outline

- Lung Anatomy
- Lung Carcinoma Classification & Morphology
- Lung Carcinoma Epidemiology
- Associated Molecular Abnormalities/Targets
Bronchial Wall
Lobule Architecture
Bronchiolar Wall
Neoplasms Involving the Lung

- Primary Neoplasms of Lung
- Metastatic Neoplasms to Lung
Primary Neoplasms of Lung: Classification by Lineage

- Epithelial – most common
- Melanocytic
- Stromal
Malignant epithelial neoplasms (Carcinomas) Classification and Morphology

- Squamous cell carcinoma
- Adenocarcinoma
- Large cell undifferentiated carcinoma
- Small cell undifferentiated carcinoma
Squamous cell carcinoma

- **Clin:** Smokers (98%)
  20-30% of common carcinomas

- **Rad:** central > > peripheral

- **Path:** Bronchi > Larynx > Trachea
  +/- Desmosomes (intercellular bridges)
  +/- Keratin production, e.g. keratin pearls
Squamous cell carcinoma
Squamous cell carcinoma
Squamous cell carcinoma in situ

Respiratory mucosa

Squamous cell carcinoma in situ
Invasive Squamous Carcinoma

Desmosomes

Keratin
Metastatic squamous cell carcinoma to lymph node

Normal lymph node lymphocytes

Mets in subcapsular sinuses
Adenocarcinoma

- Clin: 30-40% of common carcinomas
  Most common carcinoma in non-smokers

- Rad: peripheral > central

- Path: +/- glands
  +/- mucin
  Bronchiolo-alveolar carcinoma subset
Adenocarcinoma

Primary

Pleural effusion

Adapted from Slide Atlas, Diagnostic Oncology, ed. AT Skarin, Gower Med Publ, 1992
Adenocarcinoma

Mucin production (red on PASd stain)

Adenocarcinoma
Bronchioloalveolar carcinoma (BAC)

- Clin: 20-25% of adenocarcinomas
  Not associated with cigarette smoking
- Rad: Peripheral, can be multifocal and bilat
- Path: Lepidic (butterfly-like) growth pattern
  Mucinous or non-mucinous
  Unifocal or multifocal
  Distinction of multifocal 1° from mets
Bronchiolo-alveolar carcinoma

Multiple, bilateral pulmonary nodules

Adapted from Slide Atlas, Diagnostic Oncology, ed. AT Skarin, Gower Med Publ, 1992
Bronchiolo-alveolar carcinoma
Bronchiolo-alveolar carcinoma
Large cell undifferentiated carcinoma

- Clin: 10% of common carcinomas
- Rad: non-specific
- Path: H&E: Undifferentiated
  - No squamous or adeno features
  - No neuroendocrine features
Large cell undifferentiated carcinoma

Adapted from Slide Atlas, Diagnostic Oncology, ed. AT Skarin, Gower Med Publ, 1992
Large cell undifferentiated carcinoma
Non-Small Cell Lung Carcinomas: Prognostic variables

• Definitely: Stage (local, nodes, distant mets), performance status, weight loss

• Definitely not: Age, histology
Small cell (undifferentiated) carcinoma

- **Clin:** Smokers
  Ca. 20% of common carcinomas
  Ectopic hormones (e.g. ACTH, ADH)
  Commonly high stage at presentation
  Responsive to chemo/RT, but low 5 yr survival

- **Rad:** Central in >90%
  Frequent metastasates to LNs and distant sites

- **Path:** Malignant cytology
  No nucleoli
  High mitotic activity and necrosis
Small cell carcinoma

At diagnosis

Response to Chemo/radiotherapy

Adapted from Slide Atlas, Diagnostic Oncology, ed. AT Skarin, Gower Med Publ, 1992
Small cell carcinoma

Necrotic carcinoma

Viable carcinoma

Small cell carcinoma
Small cell carcinoma
Small cell carcinoma
Normal lymphocytes

Metastatic small cell carcinoma
Small Cell Lung Carcinoma: Prognostic variables

- Definitely: Stage, performance status
- Probably: Gender, age, # of metastatic sites
Malignant epithelial neoplasms (Carcinomas)

- Squamous cell carcinoma
- Adenocarcinoma
- Large cell undifferentiated carcinoma
- Small cell undifferentiated carcinoma
Metastatic Neoplasms to Lung

- Most common malignant neoplasms involving the lung
- Multiple nodules favor metastases over primary neoplasms (except BAC)
- Carcinomas
- Sarcomas
- Melanoma
Metastatic carcinomas

- Breast adenocarcinoma
- GI adenocarcinoma
- Renal adenocarcinoma
- Head/neck squamous cell carcinoma
Metastatic breast carcinoma
Metastatic colon carcinoma
Metastatic renal cell carcinoma
Metastatic ENT carcinoma
Metastatic sarcomas

- Osteosarcomas
- Soft tissue sarcomas
Metastatic Osteosarcoma
Metastatic melanoma

- Clin: Extrapulmonary 1° melanoma much more common than pulmonary 1°. No known 1° in 5-10% of cases.

- Path: Variable architecture & cytology. May be pigmented. Use immunohistochemistry to confirm.
Metastatic melanoma
Summary: Classification and Morphology of Lung Neoplasms

- **1° Lung Neoplasms** – Most are carcinomas
  - Small cell vs. Non-small cell carcinoma is a major clinical distinction
  - New drug indications mandate distinction of Squamous from Adenocarcinoma

- Metastases to Lung – All lineages possible
What is the underlying science in this morphologic spectrum of lung carcinomas?
Epidemiology
## Estimated Deaths

<table>
<thead>
<tr>
<th></th>
<th>MALES</th>
<th></th>
<th></th>
<th>FEMALES</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>90,810</td>
<td>31%</td>
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<td>Lung &amp; bronchus</td>
<td>71,030</td>
<td>26%</td>
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<tr>
<td>Prostate</td>
<td>28,660</td>
<td>10%</td>
<td></td>
<td>Breast</td>
<td>40,480</td>
<td>15%</td>
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<tr>
<td>Colon &amp; rectum</td>
<td>24,260</td>
<td>8%</td>
<td></td>
<td>Colon &amp; rectum</td>
<td>25,700</td>
<td>9%</td>
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<tr>
<td>Pancreas</td>
<td>17,500</td>
<td>6%</td>
<td></td>
<td>Pancreas</td>
<td>16,790</td>
<td>6%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>12,570</td>
<td>4%</td>
<td></td>
<td>Ovary</td>
<td>15,520</td>
<td>6%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>12,460</td>
<td>4%</td>
<td></td>
<td>Non-Hodgkin lymphoma</td>
<td>9,370</td>
<td>3%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>11,250</td>
<td>4%</td>
<td></td>
<td>Leukemia</td>
<td>9,250</td>
<td>3%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>9,950</td>
<td>3%</td>
<td></td>
<td>Uterine corpus</td>
<td>7,470</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>9,790</td>
<td>3%</td>
<td></td>
<td>Liver &amp; intrahepatic bile duct</td>
<td>5,840</td>
<td>2%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,100</td>
<td>3%</td>
<td></td>
<td>Brain &amp; other nervous system</td>
<td>5,650</td>
<td>2%</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>294,120</strong></td>
<td><strong>100%</strong></td>
<td></td>
<td><strong>All Sites</strong></td>
<td><strong>271,530</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Adapted from Jemal, A et al CA Cancer J Clin 58: 71, 2008
Male Cancer Death Rates, 1930-2004

Adapted from CA Cancer J Clin 58: 71, 2008
Female Cancer Death Rates, 1930-2004

Annual Age-adjusted Cancer Death Rates

Adapted from CA Cancer J Clin 58: 71, 2008
Lung Carcinomas: 
Epidemiology

- Estimated Incidence (2008): 215,000 (US)
- Estimated Mortality (2008): 162,000 (US)
- >85% of lung carcinoma deaths (and 30% of all cancer deaths) occur in cigarette smokers
- Risk = f(# cigarettes smoked), 15-30X in heavy smokers, 50-60X in asbestos workers who smoke
- Risk decreases with cessation of cigarette smoking: ca. 1.5X after 15 years
Smoking-Associated Lung Diseases

- Anthracosis
- Desquamative interstitial pneumonia
- Chronic bronchitis
- Emphysema $\rightarrow$ pulmonary arterial hypertension
- Langerhans cell histiocytosis
- Primary lung carcinomas – all except BAC
- ENT squamous carcinoma metastatic to lung
Benefits to the Lung of Smoking
How does smoking cause lung carcinomas?
Tobacco: Chemistry

- **Gases:** CO, CO$_2$, formaldehyde, acrolein, methanol, phenol, anthracenes, pyrenes
- **Particulates:**
  - resin cores in 0.5 $\mu$M diameter water droplets
  - est. $10^9$ particles/ml
  - 50% deposited in and cleared by cilia
  - remainder: phagocytosis, lymphatic transport
- **Overall:** 4,000 chemical compounds, of which ca. 40 are considered carcinogenic
Accumulation of Mutations

\[ K_{\text{accum}} = K_{\text{mut}} - K_{\text{repair}} \]

Related to carcinogen dose
i.e. pk-yrs of smoke exposure

Related to DNA damage repair capability
DNA Damage Recognition Mechanisms

Mismatch Repair

Adapted from Carr, Science 300: 1512, 2003
Primary Lung Carcinomas

Associated Molecular Abnormalities and Potential Therapeutic Targets
Lung Squamous Carcinomas

Cytogenetic
- 3p del (RASSF1A, FHIT)
- 9p del (p16)
- 17p del (p53)
- Gene amp EGFR (30%)

Genetic
- p53 (60-70%)

Epigenetic
- p16, FHIT (early methylation)

Protein
- Bcl-2 overexpression (25%)
Lung Adenocarcinoma (Smokers)

Cytogenetic
- Extensive amplifications and deletions
- Most common 14q amp, NKX2→ TTF1

Genetic
- KRAS mutations (10-30%)
- P53 mutations (50-70%)

Epigenetic
- p16 methylation assoc with KRAS mutations

Protein
- TTF-1 overexpression (85%)
Lung Adenocarcinoma (Non-Smokers)

Cytogenetic
- EGFR amplification

Genetic
- EGFR mutation (10-40%)
- KRAS mutation (10-30%)
- Mutually exclusive

Epigenetic
- Lower methylation index

Protein
- +/- EGFR overexpression
Small Cell Lung Carcinoma

Cytogenetic
- Extensive amps (e.g. Myc) and dels
- Del 3p (FHIT, VHL), 13q (Rb), 17p (p53)

Genetic
- Rb mutations
- P53 mutations

Epigenetic
- Methylation of CDH1 (E-cadherin)

Protein
- Bcl-2, E2F-1, telomerase overexpression
Unknown Case

- 59 y.o. ex-smoker male with 6cm R hilar mass
- Mediastinal and cervical lymphadenopathy
- Bone and brain lesions
- All lesions PET positive
- Hard R cervical lymph node, for biopsy
R Cervical LN Biopsy
R Cervical LN Biopsy – CK20
R Cervical LN Biopsy – TTF-1
R Cervical LN Biopsy – EGFR
Morphologic Diagnosis

By H&E alone:
- Metastatic non-small cell carcinoma, poorly differentiated, favor ACa

H&E + IPOX:
- Metastatic ACa, poorly differentiated, consistent with lung primary
- EGFR 1+(of 3+), 20-30% of ACa cells
Molecular Data

Exon 19 EGFR
Wildtype Sequence

Exon 19 EGFR
Patient Sequence
(delE746-A750)

Adapted from Shigematsu, JNCI 97: 339, 2005
EGFR Dimerization and Downstream Signaling
EGFR Mutations in NSCLC

Adapted from Herbst et al, NEJM 359: 1367, 2008
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Activating EGFR Mutations

Adapted from Misudomi et al, Int J Clin Oncol 11: 190, 2006
Mutations Also Predict Response to Tyrosine Kinase Inhibitors (TKIs)

Adapted from Misudomi et al, Int J Clin Oncol 11: 190, 2006
Model Structure of EGFR Kinase Domain

Del-1 deletion: codons 746-750

G719S

L858R

Adapted from Paez et al, Science 304: 1497, 2004
**EGFR Mutants Can Transform Fibroblasts**

Table 1. NIH-3T3 Cells Expressing the Lung Cancer-Derived Mutant EGFR Display Loss of Contact Inhibition

<table>
<thead>
<tr>
<th>Retrovirus</th>
<th>Normalized Foci</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBabe-Puro</td>
<td>0</td>
</tr>
<tr>
<td>Wild-type EGFR</td>
<td>0</td>
</tr>
<tr>
<td>L858R</td>
<td>1,280</td>
</tr>
<tr>
<td>G719S</td>
<td>975</td>
</tr>
<tr>
<td>D837A</td>
<td>0</td>
</tr>
</tbody>
</table>

Reproduced with permission from Greulich et al, PLoS Med 2:1167, 2005

Table 2. Clonal NIH-3T3 Cell Lines Expressing the Lung Cancer-Derived Mutant EGFR Form Tumors in Immunocompromised Mice

<table>
<thead>
<tr>
<th>Retrovirus</th>
<th>Number of Tumors</th>
<th>Average Tumor Diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBabe-Puro</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>Wild-type EGFR</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>L858R</td>
<td>6/6</td>
<td>12 ± 0.8*</td>
</tr>
<tr>
<td>G719S</td>
<td>4/4</td>
<td>7 ± 0.9*</td>
</tr>
<tr>
<td>D837A</td>
<td>0/3</td>
<td>0</td>
</tr>
</tbody>
</table>

Reproduced with permission from Greulich et al, PLoS Med 2:1167, 2005
EGFR Mutants are Active Tyr Kinases without Ligand, i.e. Constitutively Activated

(Protein Immunoblot)

Reproduced with permission from Greulich et al, PLoS Med 2: 1167, 2005
Most Activating EGFR Mutations Are Also Sensitive to TKI

Reproduced with permission from Greulich et al, PLoS Med 2: 1167, 2005
Predictive Variable:
Response to Taxol/CarboPt +/- TKI
Stratifies by EGFR Mutation Status

Adapted from Eberhard et al, J Clin Onc 23: 5900, 2005
Screening for EGFR Mutations
Different Amplicon Lengths Allow Detection of 15 bp Deletion in Exon 19

Reproduced with permission from Pan et al, J Mol Diag 7: 396, 2005
EGFR Exon 21 L858R Mutation Assay Based on RFLP Analysis

Reproduced with permission from Pan et al, J Mol Diag 7: 396, 2005
Significance of KRAS Mutations
### Mutually Exclusive Relationship of KRAS and EGFR in NSCLC

#### Summary of the EGFR and KRAS Mutational Analysis

<table>
<thead>
<tr>
<th></th>
<th>KRAS (n=274)</th>
<th>EGFR (n=274)</th>
<th>Mutant (n=29)</th>
<th>Wild-Type (n=199)</th>
<th>Indeterminate (n=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant (n=55)</td>
<td>2</td>
<td>46</td>
<td>7</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Wild-Type (n=209)</td>
<td>26</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Indeterminate (n=10)</td>
<td>1</td>
<td>3</td>
<td></td>
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<tr>
<td>Indeterminate (n=10)</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indeterminate (n=46)</td>
<td>6</td>
<td></td>
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</tr>
</tbody>
</table>

Adapted from Eberhard, D et al  J Clin Onc 2005; 23: 5900
KRAS Mutants are Not Sensitive to TKIs

Table 1. *EGFR* and *KRAS* Mutation Status in Lung Adenocarcinomas Sensitive or Refractory to Gefitinib or Erlotinib

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene Mutated</th>
<th>Proportion Sensitive</th>
<th>Proportion Refractory</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib</td>
<td><em>EGFR</em> 9/12</td>
<td>0/12</td>
<td>5/12</td>
<td>0.0034</td>
</tr>
<tr>
<td></td>
<td><em>KRAS</em> 0/12</td>
<td></td>
<td></td>
<td>0.0373</td>
</tr>
<tr>
<td>Erlotinib</td>
<td><em>EGFR</em> 8/10</td>
<td>0/26</td>
<td></td>
<td>1.487 × 10⁻⁶</td>
</tr>
<tr>
<td></td>
<td><em>KRAS</em> 0/9</td>
<td></td>
<td>4/26</td>
<td>0.5531</td>
</tr>
<tr>
<td>Gefitinib or Erlotinib</td>
<td><em>EGFR</em> 17/22</td>
<td>0/38</td>
<td></td>
<td>6.801 × 10⁻¹¹</td>
</tr>
<tr>
<td></td>
<td><em>KRAS</em> 0/21</td>
<td></td>
<td>9/38</td>
<td>0.0201</td>
</tr>
</tbody>
</table>

Reproduced with permission from Pao et al, PLoS Med 2: 57, 2005
Molecular Diagnosis and Evaluation of the Current Case

- EGFR gene mutation present, del L746-A750
  - Activating, would be TKI- and chemotherapy- sensitive
- KRAS not mutated

- Data predict that the patient should have an increased survival with either TKI or taxane/Pt chemo.
Summary

- Primary lung carcinoma is common and lethal
- Most 1° lung carcinomas assoc with smoking
- Different types treated differently
- Understanding pathogenesis of different lung carcinomas allows identification of new targets
- EGFR/KRAS mutation screening can predict response to tyrosine kinase inhibitors (TKIs)