Molecular Diagnosis in Lung Cancer Treatment

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Lung cancer development is a multi-stage process

ADENOCARCINOMA
Normal AAH BAC Invasive ca

SMALL CELL CARCINOMA

SQUAMOUS CELL CARCINOMA
Normal AAH SMALL CELL CARCINOMA Invasive ca

6 essential alterations for transformation of Normal cell to Cancer cell

Self-sufficiency in growth signals
Evasion of apoptosis
Inensitivity to anti-growth signals
Sustained angiogenesis
Tissue invasion & metastasis
Limitless replicative potential


Imbalance in cellular homeostasis

APOTOPSIS
Tumor suppressor gene inactivation
Growth inhibition
Angiogenesis
Invasion
Replication

NORMAL CELLS CANCER CELLS
Potential impacts of molecular aberrations

Clinical outcome:
• Promote metastasis and poor prognosis
• Affect response to treatment

Opportunities:
• Develop better disease/therapeutic markers
• Serve as therapeutic targets

Carcinoma cells invariably demonstrate complex chromosomal abnormalities

Normal Karyotype (2n=46)

NSCLC cell line (2n=70)

Abnormalities observed:
• Aneuploidy or unbalanced gain or loss of chromosomes
• Markers chromosomes that cannot be classified by usual G-banding technique

Abnormal (marker) chromosomes invariably represent complex translocations

SKY: Spectral Karyotyping (Chromosomal painting)

Comparative Genomic Hybridization (CGH)
CGH studies have revealed chromosomal regions that are commonly involved in genetic gains and losses.

High-throughput oncogene mutation profiling in human cancer

• 1000 human cancers were studied, including 255 lung cancers
• Tumors were profiled for 238 known mutations in 17 oncogenes
• 30% of samples carry at least one mutation
• In lung cancer, 32% had at least one mutation

Tumor type distribution of oncogene mutations

EGFR is a member of tyrosine kinase receptor family that transmit extracellular growth signals

1. Produce own growth factors (GF)
2. Increase receptor expression
3. Increase activity of receptors (by mutation)
4. Increase activity of molecules that transmit growth signals from receptors

Functionally important protein phosphorylation sites include:
- tyrosine
- serine
- threonine
Aberrant RTK signaling pathways in NSCLC

- **Amplification of RTK:**
  - EGFR (~10%), high polysomy (~30%)
  - PI3K-alpha (60% of SQCC)

- **Activating mutations:**
  - EGFR (10-60%)
  - RAS (20%; mainly ADC)
  - Met/HGF receptor (occasional)
  - Collagen (discoid domain) receptors (DDR 1&2)

- **Overexpression of RTK:**
  - EGFR (60%; 90% SQCC, 50% ADC)
  - Met/HGFR (70%, mainly ADC)

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**Frequency of high EGFR protein expression in NSCLC**

<table>
<thead>
<tr>
<th></th>
<th>SQCC</th>
<th>ADC</th>
<th>LCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rusch (1997)</td>
<td>94%</td>
<td>57%</td>
<td>63%</td>
</tr>
<tr>
<td>Fontanini (1998)</td>
<td>57%</td>
<td>35%</td>
<td>23%</td>
</tr>
<tr>
<td>Hsieh (2000)</td>
<td>92%</td>
<td>53%</td>
<td>60%</td>
</tr>
<tr>
<td>Hirsch (2003)</td>
<td>82%</td>
<td>46%</td>
<td>33%</td>
</tr>
</tbody>
</table>

SQCC: squamous cell ca, ADC: adenoca; LCC: large cell ca

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**EGFR tyrosine kinase domain mutations**

EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy.


SCIENCE April 29, 2004

Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib

Thomas J.Lynch,M.D.,Daphne W.Sell,Ph.D.,Raffaella Sordella,Ph.D.,Sarada Gurubhagavatula,M.D., Ross A.Okimoto,B.S, Brian W.Bromeran,B.A.,Patricia L.Harris,M.S., Sara M.Haserlat, B.A., Jeffrey G.Supko,Ph.D, Frank G.Haluska,M.D.,Ph.D.,David M.Louis,M.D.,David C.Christian,M.D., Jeff Settleman,Ph.D., and Daniel A.Haber,M.D.,Ph.D.

NEW ENGLAND JOURNAL OF MEDICINE, MAY 20, 2004
Mutations in kinase domain


Mutant EGFR are more sensitive to inhibition of kinase activity by EGFR inhibitors

Paez et al. Science 2005;304:1497-1500

“Oncogene addiction” theory
(Weinstein IB. Science 2002;297:63-4)

- Dependency of tumor cells on single and predominant oncogenic activity to sustain their proliferation and/or survival
- Oncogene addiction can be the achilles heel of cancer.

EGFR tyrosine kinase domain mutations

- More common in:
  - Adenocarcinoma
  - Women
  - East Asian NSCLC patients
  - Never smokers

- Tumors with mutations demonstrate significantly greater response rate to EGFR inhibitor drugs gefitinib (Iressa) or erlotinib (Tarceva)
EGFR mutation predicts higher response rate to EGFR TKI treatment

Study No. Pts Pts selection ethnicity Agent RR of WT pts RR of Mut pts Resp Criteria
Tokumo 21 recurrent disease japanese gefitinib 2/12 (17%) 8/9 (89%) ECOG
Takano 86 recurrent disease japanese gefitinib 33/71 (11%) 4/27 (15%) ECOG imaging
Kim 37 sample availability korean gefitinib 3/27 (11%) 11/11 (100%) WHO
Chua 54 sample availability chinese gefitinib 43/90 (49%) 17/23 (75%) ECOG
Mean 21/154 (14%) 74/104 (71%)
Cortes-Funes 85 sample availability spanish gefitinib 6/73 (9%) 6/10 (60%) RECIST
Cappuzzo 89 sample availability italian gefitinib 4/74 (5%) 8/17 (53%) ECOG
Eleuther 274 TRIBUTE US erlotinib+chemo 18/99 (18%) 8/15 (53%) RECIST
Mean 52/246 (11%) 31/42 (52%)

How about survival benefit from erlotinib/gefitinib?

BR.21 study: EGFR TK domain mutation is not a good predictor of differential survival benefit from erlotinib compared to placebo

Tsao MS et al. NEJM 2006;354:527-28

High increases in EGFR gene copy number also predicts response to EGFR TKI drugs

Gene copy number assessed by Fluorescent In Situ Hybridization (FISH)

Low copy number: ≤4 gene copies in <40% cells
High copy number: ≥4 gene copies in ≥40% cells or Gene/chromosome ratio >2

BR.21 study: high EGFR gene copy number is a better predictor of survival benefit from erlotinib than mutation

P value for interaction = 0.10
Tsao MS et al. NEJM 2005;353:133-44
Iressa Survival Evaluation in Lung Cancer (ISEL): Phase 3 placebo controlled trial of gefitinib (Iressa)


Survival benefit of Iressa appears mainly in EGFR high copy (by FISH) patients


Patients with high EGFR gene copy have poorest prognosis, yet they are most likely to benefit from drug treatment

Ras downstream pathways

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>PI3K</td>
<td>Mitogenesis/proliferation</td>
</tr>
<tr>
<td>Akt/PKB</td>
<td>Survival/anti-apoptosis</td>
</tr>
<tr>
<td>Ras</td>
<td>Gene expression</td>
</tr>
<tr>
<td>Raf</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>RAF</td>
<td>Motility/invasion</td>
</tr>
<tr>
<td>RalGDS</td>
<td>angiogenesis</td>
</tr>
<tr>
<td>Tiam1</td>
<td></td>
</tr>
<tr>
<td>Ras1f1A</td>
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</table>

EGFR high copy

EGFR low copy
K-ras mutations could be a negative selection marker for erlotinib therapy

**TRIBUTE TRIAL**

<table>
<thead>
<tr>
<th>K-ras Mutations</th>
<th>Placebo</th>
<th>Tarceva</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-ras Wild Type</td>
<td>50%</td>
<td>40%</td>
</tr>
<tr>
<td>K-ras Mutant</td>
<td>50%</td>
<td>60%</td>
</tr>
</tbody>
</table>

K-ras mutations: 55/264 (21%)

D. Eberhard et al, J Clin Oncol 2005;23:1-14

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BR.21: KRAS mutant patients show no benefit from erlotinib, but the number of patients is small

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Early stage NSCLC

Is there a role for molecular markers?

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**JBR.10 Trial**

Vinorelbine plus Capcitabine vs. Observation in Resected Non-Small-Cell Lung Cancer

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**HR: 0.63 (0.47-0.84), p=0.02**

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**HR: 0.61 (0.54-0.69), p=0.01**
JBR.10: Patients with ras mutation are not likely to benefit from adjuvant chemotherapy

**Ras Wild Type**

**Ras Mutant**

<table>
<thead>
<tr>
<th>Time (years)</th>
<th>Observation</th>
<th>Vin/Plat</th>
<th>Observation</th>
<th>Vin/Plat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>169</td>
<td>164</td>
<td>2</td>
<td>127</td>
</tr>
<tr>
<td>2</td>
<td>127</td>
<td>128</td>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>90</td>
<td>6</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>100</td>
<td>6</td>
<td>100</td>
</tr>
</tbody>
</table>

Hazard Ratio (95% C.I.): Vin/Plat/Observation: 0.69 (0.49, 0.97)
Medinan (95% C.I.): Observation: 6.2 (3.8, inf.), Vin/Plat: 6.2 (5.3, inf.)
Test for equality of groups: Log-Rank: p=0.0341

Summary Statistics:

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Observation</th>
<th>Vin/Plat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>20%</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>40%</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>60%</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>80%</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>100%</td>
<td>0</td>
<td>0</td>
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Interaction P value = 0.25

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**Cisplatin-DNA Adduct and Nucleotide Excision Repair (NER)**

Hypothesis: ERCC- patients are not able to repair damages from cisplatin

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**International Adjuvant Lung Trial (IALT)**

- 1867 patients with completely resected NSCLC I-II-IIIA
- HR= 0.86 [0.76-0.98], p<0.03
- Absolute benefit: 4.1% improvement of 5 year OS
- Tumor from 761 pts were available for marker analyses

Olaussen KA, et al. NEJM 2006;355:983-91
JBR.10: Patients with high class III $\beta$-tubulin expression have poorer survival (prognosis)

JBR.10: Survival benefit from adjuvant cis/vin appears greater in patients with high class III $\beta$-tubulin

**Question:** Are high tubulin expressing tumors addicted to their tubulin level?

### Biomarkers for adjuvant therapy in NSCLC patients

- **Poor prognostic markers:** to select patients with high risk for death from recurrence and possibly benefit from adjuvant therapy.
- **Predictive markers:** to select patients who are most likely to benefit from a specific adjuvant therapy

### What about microarrays?

- **Good evidence** that gene expression profiles by microarray can distinguish:
  - tumors of different histological types
  - patients with different prognosis
- **But signatures** for histological typing do not overlap with those for prognosis
- **Prognosis signatures** are more reflective of molecular pathways important for the biology of lung cancers
Metagenes classify patients for their risk of recurrence better than clinical model

Outstanding questions:
- Is metagene method sufficiently validated?
- Is microarray approach most cost-effective?
Fine mapping of chromosomal abnormalities by Array-CGH

- Chromosomes are cut into fragments that are ~100 kilobases long
- Fragments are arrayed on glass slide
- Differentially labeled DNA from tumor and normal are co-hybridized to the microarrays and signals for each type of sample are detected and compared

Chromosome 5p: telomerase (hTERT)

- Role of telomerase complex is to maintain the length of telomeres
- Progressive shortening with continuous replication can trigger apoptosis or growth arrest
- Activation of telomerase is an obligate step during carcinogenesis
CONCLUSIONS

- Currently there are several very promising predictive markers for selection of NSLC patients to receive targeted or adjuvant chemotherapy

- These markers still require additional validation before they can be implemented as routine clinical tests

- Best way to validate these markers are in patients involved in large phase 3 clinical trials

- The use of molecular markers for stratifying cancer patients to therapeutic options will likely come to reality during the next decade