Thoracic Surgeon as a Molecular Biologist in the Treatment of Lung Cancer: You Need More Than Steel to Heal!

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DISCLOSURES

• No disclosures, conflicts
• Not a consultant for any therapeutics discussed
• All therapy in compliance with the FDA
• No off-label recommendations
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Lung Cancer Staging

Objectives
• Review current staging
• Overview of molecular genomics
• Essentials of molecular biology for the radiologist
• Utilizing genomics in guiding lung cancer therapy

Background
• Lung Cancer is the leading cause of death due to cancer in the US
• There are two main types of lung cancer, based on microscopic appearance of the tissue
  • Small cell (20%)
  • Non-small cell (80%)

Lung Cancer Staging
• Typically involves obtaining detailed images, such as CT scans, and observations during surgery
• Determines type of treatment
• Determines probability of survival
Classic Lung Cancer Staging

- IA tumor less than 3 cm
- IB tumor more than 3 cm
- II tumor spread to lymph nodes
- III tumor beginning to affect vital structures of the chest, such as the airways in addition to lymph nodes
- IV distant spread of tumor

Stage I Non-Small Cell Lung Cancer

- Cancer is found only in the lung
- Surgical removal recommended
- Radiation therapy and/or chemotherapy may also be used
Stage II Non-Small Cell Lung Cancer

- The cancer has spread to lymph nodes in the lung
- Treatment is surgery to remove the tumor and nearby lymph nodes
- Chemotherapy recommended; radiation therapy sometimes given after chemotherapy

Stage III Non-Small Cell Lung Cancer

- The cancer has spread to the lymph nodes located in the center of the chest, outside the lung
- Stage IIIA cancer has spread to lymph nodes in the chest, on the same side where the cancer originated
- Stage IIIB cancer has spread to lymph nodes on the opposite side of the chest, under the clavicle, or the pleura
- Surgery or radiation therapy with chemotherapy recommended for stage IIIA
- Chemotherapy and sometimes radiation therapy recommended for stage IIIB

Stage IV Non-Small Cell Lung Cancer

- The cancer has spread to different lobes of the lung or to other organs, such as the brain, bones, and liver
- Stage IV non-small cell lung cancer is treated with chemotherapy, possible radiation
Problems with Cancer Staging

• 30-35% of patients with Stage IA cancer, who just get surgery will relapse at some point
• A subgroup of these patients may benefit from receiving chemotherapy in addition to surgery

However, existing clinical trials indicate no benefit in giving these patients chemotherapy!!

Problems with Cancer Staging

• All patients with stage IB, II, and III cancer currently receive chemotherapy
• A subgroup of these patients may be getting toxic chemotherapy that they do not need
The current system of classifying patients for staging appears to have some problems.

It would be useful to be able to identify subgroups of patients within the existing staging categories.

Gene expressions profiles may identify such patient subgroups.
Methods

- Patient division into subgroups is addressed as a classification problem
- Three cohorts of patients analyzed:
  - Training cohort of 89 patients enrolled through the Duke Lung Cancer Prognosis Laboratory
  - Two independent validation cohorts of 25 patients and 84 patients from separate data collections

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Duke Lung Cancer Prognosis Laboratory (N=89)</th>
<th>Tumor Cell Line (N=25)</th>
<th>CUP (N=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>67 (31)</td>
<td>74 (34)</td>
<td>68 (28)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male: 56 (63)</td>
<td>19 (76)</td>
<td>47 (56)</td>
</tr>
<tr>
<td>Race</td>
<td>White: 80 (89)</td>
<td>18 (72)</td>
<td>62 (74)</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>Never: 34 (38)</td>
<td>18 (72)</td>
<td>50 (59)</td>
</tr>
<tr>
<td>Tumor Grade</td>
<td>2 (23)</td>
<td>9 (36)</td>
<td>13 (15)</td>
</tr>
<tr>
<td>Tumor Size</td>
<td>8 (9)</td>
<td>7 (28)</td>
<td>18 (21)</td>
</tr>
<tr>
<td>Stage</td>
<td>IIA: 30 (44)</td>
<td>5 (20)</td>
<td>24 (28)</td>
</tr>
<tr>
<td></td>
<td>IIB: 9 (14)</td>
<td>5 (20)</td>
<td>7 (8)</td>
</tr>
<tr>
<td></td>
<td>IIIA: 4 (6)</td>
<td>2 (8)</td>
<td>7 (8)</td>
</tr>
<tr>
<td></td>
<td>IIIB: 10 (15)</td>
<td>5 (20)</td>
<td>8 (10)</td>
</tr>
<tr>
<td></td>
<td>IV: 6 (7)</td>
<td>—</td>
<td>9 (11)</td>
</tr>
<tr>
<td></td>
<td>Other: —</td>
<td>—</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Methods

- Material for gene expression experiments was obtained from tumor tissue
- A pathologist reviewed all microscopic samples to determine whether the patients had Non-small cell lung cancer
RNA obtained from tumor tissue for microarray analysis

Analysis of Gene Expression

- Genes were filtered to remove those with low levels of expression or variance:
  - Maximum expression did not exceed median expression
  - Genes did not vary more than twofold across samples

Additional filtering was performed (on remaining 20,700 genes) by computing the correlation of each gene’s expression level with the patient outcome (either recurrence of cancer or not)

The top 10% of correlated genes were selected for further analysis (2070 genes)

In the process of leave-one-out cross validation, the process of gene filtering was re-applied for each sample
Analysis of Gene Expression

- Among the remaining highly correlated genes, K-means clustering was used to create grouping of approximately 15-20 genes each.
- From each cluster, singular value decomposition was used to identify the dominant average expression pattern, called a “metagene.”

Creating a Metagene

Clusters

SVD

Metagene 1

Metagene 2

Metagene 3

Metagene 4
Analysis of Gene Expression

- The set of metagenes and clinical factors was then used in a binary tree classification analysis to recursively partition the samples into smaller subsets
- Within subgroups, predictions of recurrence were made in terms of the estimated relative probabilities

The end result was a computed probability of recurrence of cancer for each patient
- The subset of metagenes receiving the highest weight across all trees was identified as the genes that most heavily contribute to overall risk prediction

Methods

- As a comparison, a “traditional” predictive model using clinical factors was used to predict patient outcome:
  - Age
  - Gender
  - Tumor size
  - Tumor stage
  - Smoking history
  - Type of tumor
Methods

• This clinical factors were treated as principal components, or “metagenes” in a classification tree analysis to generate a clinical model of the probability of recurrence for each patient

• Probabilities of recurrence were computed using the metagenes alone, the clinical factors alone, and both the clinical factors and metagenes together

• Logistic regression for recurrence (with and without the metagene data) was also computed to assess the baseline predictive value of each clinical variable

Kaplan-Meier Survival Estimates For Training Cohort

• Accuracy of prediction including the metagenes was 93%
• Accuracy of prediction using clinical data alone was 64%
Lung Metagene Model

- Appears to be a better predictor of outcome than using clinical variables
- In addition, the metagenes appear to reflect the biology of the disease, because those metagenes with the most discriminatory power were genes previously implicated in the disease process (i.e. angiogenesis)

Validation of the Metagene Model

- Using the metagene model, the risk of recurrence was predicted for two independently collected, heterogeneous sample populations
Validation of Metagene Model

- The predictive accuracy of the model for ACOSOG samples was 72%
- The overall predictive accuracy of the model for CALGB samples was 79%

The metagene model provides an alternative way of understanding the disease process itself.
Using the metagene model may be a better way to stage patients, and determine a treatment plan, especially those with early disease.

Epidermal Growth Factor Receptor (EGFR)
Cellular Signalling Pathways

- Vital for cell cycle progression, growth, differentiation & death.
- Growth Factors – The key stone
- A delicate balance between activating and inhibitory signals needs to be maintained normally
- Alteration in this balance - Dysregulated cellular proliferation & survival of abnormal cells.

Growth Factors & Cell Cycle

Breast                               14 % - 91 %
Colon                                25 % - 77 %
Lung Cancer                     40 % - 80 %
(Non small cell)                   
Ovarian                              35 % - 70 %
Pancreatic                         30 % - 50 %
Head & Neck                      80 % - 95 %
Some Landmarks in EGFR Signalling
Stanley Cohen
EGF in mice (1960’s)
Human EGF (1970’s)
Isolation and cloning of EGFR (1980’s). Link between EGFR and malignant transformation of cells demonstrated
Mendelsohn et al.,
Blocking EGFR signalling to treat cancer
Murine monoclonal antibodies targeting EGFR-TK → Human:murine chimeric version
More than 20 anti-EGFR agents in development

Human Epidermal Growth Factor Receptor Family

EGF, TGFα, β Cellulin
Amphiregulin, HB-EGF

Heregulins

No specific ligands - often acts as dimer partner

nomedk

EGFR Structure

Extracellular Domain

Transmembrane Domain

Intracellular Domain
EGFR Homo Dimerisation

erbB1 HER1 EGFR
erbB2 HER2 neu
erbB3 HER3
erbB4 HER4

EGFR Stimulation & dimerisation

EGFR stimulation leads to dimerisation and activation of EGFR.

Hetero Dimerisation

Risk for cancer

EGFR Function in Normal Cell

Gene Transcription
Cell Cycle Progression
Antiapoptosis
Angiogenesis

Cell Proliferation
EGFR signal transduction in tumor cells

Other mechanisms of EGFR stimulation

How EGFR variant differs from the wild type

<table>
<thead>
<tr>
<th>EGFR - Variant III</th>
<th>EGFR – Wild Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>No extracellular domain</td>
<td>Present</td>
</tr>
<tr>
<td>Ligand cannot bind</td>
<td>Can bind</td>
</tr>
<tr>
<td>TK constitutively active</td>
<td>TK activated by ligand binding</td>
</tr>
<tr>
<td>Cannot dimerise</td>
<td>Can dimerise</td>
</tr>
<tr>
<td>Not found in normal cells</td>
<td>Found normally</td>
</tr>
<tr>
<td>More propensity for cancer</td>
<td>Up regulation leads to cancer</td>
</tr>
</tbody>
</table>
Gene transcription
Cell Cycle Progression
Cell Proliferation
Anti Apoptosis
Metastasis
Cancer

Consequence of proliferation of EGFR receptors

Normal Cell
Up Regulation
Cancerous Cell

EGFR – A good target for lung cancer (non small cell)

High level of receptor expression compared with healthy tissue.
EGFR - Key role in tumour cell growth & function.
EGFR inhibition can inhibit downstream activity.
EGFR inhibitors have no severe toxicity.
Strategies to inhibit EGFR signaling

EGFR tyrosine kinase inhibitors
Anti-EGFR mAbs
Anti-ligand mAbs
Bispecific Abs

Drugs Available

- Gefitinib: Highly selective, potent & reversible EGFR Tyrosine Kinase Inhibitor
- Erlotinib
- Cetuximab - Monoclonal Anti EGFR antibody
  - H 447
  - MDX 210 - Bispecific Anti EGFR antibody linked to Anti CD 64

Indications

- Gefitinib & Erlotinib:
  - Monotherapy in advanced stage of NSCLC
- Cetuximab
  - Metastatic colorectal cancer with/without Irinotecan
    - Dose:
      - Gefitinib 250 mg O.D. oral
      - Erlotinib 150 mg O.D. oral
      - Cetuximab 400 mg/m² i.v. → 200 mg/m² i.v. wkly
Advantages of EGFR Inhibitors

Orally effective
Better quality of life.
Can be used as monotherapy.
No need for premedication or dose monitoring.
No hematological toxicity.
Potential for long term treatment.
Reduced resistance to radiation or hormone therapy

Conclusion...
EGFR inhibitors- a definite role in treatment of cancer
Combination chemotherapy – Further studies needed
Improves QOL with minimal adverse effects
- Can be administered at optimal biological dose
- Potential for use in multiple tumors

Conclusion...
Role in early stage of cancer needs to be ascertained
Survival not significantly prolonged
Costly
Review Articles


Mini Review


Original Articles

THANK YOU