Molecular pathogenesis of lung cancer

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Lung epithelial cells

Carcinogen exposure
genetic predisposition
Auto- and paracrine effects
on cell proliferation

Preneoplastic stage

Somatic mutation
Ras / EGFR
3p, 9p
P53
p16, cyclins
telomerase
other

Somatic deregulation
Matrix proteases
Adhesion-Migration
Angiogenesis

Invasive and metastatic tumor

Somatic mutation
chromosome deletion
methylation
point mutation
Key pathways altered in lung cancer

- Tyrosine-kinase receptors pathway
  EGF-R / Raf / Ras / MAP-K...
  PI3K / AKT / PTEN

- P53 pathway: apoptosis/senescence
  P53 / Bcl2 / Bax
  P14ARF / MDM2 / P53

- Rb pathway: G1 arrest
  Rb / P16INK4 / CyclinD1,E

- G2 arrest pathway
  P14ARF / ATM – P / CHK2-P
Atypical alveolar hyperplasia

Bronchioloalveolar carcinoma

Adenocarcinoma mixed type

Adenocarcinoma acinar
Epidermal growth factor receptor signaling: multi altered pathway

Mendelsohn, J. et al. J Clin Oncol
Summary of mutations reported in the TK domain of EGFR in NSCLCs

EGF-R mutations in NSCLC patients

PAO et al PNAS 2004. MARCHETTI et al J. C. O. 2005

- 10 to 15% in adenocarcinoma
- Few EGF-R mutations in squamous cell and large cell carcinomas
- EGF-R mutation in
  - 26% in adenocarcinoma with "BAC" component
  - 20% in papillary adenocarcinoma
- Adenocarcinoma of smokers: 5%; non smokers: 50% (OR:3.6)
- More frequent in females than males (OR:2.8)
- More frequent in asian than caucasian patients

 ➔ EGF-R mutation is a better predictor of Gefitinib efficacy than clinicopathological variables
Disomy 7 / EGFR: 40%  
Trisomy: 40%

High polysomy: 13%  
Amplification EGFR: 9%
Epidermal growth factor receptor signaling:
multi altered pathway
Transduction of growth signal

tyrosine-kinase receptors

- Ras-GTP → Ras-GDP
- Gene activation
  * Ki-ras gene mutation (codon 12)
  * 15% in adenocarcinomas
  * 15% in atypical alveolar hyperplasia

➢ Early event in the sequence of AAH → adenocarcinoma
K-RAS 2

Exon 1

GGT  GGC  GCA  CCA

Codon 12  Glycin

Codon 13

Exon 2

GCA  CCA

Codon 59

Codon 61

Unique missense mutation
First or 2\textsuperscript{nd}, never the third

TGT  Cystin  CGT  Arginine
GTT  Valine  AGT  Serine
GAT  Aspartate  GCT  Alanine
Mutual exclusion between EGF-R, Her2 and K Ras mutation

- No patient with more than one of these mutations
- Her2 mutation in 2.8% of ADC
  
  H Shigematsu et al Cancer Res 2005

- Ras mutation early : AAH / BAC

  In normal lung around ADC with mutation
  Adenoc. Mixed with BAC: 40%
  Acinar / Papillary: 20%

Marchetti et al J. C. O. 2005
<table>
<thead>
<tr>
<th></th>
<th>AAH</th>
<th>BAC</th>
<th>ADC mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>KiRas Mut.</td>
<td>15%</td>
<td>15%</td>
<td>15% smokers</td>
</tr>
<tr>
<td>R-EGF Mut.</td>
<td>?</td>
<td>20%</td>
<td>40% no smokers</td>
</tr>
<tr>
<td>P53 Mut.</td>
<td>0</td>
<td>10%</td>
<td>30%</td>
</tr>
<tr>
<td>LOH &gt; 1</td>
<td>0</td>
<td>25%</td>
<td>46%</td>
</tr>
</tbody>
</table>

AAH → BAC → ADC mixed?
Unsupervised hierarchical clustering and analysis of three major genetic changes in 90 patients with adenocarcinoma
“Field cancerization”

- Preneoplastic and preinvasive lesions are multifocal in smokers
- They may randomly affect any anatomical location
- Their individual potential for progression depends on chronology and rate of accumulation of molecular and genetic abnormalities
DNA damage

Mdm2

P53

p14

Oncogenic stimuli
Myc, Ras, E2F1

waf1  p21

cdk  cyclD1

Rb  E2F

G1 arrest

G1 → S

p16

Bax  DR5  Fas

apoptosis  cell survival

p14

Rb - P

E2F

+  +  -

DNA damage

Mdm2

p14

Oncogenic stimuli
Myc, Ras, E2F1

waf1  p21

cdk  cyclD1

Rb  E2F

G1 arrest

G1 → S

p16

Bax  DR5  Fas

apoptosis  cell survival

p14

Rb - P

E2F
P53

Waf1 (p21)

mdm2

Cdk2 - cyclin E
Cdk4 - cyclin D1

G1 arrest

Rb
E2F

P16 (mts1)
P15 (mts2)

Rb

PP

S entry

E2F
Phenotypic changes associated with cell cycle deregulation (G1 arrest) via RB phosphorylation / inactivation

- RB: 10% loss
- P16 \(^{INK4}\): 50% loss > SCC
- Cyclin D1: 70% over expression > ADC
- Cyclin E: 40% over expression > SCC

E. Brambilla et al. J Pathol 1999
Pre- and neoplastic bronchial lesions

Hyperplasia
↓ ?

Metaplasia
↓ ?

Dysplasia
↓ ↓ ?

In situ carcinoma
↓ ↓ ↓ ?

Invasive carcinoma

Molecular Identity

- Cell cycle regulation
- Apoptosis / Senescence
- Angiogenesis / Migration
Cyclin D1 and E overexpression in preinvasive bronchial lesions (n=80)

Graph showing the percentage of Cyclin D1 and Cyclin E overexpression across different stages of bronchial lesions (Normal, Hyperplasia, Metaplasia, Mild dysplasia, Moderate dysplasia, Severe dysplasia, CIS). The graph indicates a significant difference with $P < 0.001$. The y-axis represents the percentage from 0% to 100%, and the x-axis represents the different stages of lesions.
P53 BCL2 : BAX > 1

Cyclin D1
Cyclin E

P = 0.02

Tumor suppressor genes inactivated by methylation

LOH ✓
✓ P16<sup>INK4</sup>
✓ RAR<sub>β</sub>
✓ RASSF1

DAP-kinase Caspase 8

✓ FHIT

0<sup>6</sup>MGMT GST<sub>π1</sub>

✓ E Cadherin APC TIMP 3

CELL CYCLE REGULATION
APOPTOSIS
DNA REPAIR
MIGRATION
DNA damage

DNA damage

p14

p14

Mdm2

p14

Oncogenic stimuli
Myc, Ras, E2F1

P53

? +

waf 1  p21

+ -

p21

p14

bcl2

bcl2

Bax

DR5

Fas

apoptosis cell survival

p16

p16

cyclD1

cyclD1

cdk

cdk

Rb

Rb

Rb - P

Rb - P

E2F

E2F

E2F

E2F

G1 arrest

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S

G1 S
p53 stabilization

Transactivation
## P53 upstream pathway

<table>
<thead>
<tr>
<th>Gene</th>
<th>NSCLC</th>
<th>SCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53: mutation</td>
<td>50%</td>
<td>70%</td>
</tr>
<tr>
<td>Mdm2: overexpression</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>P14ARF: loss of expression</td>
<td>25%</td>
<td>40%</td>
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</tbody>
</table>

Eymin et al. Oncogene 2002

- No lung cancer have an intact P53 – Rb pathway
  - no prognostic value
Oncogenic stimuli
Myc, ras, E2F1

DNA damage
Telomere shortening

P53 dependant pathway

P14^ARF

H2AX → P-H2AX

Rb

CHK2

P-CHK2

G2 arrest
apoptosis
**P14\textsuperscript{ARF} tumor suppressor functions**

**response to DNA damage \rightarrow G2 arrest**

- \(p14\textsuperscript{ARF}\) induces G2 arrest and inhibits cell growth independently of p53
  
  Eymin et al, Oncogene, 2003

- Activation of checkpoint kinases CHK1/2 is involved in \(p14\textsuperscript{ARF}\) induced G\(_2\) arrest
  

- \(P14\textsuperscript{ARF}\) activates a TIP60 dependent ATM/CHK2 pathway to induce G\(_2\) arrest

- **Direct correlation between** \(P14\textsuperscript{ARF}\) loss and lack of CHK2 phosphorylation \(\ p: 0.0006\)
  
  Eymin et al Mol Cell Biol, 2006
Alkylants
Tobacco carcinogens

\[ \text{Small Cell Carcinoma, Adenocarcinoma} \]
Gazzeri et al, Cancer Research, 1998

\[ \text{p14ARF Tip60} \]

\[ \text{ATM'CHK2} \]
Response to DNA damage

Cell cycle arrest

DNA repair
Chemoresistance?

Apoptosis
Conclusion

- Lung tumorigenesis is dependent of more than one genetic alteration: **P53 pathway**
- Adenocarcinoma target preferentially the Tyrosine Kinase pathway
- Evasion from cell cycle checkpoints and DNA damage response are critical and early events
- **P14ARF** is a key player in control of oncogenic stimuli and regulation of response to DNA damage → dominant policing tumor suppressor
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