Finally some good news about lung squamous cell carcinoma molecular pathology

Alex Soltermann
Lung squamous cell carcinoma, SCC

Definition

- Malignant epithelial tumor showing **keratinization** and/or **intercellular bridges** (=desmosomes).
- **Extracellular** vs. **intracellular** keratin (pink cytoplasm, dyskeratotic cells)
- „**Squamoid features“** (e.g. of solid adenocarcinoma) do not count!

Risk factors

- > 90% in **cigarette** smokers
- HPV in 20% in Europe

Precursor

- High grade **dysplasia** in adjacent bronchial epithelium
Macroscopy

Localisation

• Majority centrally in mainstem, lobar or segmental bronchi

Spread

• Central: intraepithelial, endobronchial, submucosa following lung parenchyma, lymph nodes per continuitatem

• Periphery: Solid nodule or mass, rarely „alveolar space filling type (BAC)“

Biomarkers

• Bevacizumab: fatal hemorrhage in cavitating SCC. EGRF mut rare
Histotypes of NSCLC based on morphology

**Large Cell Ca:**
- Adeno Ca
- Squam. Cell Ca
- „True Large Cell“

**AB-PAS + 4-marker IHC panel:**
- Adeno Ca: TTF1 and CK7
- Squam. Cell Ca: CK5/6 and p63

**Adenocarcinoma**
Mucine, Glands

**Large Cell Ca**

**Squam. Cell Carcinoma**
Keratine, Desmosomes
Classification with immunohistochemistry

Second line markers: **Napsin A** for Adeno, **Desmocollin-3** for Squamous, but:

- Both TTF1- Napsin A+ Adeno and CK5/6- DSC3+ Squamous do not exist?
- What is the gold standard: TMA cores as „biopsy surrogate“ following OP specimen?
- Extensive algorithms like Pulmotype®?

*Chen, Oncology 2011; Ring, Mod Pathol 2009*
Molecular alterations in 2004 WHO classification

**Cytogenetics and CGH**
- Alterations of chromosome 3, involving PI3KCA (catalytic subunit)

**Molecular genetics**
- > 80% of SCC EGFR protein (HER1) positive, HER2 rare
- Activating mutations of KRAS and EGFR rare
- Disruption of RB pathway via upstream inactivation of p16Ink4
- Disruption of p53 gene function via mutation
EGFR = HER1 protein, Cetuximab

**H-Score, formerly IRS (immunoreactivity score)**

- **Intensity** 0, 1, 2 or 3 multiplied by **frequency** of stained cells (0, 10 for 1-10%, 50 for 11-50% and 100 for 51-100% positive cells (from ERCC1))

- Internal control: Basal bronchial epithelium

- Immunoreactivity in hypoxic areas increased but towards keratinization decreased
Flex Study: AdenoCa vs. Squam Cell Ca

Neck dissection: AdenoCa

FLEX survival: high EGFR expression – histology subtypes

<table>
<thead>
<tr>
<th></th>
<th>Adenocarcinoma</th>
<th>Squamous cell carcinoma</th>
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<tbody>
<tr>
<td>CT + cetuximab</td>
<td>20.2 mo, 65%</td>
<td>11.2 mo, 44%</td>
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<tr>
<td>CT</td>
<td>13.6 mo, 52%</td>
<td>8.9 mo, 25%</td>
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Merck-Serono NSCLC Advisory Board 21.11.2011:

Benefit for adenocarcinoma or rather squamous cell carcinoma?
Fibroblast growth factor receptor 1

Clinical Lung Cancer Genome Project Cologne, CLCGP, Roman Thomas

- > 1500 frozen tumors, including carcinoids, SCLC, LC and LCNEC

- High resolution gene copy number analyses by Affymetrix 6.0 SNP/CNV

- Validation on Zurich NSCLC TMA: 22% of lung SCC are FGFR1 amplified

- Lung cancer cell line panel (n=83): FGFR inhibitor PD173074 inhibited growth in FGFR1_{amp} cell lines

- Cytotoxicity rescued by FGFR1^{V561M} resistance allele

Weiss, Sci Transl Med 2010
Fibroblast growth factor receptor 1

**FRS2** (fibroblast growth factor receptor substrate) attenuates FGFR signaling by Grb2-mediated recruitment of the ubiquitin ligase Cbl

*Hynes, Cancer Res 2010; Wong, PNAS 2002; Beenken, Nat Rev Drug Discov 2009*
Discoidin Domain Receptor 2 = DDR2

A

Primary screen

201 genes sequenced in 20 SCCs

25 total coding mutations
Recurrent mutations in TP53 (8)
Mutations in six tyrosine kinase genes including DDR2 (2)

Secondary screen

Six tyrosine kinase genes sequenced in 48 SCCs

4 DDR2 mutations

Validation screen

DDR2 sequenced in 222 SCCs

5 DDR2 mutations

Total of 11 DDR2 mutations in 290 samples
9 in 277 primary SCCs
2 in 13 cell lines (L239R in HCC-366 and I638F in NCI-H2286)

B

DDR2


0 100 200 300 400 500 600 700 800

- Discoidin
- Kinase

Missense mutation

Hammerman, Cancer Discovery 2011
Discoidin Domain Receptor 2

Receptortyrosin-Kinase, collagen receptor (CD167b)

- Communication with microenvironment
- Sanger sequencing of tyrosine kinome: Mutations in DDR2 kinase gene in 3.8% of lung SCCs and cell lines
- DDR2\textsuperscript{mut} cell lines killed by multitarge-ted kinase inhibitor \textit{dasatinib} (Sprycel®)
- Growth inhibition in mouse xenograft model
- Response to \textit{dasatinib plus erlotinib} in one patient with DDR2\textsuperscript{mut} lung SCC

- Dictyostelium discoideum: Soil-living amoeba = slime mold, transitions from unicellular into a multicellular slug and then into a fruiting body
Phosphatidyl-Inositol-3 Kinase (PI3K)

**Massion, Cancer Res 2002; Array CGH in NSCLC:**
- In SCC but not AC gain of chromosome 3q22-q26, loss of chromosome 3p
- Including PI3KCA, the alpha catalytic subunit of PI3K
- Activity of the downstream PI3K effector protein kinase B higher in SCC, correlated with PI3KCA copy number increase (FISH)

**Sos, PNAS 2009; Single and combined PI3K- and MAPK-pathway inhibition**
- Cell lines with RTK lesions depend on PI3K, with RAS mutations on MAPK

**Genentech, San Francisco**
- PI3KCA amplification in 30% of lung SCC, rare in adeno. Reduced PTEN expression in both SCC and AC.
- Study plan: PI3KCA FISH and PTEN IHC
Predictive molecular biomarkers 2011/12

Adenocarcinoma

- KRAS
- EGFR
- PI3K
- FGFR4
- MEK
- BRAF
- EML4-ALK
- HER2

Squamous Cell Ca

- FGFR1
- DDR2
- PI3KCA

FGFR1: Fibroblast growth factor receptor 1
DDR2: Discoidin domain receptor 2
PI3KCA: Phosphatidyl-Inositol-3 Kinase, alpha catalytic subunit

M. Kris, ASCO 2011
**Metastasis of Head Neck SCC: HPV, p16?**

Disruption of RB via methylation of p16, not p16 induction by HPV E6/7

**HPV prevalence in lung cancer**
- 4/218 (Coissard, Mod Pathol 2005)
- 17% in Europe, up to 80% in Japan (Kobt, Pathol Res Pract 2011)

„Comparative HPV PCR“
- 14% identical, 43% discordant HPV genotype lung + head neck SCC (Weichert, Am J Surg Pathol 2009)

Dysplasia in adjacent bronchus

Gene expression profiles, LOH (Geurts, Clin Cancer Res 2005)
Tumor microenvironment

Hofmeister, Cancer Immunol Immunother 2008

Anoikis
Programmed cell death induced by anchorage-dependent cells detaching from the surrounding extracellular matrix (ECM)

CAF Cancer-associated fibroblast
TEC Tumor endothelial cell
TAM Tumor-associated macrophage
ECM Extracellular matrix with soluble factors
Targeting oncofetal isoforms of stroma proteins

- Darleukin (L19-IL2) + dacarbazine in metastatic melanoma (CR)
- Teleukin (F16-IL2) + doxorubicin/paclitaxel in solid tumors
- Tenarad (F16-I^{131}) in refractory Hodgkin’s Disease
- Radretumab (L19-I^{131}) + irradiation in solid tumor brain metastases

Rybak and Neri, ChemMedChem 2007

Philogen
innovating targeting

• L19 sc-Fv: extracellular domain B of fibronectin
• F16 sc-Fv: extra-domain A1 of tenascin C

Palumbo, Br J Cancer 2011

Balb-c nude mice + F9 Terato-Ca
L19 small immune protein (SIP) coupled to photosensitizer porphyrin + laser

Philogen
innovating targeting
N-glycoproteins in pleural effusions of lung adenocarcinoma

N-glycoprotein capturing following mass spectrometry:

- 170 N-glycoproteins identified (5 malignant, 5 benign effusions), mostly belonging to serum factors and extracellular matrix

- **Periostin**: Secreted matricellular protein of the ECM, identified with probability 0.99 in the malignant. Epithelial-mesenchymal transition marker.

Validation on NSCLC TMA 1993-2002 (n=532):

- High stromal periostin correlated with squamous cell histology, larger size, higher pT, higher stage, pM1, vascular invasion, tumor relapse and decreased survival

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Tumor micro-architecture on whole sections

**Fibrous stroma (bad):** Stroma larger as some cancer nests, plump fibroblasts and visible collagen fibers

**Thin stroma (good):** Good Narrow stroma, thin collagen fibers

**Stage 1 lung SCC (n=220):** Fibrous stroma correlated with vascular invasion and decreased survival, low E-cad but high laminin, fibronectin, MMP7 and C-met

*Takahashi, J Thorac Oncol 2011*
Take home message

- Histo-diagnostic SCC biomarkers: p63 and CK5/6

- Predictive biomarkers: EGFR, FGFR1, DDR2, PI3KCA

- Differential lung vs. head neck SCC: Comparative HPV PCR

- Tumor microenvironment: Anti-stroma sc-Fv antibodies
Thanks

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