New targets in squamous cell carcinoma of the head and neck

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Presentation outline

• EGFR dependent disease
• Genetic alterations
• Targeting selected pathways
• Targeting the microenvironment
• Clinical considerations and conclusions
Significantly selected mutated genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>44-72%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>9-22%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>6-22%</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>9-19%</td>
</tr>
<tr>
<td>FAT1</td>
<td>23%</td>
</tr>
<tr>
<td>CASP8</td>
<td>8-10%</td>
</tr>
<tr>
<td>FBXW7</td>
<td>5%</td>
</tr>
<tr>
<td>MLL2</td>
<td>7-18%</td>
</tr>
<tr>
<td>HLA-A</td>
<td>3%</td>
</tr>
<tr>
<td>TGFBFR2</td>
<td>4%</td>
</tr>
<tr>
<td>HRAS</td>
<td>4-8%</td>
</tr>
<tr>
<td>EPHA2</td>
<td>4%</td>
</tr>
<tr>
<td>NFE2L2</td>
<td></td>
</tr>
</tbody>
</table>

Stransky et al. Science 2011
Agrawal Science 2011
Hayes et al. ASCO 2013
Pickering et al. Cancer Discov 2013
Candidate Therapeutic Targets

Analysis – Tanguy Seiwert, Niki Schultz

<table>
<thead>
<tr>
<th>Receptor Tyrosine Kinases</th>
<th>HPV(-) N=244</th>
<th>HPV(+) N=35</th>
</tr>
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<tbody>
<tr>
<td>EGFR</td>
<td>16%</td>
<td>6%</td>
</tr>
<tr>
<td>FGFR1</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>MET</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>ERBB2</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>IGF1R</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>EPHA2</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>DDR2</td>
<td>3%</td>
<td>6%</td>
</tr>
<tr>
<td>FGFR2</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>FGFR3</td>
<td>2%</td>
<td>6%</td>
</tr>
<tr>
<td>CCND1</td>
<td>30%</td>
<td>3%</td>
</tr>
<tr>
<td>MYC</td>
<td>14%</td>
<td>3%</td>
</tr>
<tr>
<td>HRAS</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>18%</td>
<td>37%</td>
</tr>
<tr>
<td>PTEN</td>
<td>31%</td>
<td>20%</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>NF1</td>
<td>14%</td>
<td>9%</td>
</tr>
<tr>
<td>TP53</td>
<td>82%</td>
<td>3%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>89%</td>
<td>9%</td>
</tr>
</tbody>
</table>

Amplification  Homozygous Deletion  Heterozygous Deletion  mRNA Downregulation  Mutation  RPPA Downregulation  RPPA Upregulation

Courtesy of Dr. Hayes et al. ASCO 2013
HPV positive


26-30 September 2014, Madrid, Spain
Presentation outline

• EGFR dependent disease
• Genetic alterations
• Targeting selected pathways:
  - Cell cycle
  - MAPK, JAK/STAT, and PIK3/mTOR pathways
  - The differentiation pathways
  - Others
Presentation outline

- EGFR dependent disease
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  - Others
HPV positive

Leemans R et al, Nature Reviews 2011
HPV negative

DNA damage
- Mutated in 70%

Cyclin A CDK1

Cyclin B CDK1

Senescence and differentiation
- Inactivated in 90%

Mitogens
- Amplified in 30%

Leemans R et al, Nature Reviews 2011
HPV negative

Mutated in 70%

Inactivated in 90%

Amplified in 30%

P53 gene therapy: Advexin ONYX-015

CDK inhibitors: Palbociclib LEE011, LY2835219

Leemans R et al, Nature Reviews 2011
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HPV positive versus HPV negative

- More frequent
- Canonical mutations (TCGA)

Hayes et al. aSCC 2013
PI3K/AKT/mTOR

- PIK3CA amplification: 18-22%
- PTEN gene copy loss: 8%
- PTEN mutation: 5%
PI3K inhibitors

- Selection of patients?
  - PIK3CA mutated seem to respond better (*H1047R*)
  - BUT some patients with no mutations respond also

- Which inhibitors?
  - PI3K/mTOR: BEZ or GDC-0980
  - Pan-Class I PI3K inhibitor: BKM120, GDC-0941, PX-866
  - alpha-specific (p110alpha-specific) inhibitors: BYL719

Janku F et al. Cancer Res 2013,
PI3K/mTOR inhibitors

• May have a role to overcome cetuximab resistance (AACR 2014)

• mTOR inhibitor: Temsirolimus gave PFS rate at 12 weeks of 40% (CI95% 25-55%) in progressive patients after platinum and cetuximab (n=42)

AACR 2014
Grunwald et al et al. ESMO 2012
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  - Others
NOTCH pathway

- In mammalian cells, Notch activation maintains stem cell potential and inhibit differentiation, promoting carcinogenesis
- Activating NOTCH mutation is found in 50% of T-ALL
- In keratinocytes, increased Notch activity causes exit from the cell cycle and induces differentiation
- In head and neck cancer, NOTCH could be a tumor suppressor gene
- Down-modulation or loss function of NOTCH by mutations promote carcinogenesis
NOTCH pathway is defective in 66%

Pickering et al. Cancer Discovery 2013
NOTCH pathway not so simple

Aster J C, and Blacklow S C JCO 2012;30:2418-2420

26-30 September 2014, Madrid, Spain

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Differentiation pathways

- FAT1 role in differentiation, ↓ tumor growth, and ↓ invasion
- FAT1 inactivated in 20-30%
- FAT1 could also stimulate the Wnt pathway.
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  - The differentiation pathways
  - Others
Others

- MLL2 (11-17%), MLL3 (7.3%), NSD1 (10%), and EZH2 (0.35%) (methylation and histone modification) (Inhibitors of DNA methyl-transferases: azacitidine, decitabine or histone deacetylases: vorinostat, romidepsin).
- DDR2 (2-5%), EPHA2 kinases (2-5%) (STAT and Src activation)
- CASP8 (8-10%)
- CUL3, NFE2L2, KEAP1 (oxidative stress)
- DDX3X, CYLD (HPV+)

Stransky et al Science 2011
Agrawal Science 2011
Hayes et al. ASCO 2013
Pickering et al Cancer Discov 2013
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Stromal fibroblasts, T cells, macrophages, and other cell types develop abnormal phenotypes in a disorganized response to the cancer.

These non-cancerous cells provide many of the paracrine signals necessary to promote cancer growth.
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Markers</th>
<th>Secreted Factors</th>
<th>Metabolism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous Cell Carcinoma</td>
<td>E-cadherin, cytokeratins, PD-L1, FasL</td>
<td>MMP 2, MMP 9, MMP 13, ROS, VEGF, CXCL1, CXCL8, PDGF, IL-8, FGF-2, TGF-β, TNF-α, IL-1, GMCSF</td>
<td>Glycolytic: (MCT4⁺, MCT1⁻, TOMM20⁻, COX⁻) OXPHOS: (MCT1⁺, MCT4⁻, TOMM20⁺, COX⁺)</td>
<td>Tan et al, Koontongkaew S, Zhang Z et al, Smith A et al, Curry J et al, Feron O.</td>
</tr>
<tr>
<td>Central tumor compartment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leading edge/invasive front, perivascular niche (proliferative cancer cells: high Ki-67)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer stem cells</td>
<td>CD33, CD144, ALDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial to mesenchymal transition</td>
<td>N-cadherin, vimentin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cancer-Associated Fibroblast</td>
<td>α-SMA, integrin α6</td>
<td>HGF, CXCL12, TGF-β, MMP2, MMP9, PMF, PDGF, Type IV collagen, Coll5-binding integrins, PGE2</td>
<td>Glycolytic: (MCT4⁺, MCT1-LDH-B⁺)</td>
<td>Leef G, Curry J et al, Wheeler SE et al, Marsh D et al</td>
</tr>
<tr>
<td>Tumor-Infiltrating Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Regulatory T cells</td>
<td>CD4⁺CD25⁺FoxP3⁺</td>
<td>IL-10, IL 12, TGF-β</td>
<td></td>
<td>Young MR, Ferris RL et al, Whiteside TL</td>
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<tr>
<td>Cytotoxic T cells</td>
<td>CD8⁺, TCR, Fas, PD-1</td>
<td>Perforin, granzymes, granulysin</td>
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<td></td>
</tr>
<tr>
<td>Th2 suppressor cells</td>
<td>CD4⁺</td>
<td>IL-4, IL-6, IL-10</td>
<td></td>
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</tr>
<tr>
<td>Myeloid progenitor cells</td>
<td>CD34⁺</td>
<td>TGF-β</td>
<td></td>
<td></td>
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<tr>
<td>Tumor-Associated Macrophages (M2)</td>
<td>IL-10, TGF-β, MIF, EGF, CSF-1, MMP9, CXCL2, CXCL8, VEGF, ROS, RNS, PGEs</td>
<td></td>
<td></td>
<td>Lago Costa N et al, Dumitru C et al, Galdiero MR et al, Galdiero MR et al, Dumitru C et al, Neiva KG et al</td>
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<tr>
<td>Tumor-Associated Neutrophils</td>
<td>IL-10, TGF-β, MIF, EGF, CSF-1, MMP9, CXCL2, CXCL8, VEGF, ROS, RNS, PGEs</td>
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</tr>
<tr>
<td>Endothelial Cells</td>
<td>E-cadherin, vimentin</td>
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</tr>
</tbody>
</table>

**Abbreviations:** Rb, retinoblastoma gene; EGFR, epidermal growth factor receptor; CDKN2a, cyclin-dependent kinase inhibitor 2a; STAT3, signal transducer and activator of transcription 3; PD-L1, programmed death ligand-1; FasL, Fas ligand; MMP, matrix metalloprotein; ALDH, aldehyde dehydrogenase; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; IL, interleukin; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; PDGF, platelet-derived growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; EGF, epidermal growth factor; CSF-1, colony-stimulating factor-1; TCR, T-cell receptor; FoxP3, forked/winghead transcription factor; RNS, reactive nitrogen species; MCT, monocarboxylate transporter; PGE, prostaglandin; PD-1, programmed death-1; TOMM20, translocase of outer mitochondrial membrane 20; COX, cytochrome C oxidase complex; LDH-B, lactate dehydrogenase B.
These factors have the potential to promote:

- Angiogenesis
- Tumor growth
- Immune suppression
- Epithelial-mesenchymal transition
- Tumor invasion and metastasis

....
Preoperative window opportunity study with cetuximab

Day

-15
-8
-1

Surgery

Therapy

Cetuximab loading dose 400mg/m²

18 FDG Pet-CT scan, Tumor biopsy, blood and plasma samples

Cetuximab 250mg/m²

*18 FDG Pet-CT scan and plasma samples

Tumor biopsy and plasma samples

Schmitz et al, Annals of Oncology 2013
Tumour after Cetuximab infusion

Treatment naive tumour

26-30 September 2014, Madrid, Spain
**Microarray data**: individual analysis

Selection criteria: | Fold Change | > 1.75 (=0.8 in log2 scale) and (FDR B&H) corrected p-value <= 0.05

<table>
<thead>
<tr>
<th>ECM</th>
<th>T growth &amp; signaling</th>
<th>Vascularization</th>
<th>EMT</th>
<th>CAF</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCN</td>
<td>DCN (-)</td>
<td>RGS5 (+) (abnormal vasc)</td>
<td>OLFM1 (+)</td>
<td>ASPN (+)</td>
<td>ADAMDEC1</td>
</tr>
<tr>
<td>Selp</td>
<td>LPAR3 (+)</td>
<td>MGP (-)</td>
<td>OLFML3 (+)</td>
<td>OLFML3 (+)</td>
<td>APOC1</td>
</tr>
<tr>
<td>Sparcl1</td>
<td>SCARA5 (-)</td>
<td></td>
<td>CXCL12 (+)</td>
<td>CXCL12 (+)</td>
<td>C3</td>
</tr>
<tr>
<td>Aspn</td>
<td>TGFA (+)</td>
<td></td>
<td></td>
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<td>CD163</td>
</tr>
<tr>
<td>Clec3b</td>
<td>TXNIP (-)</td>
<td></td>
<td></td>
<td></td>
<td>DARC</td>
</tr>
<tr>
<td>COL3A1</td>
<td>ZNF521 (+)</td>
<td></td>
<td></td>
<td></td>
<td>VSIG4</td>
</tr>
</tbody>
</table>

**Growth inhibition**

* Affymetrix HG U133 Plus 2.0; criteria: quantity: ≥ 50ng/ul, RIN≥6; baseline: 15 samples, post-C: 19 samples
What did we learn?

- Intertumor heterogeneity
- Altered genes are tumor suppressor genes
- HPV + and HPV – are different diseases
- Subset with targetable genes (HPV+ and PIK3CA, ....)
Limitations

- Feedback loop and cross-talk between pathways
- Tumor heterogeneity
- Bioinformatic
- Signification of some mutations?