Biologic Subtypes of SCLC and their Clinical Significance

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Disclosure: Dr. Gazdar receives licensing fees for the cell lines he has established
Acknowledgments

Gazdar Lab
• Wei Zhang
• Mahboubeh Papari-Zareii
• Victor Stastny
• Yu-An Zhan

Department of Clinical Sciences
• Gaoxiang (Douglas) Jia
• Guanghua (Andy) Xiao
• Tao Wang

Minna Lab
• John Minna
• Luc Girard
• Michael Peyton
• Buddy Huffman
• Brenda Timmons

MD Anderson
• Ignacio Wistuba

Other UT Southwestern
• Jane Johnson (Neuroscience)
• Melanie Cobb (Pharmacology)
• Karine Pozo (Neuroscience)

Other
• Hans Ghayee (U Florida)
• Karel Pacak (NIH)
• Trudy Oliver (U. Utah)
Mutated genes in SCLC tumors (n = 81)


Key Points

- TP53 and RB1 universally inactivated
- Histone acetyltransferase genes EP300 & CREBBP
- Notch genes
- FMN2 and COL22A1 involve in collagen and actin binding and cytoskeleton
- KIAA1211 - not much known
Is SCLC a Homogenous Cancer?

• Only one morphologic type recognized by the WHO Classification 2015, although it may be combined with non-SCLC elements
• Clinically it is treated uniformly, with adjustments for tumor extent/staging
• No approved targeted therapies

SCLC

Combined SCLC (>10% NSCLC)
**“Classic” and “Variant” forms of SCLC**

<table>
<thead>
<tr>
<th>Classic SCLC</th>
<th>Variant SCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H128 (NE=0.89)</td>
<td>NCI-H82 (NE=0.21)</td>
</tr>
</tbody>
</table>

### Properties of variant SCLC
- Morphology
- Substrate attachment
- Increased growth & cloning
- MYC overexpression
- Radioresistance
- Decreased NE properties

Gazdar et al, Cancer Res 1985
Radiation survival curves for classic and variant SCLC

Classic SCLC

Surviving fraction at 200 rads 0.12

Variant SCLC
Myc family amplified

Surviving fraction at 200 rads 0.58

Surviving fraction at 200 rads 0.56

Carney, DN et al, Cancer Res, 1983
SCLC Cell Lines and Tumors Segregate in Low and High NE Subgroups

**k-Means Clustering (k = 2)**

For SCLC Cell Lines (n = 70):
- **Low NE Scores (11%)**
- **High NE Scores (89%)**

For George Tumors (n = 81):
- **Low NE Scores (20%)**
- **High NE Scores (80%)**
**Genes of interest and their relationship to NE differentiation in SCLC tumors (n = 81)**

<table>
<thead>
<tr>
<th>Correlation with NE score</th>
<th>Gene</th>
<th>r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Correlation</td>
<td>ASCL1</td>
<td>r = 0.68</td>
<td>1.6E-6</td>
</tr>
<tr>
<td></td>
<td>NEUROD1</td>
<td>r = 0.31</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>DLL3</td>
<td>r = 0.64</td>
<td>7.4E-08</td>
</tr>
<tr>
<td></td>
<td>SOX2</td>
<td>r = 0.31</td>
<td>8.0E-03</td>
</tr>
<tr>
<td>Negative Correlation</td>
<td>REST</td>
<td>r = -0.85</td>
<td>2.8E-09</td>
</tr>
<tr>
<td></td>
<td>Hippo pathway (Ajuba)</td>
<td>r = -0.81</td>
<td>4.5E-05</td>
</tr>
<tr>
<td></td>
<td>NOTCH Pathway (NOTCH1)</td>
<td>r = -0.46</td>
<td>9.4E-07</td>
</tr>
<tr>
<td></td>
<td>MYC</td>
<td>r = -0.66</td>
<td>1.8E-06</td>
</tr>
<tr>
<td></td>
<td>TGF Pathway (TGFB1)</td>
<td>r = -0.47</td>
<td>9.9E-04</td>
</tr>
<tr>
<td></td>
<td>Stem cells (ALDH1A3)</td>
<td>r = 0.72</td>
<td>9.9E-06</td>
</tr>
<tr>
<td></td>
<td>FGFR1</td>
<td>r = -0.52</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>JAK/STAT Signaling (JAK2)</td>
<td>r = -0.44</td>
<td>8.5E-07</td>
</tr>
<tr>
<td>No Correlation</td>
<td>SLFN11, NFIB, MYCL, MYCN, EZH2, DDR1, ATR, CDK7, KDM1A (LSD1A)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Molecular Changes Associated with Loss of NE Differentiation in SCLC

Zhang et al, Transl. Lung Cancer Res, On line 2018
Heterogeneity and Plasticity in SCLC Cell Line NCI-H69

Early passage

NE score 0.88

Spheroid Subline
NE score 0.87

• Morphology Classic
• ASCL1 High
• DLL3 High
• REST Absent
• MYC Low

Attached Subline
NE score -0.2

• Morphology Different
• ASCL1 Low
• DLL3 Low
• REST High
• MYC High
**MYC family and FGFR1 amplification in SCLC**

**Frequency of MYC amplification**

<table>
<thead>
<tr>
<th></th>
<th>Pretherapy</th>
<th>Post therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7/52 (11%)</td>
<td>16/44 (36%)</td>
</tr>
</tbody>
</table>

**Johnson, Gazdar, Minna et al, J Cell Biochem 1996**

**Myc Family Overexpression & Amplification**

### Gene Correlation with NE Score

<table>
<thead>
<tr>
<th>Gene</th>
<th>Correlation</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC</td>
<td>$r = -0.66$, $p = 7.0E-05$</td>
<td></td>
</tr>
<tr>
<td>MYCL</td>
<td>$r = 0.12$, $p = 0.5$</td>
<td></td>
</tr>
<tr>
<td>MYCN</td>
<td>$r = -0.21$, $p &gt; 0.5$</td>
<td></td>
</tr>
</tbody>
</table>
**Genes whose expression is positively correlated with NE differentiation**

**SCLC Tumors (n = 81)**

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>DLL3</th>
<th>CHGA</th>
<th>NKX2-1</th>
</tr>
</thead>
</table>

- **DLL3**  \( r = 0.64, \ p = 5.9E-06 
- **CHGA**  \( r = -0.77, \ p = 6.9E-07 
- **NKX2-1**  \( r = 0.64, \ p = 1.5E-04 

**Figure:**

- Tumors are ranked based on NE Score:
  - NE Score High
  - NE Score Low

- Genes shown include:
  - DLL3
  - CHGA
  - NKX2-1

- The correlation coefficients and p-values indicate the strength and significance of the correlations with NE differentiation.
<table>
<thead>
<tr>
<th>First author (Year)</th>
<th>Specimens studied</th>
<th>Nomenclature for NE High</th>
<th>Nomenclature for NE low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang (2018)</td>
<td>Tumors/Cell lines/GEMM</td>
<td>NE High</td>
<td>NE Low</td>
</tr>
<tr>
<td>George (2015)</td>
<td>Tumors/GEMM</td>
<td>NE High</td>
<td>NE Low</td>
</tr>
<tr>
<td>Cardnell (2017)</td>
<td>Tumors/Cell lines</td>
<td>High TTF1/Low cMYC</td>
<td>High cMYC/Low TTF1</td>
</tr>
<tr>
<td>Udyavar (2017)</td>
<td>Tumors/Cell lines</td>
<td>NE/epithelial</td>
<td>Non NE/mesenchymal</td>
</tr>
<tr>
<td>Ito (2016)</td>
<td>Cell lines</td>
<td>NE positive/YAP1 negative</td>
<td>NE negative/YAP1 positive</td>
</tr>
<tr>
<td>Mollaoglu (2017)</td>
<td>GEMM/Tumors/Cell lines</td>
<td>NE</td>
<td>NE low</td>
</tr>
<tr>
<td>Lim (2017)</td>
<td>GEMM</td>
<td>NE</td>
<td>Notch active, non-NE</td>
</tr>
<tr>
<td>Calbo (2011)</td>
<td>GEMM</td>
<td>Neuroendocrine profile</td>
<td>Mesenchymal profile</td>
</tr>
<tr>
<td>Stewart (2017)</td>
<td>Cell lines, PDXs</td>
<td>(High E-cadherin)</td>
<td>(EMT)</td>
</tr>
</tbody>
</table>
Adaptive and Innate Immunity

• Adaptive immunity
  Antigen specific responses
  (3-5 days+)

• Innate immunity
  Non-specific defenses activated by the antigen
  (Within hours)
Components of Innate Immunity
(Tumor Microenvironment is Different in Low and High NE SCLCs)

Punch line

- All of these components of the Innate Immune system (22 cell types, >200 genes) are highly up regulated in the low NE subtype of SCLC (p values 5.8E-03 to 2.6E-11)
- Cytokines signal through JAK/STAT, which is highly up regulated in the low NE subgroup

• Toll-like Receptors
• Cytokines (4 major families)
  • Interferon family including IFNγ
  • Interleukin family
  • Chemokine family
  • Tumor Necrosis Family
• Complement System
• Major Histocompatibility Complex (HLA antigens)
• The Inflammasome
• Cellular responses – immune & Inflammatory cells
  • Neutrophils
  • Macrophages
  • NK cells
  • Dendritic cells
  • Eosinophils
Proposed SCLC Therapeutic Target: CD47 - The “Don’t Eat Me!” Antigen (Upregulated in Low-NE Subtype)

- CD47 is expressed at variable levels on the surface of most cells, including SCLC
- It binds to the SIRPα receptor on macrophages sending a “Don’t Eat Me!” signal
- Blocking this interaction results in greater cancer cell kill

\[ r = -0.54, p = 2.0 \times 10^{-5} \]
NE Differentiation and Checkpoint Inhibitor Immunotherapy

Higher in NE Low Subtype

<table>
<thead>
<tr>
<th>Gene</th>
<th>Symbol</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1</td>
<td>PDCD1</td>
<td>-0.4</td>
<td>6.0E-03</td>
</tr>
<tr>
<td>PD-L1</td>
<td>CD274</td>
<td>-0.30</td>
<td>0.09</td>
</tr>
<tr>
<td>PD-L2</td>
<td>PDCD1LG2</td>
<td>-0.51</td>
<td>0.9</td>
</tr>
<tr>
<td>Interferon γ (IFNγ)</td>
<td>IFNG</td>
<td>-0.26</td>
<td>6.0E-03</td>
</tr>
<tr>
<td>IFNγ Signature (18 gene)</td>
<td>NA</td>
<td>NA</td>
<td>4.9E-04</td>
</tr>
<tr>
<td>CD44</td>
<td>CD44</td>
<td>-0.52</td>
<td>7.5E-03</td>
</tr>
<tr>
<td>No correlation</td>
<td>CTLA4, CD133</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Meng et al, Cancer Treat Rep 2015
<table>
<thead>
<tr>
<th>Therapy</th>
<th>Predicted Response NE High</th>
<th>Predicted response NE Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>PARP Inhibitors</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>ROVA-T</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Topoisomerase inhibitors</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Polo-like kinase inhibitors</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>HIPPO pathway Inhibition</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>FGFR1 inhibitors</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>JAK-STAT Inhibitors</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Immunotherapy (PD-1 blockage)</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>CD47 Inhibitors</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>
Recently, many new molecular targets have been identified for SCLC and several are in clinical trial.

SCLC shows considerable inter-tumor heterogeneity, especially for expression of NE properties.

Inter-tumor heterogeneity extends to the infiltrating immune & inflammatory cells.

This heterogeneity must be considered for selection of subgroups more likely to respond to specific therapies.
What remains to be done?

• Heterogeneity observations must be extended to another large, fully characterized dataset
• Predictions for differential responses to targeted therapies need to be tested in prospective and retrospective trials
• Predictions for differential responses to PD-1 blockade must be confirmed in clinical trials