Advances in understanding the biology of lung cancer

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"We have reached a point where there is a confluence of technological advances and significant conceptual breakthroughs and clinical proof of concept, that if we organize ourselves in a comprehensive integrated way, we can significantly reduce mortality in this decade for certain cancers"

President Ron DePinho,
MD Anderson Cancer Center, Houston 2012

"I believe that this nation should commit itself to achieving the goal, before this decade is out, of landing a man on the moon and returning him safely to Earth."

President John Kennedy, Houston, 1962
Major recent advances in cancer

• Technological advances in “omics”:
  ▪ Genome sequencing
  ▪ RNA expression
  ▪ MicroRNA expression
  ▪ Copy number variation and SNPs
  ▪ MicroRNA expression
  ▪ Proteomics
  ▪ Etc

• Freely accessible large public data sets

• Systems biology – the ability to integrate and translate this enormous knowledge base
The Cancer Genome Atlas (TCGA)

- Major US Government initiative to fully characterize common and not so common tumors
- First stage (funded): 25 common types, 500 frozen specimens for each type: Lung squamous & adenoca included
- Second stage (unfunded): Less common tumors, smaller numbers, frozen biopsies and FFPE specimens acceptable: SCLC included
- Exacting standards for tissue & data acquisition, processing, storage, pathology review
- Fully characterized tumors using multiple platforms
- Integrate molecular and clinical data
- Make all data publically available ASAP
The Cancer Genome ATLAS (TCGA)
Molecular characterization of lung cancer

<table>
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<tr>
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<td>DNA sequencing</td>
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Lung cancer (goals)
- Squamous cell ~500
- Adenoca ~500
- SCLC ~50
The Encyclopedia of DNA Elements (ENCODE) Project
“Beyond the sequence”

Funded by National Human Genome Research Institute (NHGRI)
All data freely available
Entire genome covered
• Gene structure
• Transcription and its regulation
• Chromatin states
• Methylation
Clinically actionable targets for NSCLC

Then

Adenocarcinoma
- EGFR
- KRAS
- Unknown

Squamous cell carcinoma
- Terra Incognita

Now

Adenocarcinoma
- EGFR
- KRAS
- Unknown

Squamous cell carcinoma
- DDR2
- PIK3CA
- AKT
- PTEN
- NFE2L2
- SOX2
- FGFR1 Amplification
- Unknown

Note: “Unknown” frequencies assume that all mutations are mutually exclusionary, and the true figure may be higher.

Gazdar 2012 based on:
- Pao & Girard, 2011
- Drilon et al, 2012
Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network

Nature Sept 2012

(300+ authors)
Significantly mutated genes in squamous cell cancers

TCGA Sept 2012
Alterations in targetable oncogenic pathways in lung squamous cancers

TCGA Sept 2012
SCLC - the forgotten cancer
A small-cell lung cancer genome with complex signatures of tobacco exposure

Erin D. Pleasance1, Philip J. Stephens1, Sarah O’Meara1,2, David J. McBride1, Alison Meynert3, David Jones1, Meng-Lay Lin1, David Beare1, King Wai Lau1, Chris Greenman1, Ignacio Varela1, Serena Nik-Zainal1, Helen R. Davies1, Gonzalo R. Ordoñez1, Laura J. Mudie1, Calli Latimer1, Sarah Edkins1, Lucy Stebbings1, Lina Chen1, Mingming Jia1, Catherine Leroy1, John Marshall1, Andrew Menzies1, Adam Butler1, Jon W. Teague1, Jonathon Mangion2, Yongming A. Sun4, Stephen F. McLaughlin5, Heather E. Peckham5, Eric F. Tsung5, Gina L. Costa5, Clarence C. Lee5, John D. Minna6, Adi Gazdar6, Ewan Birney3, Michael D. Rhodes4, Kevin J. McKernan5, Michael R. Stratton1,7, P. Andrew Futreal1 & Peter J. Campbell1,8

One paired SCLC - lymphoblastoid cell line

Nature Jan 2010

Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer

Charles M Rudin1,8, Steffen Durinck2,3,8, Eric W Stawiski2,3,8, John T Poirier1,8, Zora Modrusan2,8, David S Shames4,8, Emily A Bergbower1, Yinghui Guan2, James Shin1, Joseph Guillory2, Celina Sanchez Rivers2, Catherine K Foo2, Deepali Bhatt2, Jeremy Stinson2, Florian Gnad3, Peter M Haverty3, Robert Gentleman3, Subhra Chaudhuri2, Vasantharajan Janakiraman2, Bijay S Jaiswal2, Chaitali Parikh2, Wenlin Yuan2, Zemin Zhang3, Hartmut Koeppen5, Thomas D Wu3, Howard M Stern5, Robert L Yauch4, Kenneth E Huffman6, Diego D Paskulin7, Peter B Illei1, Marileila Varella-Garcia7, Adi F Gazdar6, Frederic J de Sauvage2, Richard Bourgon3, John D Minna6, Malcolm V Brock1 & Somasekhar Seshagiri2

80 samples – tumors, xenografts, cell lines

Nature Genetics, Sept 2012
SCLC Lung Cancer:
Numerous molecular changes and predominance of G>T transversions

Rudin et al Nature Genetics 2012
Recurrent genomic changes in SCLC

*Rudin et al Nature Genetics 2012*

Paired samples: 42
Somatic mutations: 24406
Protein altering: 7977

Newly identified mutational hotspots

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<td>G protein receptors</td>
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SOX2 is amplified in SCLC & drives proliferation: A potential clinically actionable target

Immunostaining of SCLC tumors

Doxycycline inducible shRNA targeting of SOX2

Correlation of IHC and FISH scores

Rudin et al Nature Genetics 2012
SMARCA4 loss in lung cancer
A potentially important new clinically actionable target for lung cancer
Chromatin remodeling – Two major components

- Histone modification enzymes
- Chromatin remodeling complexes (remodelers)

The remodeling complex families

~90 genes

~20 genes
Each SWI/SNF complex must contain one of two essential ATPases

- SMARCA2 (gene) - BRM (protein)
- SMARCA4 (gene) – BRG1 (protein)
- Mutations of SMARCA4 found in many tumors
- Mutations frequent in lung cancer cell lines but technically difficult to demonstrate in tumors
SMARCA$ (BRG1) is a transcriptional coregulator

BRG1-interacting proteins

Trotter and Archer. Nuc Rec Sigl, 2008
NextGen sequencing of SMARCA4: An update

NSCLC tumors

• >10% frequency
• Adeno > Squamous
• Large deletions difficult to identify

Peter Hammerman & Matt Meyerson, personal communication

NSCLC cell lines

• ~45% frequency (true frequency ~35%)
• Large deletions frequently missed

Our data
### BRG1 (SMARCA4) abnormalities in NSCLC cell lines

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<th>Total (n=64)</th>
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<td>Wild type - protein present, no mutation detected</td>
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<td>Group 1 – protein absent</td>
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<td>Homozygous frame shift deletions</td>
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<td>Any abnormality</td>
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*SMARCA4 abnormality rate is artificially high because we included all lines known or suspected to have protein loss or mutation from preliminary data or from the literature. The unbiased rate is about 35%.

NB. Mutations distributed throughout the large gene BRG1 (SMARCA4) abnormalities in NSCLC cell lines (all cell line findings confirmed by at least 2 methods)
Detection of large Homozygous deletions by SNP analysis

Chromosome 19p13.2

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- **Normal diploid**
- **Amplification or gain**
- **Hemizygous loss**
- **Homozygous loss**

**NCI-H1355** (exon18-22 deletion)

**NCI-H1944** (exon33-37 deletion)
Volcano plots demonstrating that loss of BRG1 (SMARCA4) protein has profound effects on the transcriptome and methylome of NSCLC cell lines.

**BRG1 protein loss vs WT**

**BRM protein loss vs WT**

**KRAS mutant vs WT**

**X axes:** expression or methylation value difference between Protein loss (or HD) and WT

**P value (Log10)**
Chromosome 19: A tale of two tumor suppressor genes

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Concordance = 71.4%, p = 0.035
Downstream effects: SMARCA4 loss vs LKB1 (STK11) loss

Gene expression

- Loss of both SMARCA4 & LKB1
- Loss of SMARCA4 alone
- Loss of LKB1 alone

Methylation
Can NSCLC cells with SMARCA4/BRG1 abnormalities be selectively targeted?

1. Targeted therapies have been successfully applied to tumors having specific oncogene mutations
2. To date, these clinically actionable mutations have all been dominant oncogenes, and tumor suppressor genes have not been specifically targeted
3. The numerous downstream effects of SMARCA4/BRG1 inactivation offer multiple potential targets
4. Our UT Southwestern collaborators Michael Roth and Vural Tagal have identified a class of compounds currently in advanced clinical trials that inhibit BRG1 inactive cells
NSCLC cell lines with BRG1 abnormalities can be selectively targeted

NSCLC cell lines with BRG1 protein loss are 100 fold more sensitive to a drug in clinical trial

Tagal V and Roth M
Unpublished data
Successful application of targeted therapy for a clinically actionable mutation

- Identify a clinically actionable target - SMARCA4/BRG1
- Identify a selective agent against the target – done
- Identify tumors that carry the target - In progress
Are SMARCA4 (BRG1) abnormalities present in lung and other tumors?

Immunostaining: 30% loss in NSCLC  
Reisman DN. et al. Cancer Res 2003

SMARCA4 mutations present in NSCLC by massive parallel DNA pyrosequencing analysis, but true frequency difficult to determine.  
Nieto SR. Human mutation, 2010

Loss of expression of SMARCA4/BRG1 is frequently observed in intraductal papillary mucinous neoplasms of the pancreas  
Marco DM. et al. Human Pathology, 2012

Biallelic inactivating mutations of SMARCA4 has been reported in about 2% of pancreatic cancer.  
Jones, S. et al. Science 2008

SMARCA4/BRG1 mutations found in lung, ovary, liver and brain tumors.  
Sanger Institution
BRG1 Immunostaining in NSCLC tumors (n = 512)  
Tissue microarray, MD Anderson cases

Positive (~90%)

Weak or Negative (~10%)

Adenocarcinoma  
Squamous cell carcinoma

BRG1 low expression: adenoca > squamous cell ca

Solis L. and Wistuba I.
SMARCA4 expression in lung cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>SMARCA4 low exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>29%</td>
</tr>
<tr>
<td>SCLC</td>
<td>3%</td>
</tr>
<tr>
<td>HBEC/HSAEC</td>
<td>0%</td>
</tr>
</tbody>
</table>

[Graphs showing density distributions for NSCLC, SCLC, and HBEC/HSAEC cell lines]
SMARCA4 expression in TCGA tumors (n=352)

Resected Tumors

Squamous cell ca
(n = 224)

Adenocarcinoa
(n = 128)

Low expression
(7%)

Low expression
(13%)

Corresponding non-malignant lung

LUSC; RNASeqV1; Normal

LUAD; RNASeqV1; Normal

Cases of low expression: adenoca > squamous cell ca
Lung adenocarcinomas with low SMARCA4 expression have worse prognosis (n = 440)

Publicly available data set of 440 adenocarcinoma (Director’s challenge data)
Challenges

• Despite of frequent low or absent BRG1 expression in NSCLC tumors, and high mutation rates of SMARCA4 in NSCLC cell lines, mutations (esp. deletions) are difficult to demonstrate in tumors by sequencing or SNP analysis.

• With the identification of a potentially therapeutic class of agents, there is a pressing need to identify BRG1 protein loss/mutations in NSCLC tumors.

• Our approach
  • Establish a combined expression/methylation signature from cell lines and apply to tumors
  • Validate signature with SMARCA4 gene expression and IHC
BRG1 gene expression signature

BRG1 protein loss  WT BRG1
Prediction of BRG1 status in NSCLC cell lines using a combined BRG1 expression and methylation signature

WT, BRG1 protein present, no BRG1 mutations founded
Group 1 abnormality, BRG1 protein absent
Group 2 abnormality, BRG1 protein present, mutation founded
BRG1 status unknown
Summary of findings

• Technological advances have resulted in the complete genomic characterization of many tumors

• Integration of these large data bases combined with computational biology have permitted us to explore the entire genomic landscape of cancers

• As a result of this knowledge of two “black boxes” (squamous cell and small cell carcinomas) has identified several clinically actionable targets

• Other advances include identification of SMARCA4 as a frequently mutated gene in NSCLC and all lung cancers respectively

• However our data mining is at its infancy, with many more potential clinically relevant discoveries to follow
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Support

SPORE in Lung Cancer
Early Detection Research Network
Canary Foundation
CSMD3 gene mutations in lung cancer
# CSMD3 mutations in lung cancer

CSMD3 gene mutations are very common in all types of lung cancer

- Gazdar Lab, unpublished, 2012

<table>
<thead>
<tr>
<th>Sample type (n)</th>
<th>Source</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSCLC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumors, paired (31)</td>
<td>Liu</td>
<td>19.4%</td>
</tr>
<tr>
<td>Cell lines, unpaired (73)</td>
<td>Gazdar lab</td>
<td>56%</td>
</tr>
<tr>
<td><strong>SCLC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumors, paired (30)</td>
<td>Rudin</td>
<td>40%</td>
</tr>
<tr>
<td>Cell lines, unpaired (28)</td>
<td>Gazdar lab</td>
<td>46%</td>
</tr>
</tbody>
</table>
The CSMD3 gene
(CUB and sushi multiple domains 3)

- CSMD3 is located in chromosome 8q 23.3, and consists of 73 exons over 1.2 Mb on the genomic DNA region
- CSMD3 encodes a transmembrane protein with CUB and sushi multiple domains
- Remarkably little known about the gene – less than 10 citations in PubMed
- CSMD3 was reported to play a role in autism
  

- Germline mutations of CSMD3 demonstrated in familial colorectal cancer
  
CSMD3 gene mutations in lung cancer
Are they of any significance?

• Many of the mutations are heterozygous
• Enormous gene – mutations may reflect genomic instability
• Very little known about gene function – difficult to evaluate
CSMD3 knockdown increase proliferation of immortalized bronchial epithelial cells

Liu et al, Carcinogenesis 2012
Methylation status of CSMD3 in squamous cell carcinomas

TCGA data

Gene body

Promoter 2

Promoter 1
Methylation in gene body of CSMD3 in cancers
TCGA data

Lung squamous cell

Lung adenca

Prostate carcinoma

Head & Neck

Colon carcinoma

Breast cancer

Non malignant
Malignant