Gene Expression and Epigenetics
of Lung Cancer

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Princess Margaret Cancer Centre
University of Toronto
Disclosure slide

• Patent holder on the 15-gene signature
• Honorarium from Precision Therapeutics
Topics of Discussion

1. Central Dogma in Biology
2. Epigenetic regulation of gene expression
3. Lung cancer epigenome and microRNA
4. Gene expression in lung cancers
5. Prognostic gene expression signature
Central Dogma in Biology

Biological Functions
Epigenetics: A mechanism for regulating gene activity independent of gene sequences that determine which genes are turned on and off in:

- specific cell type
- different disease state
- response to specific physiological stimulus
Epigenetic Mechanisms

Epigenetic States

A

ENHANCER

PROMOTER

GENE BODY

Inducible gene (CpG-poor promoter)

Active

Inactive

B

Constitutively expressed gene (CpG-island promoter)

C

Poised Polycomb repressed gene (CpG-island promoter)

D

Aberrantly silenced gene with promoter CpG-island methylation
Histone H3 (H3F3A)

**Writer:** establish the epigenetic marks

**Eraser:** remove the epigenetic marks

**Reader:** interpret the epigenetic marks

Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer

Martin Peifer\textsuperscript{1,2,57}, Lynnette Fernández-Cuesta\textsuperscript{1,2,57}, Martin L Sos\textsuperscript{1-4}, Julie George\textsuperscript{1,2}, Danila Seidel\textsuperscript{1,2,5}, et al.

**Putative Mutated Driver Genes**

![Diagram showing altered samples, significance, COSMIC, clustered, and fusion events for various genes.](image-url)
Genomic Deregulation of the E2F/Rb Pathway Leads to Activation of the Oncogene EZH2 in Small Cell Lung Cancer

Bradley P. Coe, Kelsie L. Thu, Sarit Aviel-Ronen, Emily A. Vucic, Adi F. Gazdar, Stephen Lam, Ming-Sound Tsao, Wan L. Lam

![Graph showing EZH2 expression levels in NSCLC and SCLC cell lines.](image1)

Normalized bronchiole: +2
Carcinoid: +1
Carcinoid: +2
Small cell carcinoma: +3

Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network*

[Graph and data as shown in the image]
CDKN2A Silencing in SqCC

Nature 2012;489:519–525 (published on line September 9)
Mapping the Hallmarks of Lung Adenocarcinoma with Massively Parallel Sequencing

Marcin Imielinski,1,2,3,5,18 Alice H. Berger,1,5,18 Peter S. Hammerman,1,5,18 Bryan Hernandez,1,18 Trevor J. Pugh,1,5,18 et al.

26-29 March 2014, Geneva, Switzerland

Organisers

Cell 2012;150 (September), 1107–1120
Hallmark of Lung Adenocarcinoma

Cell 2012;150 (September), 1107–1120
Micro-RNA (miRNA)

- Small non-coding RNA (18-22 nucleotides long)
- Key regulators in many biological processes
- Negatively regulate gene expression
Diagnostic Assay Based on hsa-miR-205 Expression Distinguishes Squamous From Nonsquamous Non–Small-Cell Lung Carcinoma

Danit Lebanony, Hila Benjamin, Shlomit Gilad, Meital Ezagouri, Avital Dov, Karin Ashkenazi, Nir Gefen, Shai Izraeli, Gideon Rechavi, Harvey Pass, Daisuke Nonaka, Junjie Li, Yael Spector, Nitzan Rosenfeld, Ayelet Chajut, Dalia Cohen, Ranit Aharonov, and Mahesh Mansukhani
miRNA May Predict Prognosis of NSCL Patients

Suggested to be superior biomarkers compared to mRNAs;

- Lower complexity (~2,100 compared to ~30,000 coding mRNAs)
- Stable against enzymatic degradation and in FFPE specimen

Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses


PNAS 2001;98:13791-95
Lung Squamous Cell Carcinoma mRNA Expression Subtypes Are Reproducible, Clinically Important, and Correspond to Normal Cell Types

Matthew D. Wilkerson, Xiaoying Yin, Katherine A. Hoadley, et al.

Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network*

Nature 2012;489:519–525 (published on line September 9)
Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses

ALL STAGES

P = 0.00476

non-C2

C2

STAGE IA & IB

P = 0.0753

non-C2

C2

PNAS 2001;98:13791-95
### Micro-array Prognostic Signatures (2005-07)

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Sample Size</th>
<th>Gene Set</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raponi (2006)</td>
<td>SQC</td>
<td>129</td>
<td>50</td>
</tr>
</tbody>
</table>
| Lu (2006) | NSCLC | 197 | 64 | MSKCC (n=63; stage I only p=1.5 x 10^{-6})
Duke (n=64; stage I only p=6 x 10^{-11}) |
| Larsen (2007) | ADC | 48 | 54 | Independent cohort (n=55, p=0.039) |
| Larsen (2007) | SQC | 51 | 111 | Independent cohort (n=58; p=0.0008) |
| Raponi (2006) | ADC | 86 | 47 | Duke validation cohort (n=36; p=0.0008) |

Gene expression–based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study

Director’s Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma:*1
Kerby Shedden2,3,17, Jeremy M G Taylor3,4,17, Steven A Enkemann5,17, Ming-Sound Tsao6,17, Timothy J Yeatman5,17, William L Gerald7,17, Steven Eschrich5,17, Igor Jurisica6,17, Thomas J Giordano8, David E Misek3,9, Andrew C Chang3,9, Chang Qi Zhu6, Daniel Strumpf6, Samir Hanash3, Frances A Shepherd6, Keyue Ding10, Lesley Seymour10, Katsuhiko Naoki11, Nathan Pennell11, Barbara Weir11, Roel Verhaak11, Christine Ladd-Acosta12, Todd Golub12, Michael Gruidi5, Anupama Sharma5, Janos Szoke7, Maureen Zakowski7, Valerie Rusch7, Mark Kris7, Agnes Viale7, Noriko Motoi7, William Travis7, Barbara Conley13, Venkatraman E Seshan14,17, Matthew Meyerson11,12,17, Rork Kuick3,17, Kevin K Dobbin15,17, Tracy Lively16,17, James W Jacobson16,17 & David G Beer3,9,17

Nature Medicine 2008;14:822-827

Provided the largest publicly available multi-institutional derived microarray dataset for future gene expression studies in lung adenocarcinoma
Gene Signatures with Potential Predictiveness for Adjuvant Chemotherapy Benefit


Prognostic and Predictive Gene Signature for Adjuvant Chemotherapy in Resected Non–Small-Cell Lung cancer

Chang-Qi Zhu, Keyue Ding, Dan Strumpf, Barbara A. Weir, Matthew Meyerson, Nathan Pennell, Roman K. Thomas, Katsuhiko Naoki, Christine Ladd-Acosta, Ni Liu, Melania Pintilie, Sandy Der, Lesley Seymour, Igor Jurisica, Frances A. Shepherd, and Ming-Sound Tsao

Training in JBR10

Testing in 4 other independent datasets

Director’s Challenge Patients (n=96)

Duke University Patients (n=48)

Univ. of Michigan Patients (n=79)

Netherland Cancer Institute (n=133)
Prognostic and Predictive Gene Signature for Adjuvant Chemotherapy in Resected Non–Small-Cell Lung cancer

Chang-Qi Zhu, Keyue Ding, Dan Strumpf, Barbara A. Weir, Matthew Meyerson, Nathan Pennell, Roman K. Thomas, Katsuhiko Naoki, Christine Ladd-Acosta, Ni Liu, Melanía Pintilie, Sandy Der, Lesley Seymour, Igor Jurisica, Frances A. Shepherd, and Ming-Sound Tsao

Predicted as High Risk (Poor Survival) by Signature (Stage IB-II, n=67)

Predicted as Low Risk (Good Survival) by Signature (Stage IB-II, n=67)

PREDICTIVENESS OF THE SIGNATURE STILL REQUIRES VALIDATION
Gene expression–based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study
Prognostic Validation in Patients with no Adjuvant Chemotherapy

Director’s Challenge consortium

Japanese cohort

JBR.10 cohort
Predictiveness Tested in JBR.10 Patients

A

Patients from high malignancy-risk group in the GSE14814 dataset

Log-rank P=.03

Survival Probability

OBS MST=3.1 y, 95% CI=2.06 to Inf y
5-year survival rate=39.2%, 95% CI=25.4% to 60.4%

ACT MST=Inf y, 95% CI=5.81 to Inf y
5-year survival rate=72.7%, 95% CI=59% to 89.6%

Number at Risk

OBS 34 27 23 18 13 12 9 3
ACT 33 30 24 24 24 19 11

B

Patients from low malignancy-risk group in the GSE14814 dataset

Log-rank P=.24

Survival Probability

OBS MST=Inf y, 95% CI=Inf to Inf y
5-year survival rate=71.4%, 95% CI=56.5% to 90.3%

ACT MST=6.66 y, 95% CI=5.63 to Inf y
5-year survival rate=70.4%, 95% CI=57.1% to 86.8%

Number at Risk

OBS 28 28 23 19 18 17 14 10
ACT 38 32 29 29 26 20 15 9

26-29 March 2014, Geneva
Stromal fibroblasts in cancer initiation and progression

Neil A. Bhowmick\textsuperscript{1,2,3}, Eric G. Neilson\textsuperscript{3,4} & Harold L. Moses\textsuperscript{1,3,4}

\textsuperscript{1}Department of Cancer Biology, \textsuperscript{2}Department of Urologic Surgery, \textsuperscript{3}The Vanderbilt–Ingram Cancer Center and \textsuperscript{4}Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA (e-mail: hal.moses@vanderbilt.edu)

It is widely accepted that the development of carcinoma — the most common form of human cancer — is due to the accumulation of somatic mutations in epithelial cells. The behaviour of carcinomas is also influenced by the tumour microenvironment, which includes extracellular matrix, blood vasculature, inflammatory cells and fibroblasts. Recent studies reveal that fibroblasts have a more profound influence on the development and progression of carcinomas than was previously appreciated. These new findings have important therapeutic implications.

Nature 432 (18 November 2004): 332-337
A Molecular Signature of Metastasis in Primary Solid Tumors

Table 1 • The 17-gene signature associated with metastasis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene name</th>
<th>GenBank ID</th>
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</thead>
<tbody>
<tr>
<td><strong>Upregulated in metastases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNRPF</td>
<td>Small nuclear ribonucleoprotein F</td>
<td>AI032612</td>
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<tr>
<td>EIF4EL3</td>
<td>Elongation initiation factor 4E-like 3</td>
<td>AF038957</td>
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<tr>
<td>HNRPA2B</td>
<td>Heterogeneous nuclear ribonucleoprotein A/B</td>
<td>M65028</td>
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<tr>
<td>DHPS</td>
<td>Deoxyhypusine synthase</td>
<td>U79262</td>
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<tr>
<td>PTTG1</td>
<td>Securin</td>
<td>AA203476</td>
</tr>
<tr>
<td>COL1A1</td>
<td>Type 1 collagen, α1</td>
<td>Y15915</td>
</tr>
<tr>
<td>COL1A2</td>
<td>Type 1 collagen, α2</td>
<td>J03464</td>
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<tr>
<td>LMNB1</td>
<td>Lamin B1</td>
<td>L37747</td>
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<tr>
<td><strong>Downregulated in metastases</strong></td>
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<tr>
<td>ACTG2</td>
<td>Actin, γ2</td>
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<tr>
<td>MYLK</td>
<td>Myosin light chain kinase</td>
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<td>MYH11</td>
<td>Myosin, heavy chain 11</td>
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<td>CNN1</td>
<td>Calponin 1</td>
<td>D17408</td>
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<td>HLA-DPB1</td>
<td>MHC Class II, DPβ1</td>
<td>M83664</td>
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<td>RUNX1</td>
<td>Runt-related transcription factor 1</td>
<td>D43969</td>
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<td>MT3</td>
<td>Metallothionein 3</td>
<td>S72043</td>
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<tr>
<td>NR4A1</td>
<td>Nuclear hormone receptor TR3</td>
<td>L13740</td>
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<tr>
<td>RBM5</td>
<td>RNA binding motif 5</td>
<td>AF091263</td>
</tr>
</tbody>
</table>

Fibroblasts Cultured from Resected NSCLC and Corresponding Normal Lung
Prognostic gene-expression signature of carcinoma-associated fibroblasts in non-small cell lung cancer

Roya Navab,1, Dan Strumpf,1, Bizhan Bandarchi,1, Chang-Qi Zhu,1, Melania Pintilie, Varune Rohan Ramnarine, Emin Ibrahimov, Nikolina Radulovich, Lisa Leung, Malgorzata Barczyk, Devang Panchal, Christine To, James J. Yun, Sandy Der, Frances A. Shepherd, Igor Jurisica, and Ming-Sound Tsao

11-gene prognostic signature

A. DCC Training (n=218)

B. DCC Test (n=218)

C. Duke (n=89)

D. SKKU (n=138)

Proc Natl Acad Sci USA 2011 Apr 26;108(17):7160-5
Ectopic Activation of Germline and Placental Genes Identifies Aggressive Metastasis-Prone Lung Cancers

Sophie Rousseaux et al.

Sci Transl Med 5, 186ra66 (2013);
DOI: 10.1126/scitranslmed.3005723
A gene expression signature of RAS pathway dependence predicts response to PI3K and RAS pathway inhibitors and expands the population of RAS pathway activated tumors

Andrey Loboda¹, Michael Nebozhyn¹, Rich Klinghoffer², Jason Frazier², Michael Chastain², William Arthur²,

BMC Medical Genomics 2010, 3:26
Prognostic Immune Markers in Non–Small Cell Lung Cancer

Kei Suzuki, Stefan S. Kachala, Kyuichi Kadota, Ronglai Shen, Qianxing Mo, David G. Beer, Valerie W. Rusch, William D. Travis, and Prasad S. Adusumilli

Clin Cancer Res; 17(16); 5247–56.

Predictive Gene Signature in MAGE-A3 Antigen-Specific Cancer Immunotherapy

Fernando Ulloa-Montoya, Jamila Louahed, Benjamin Dizier, Olivier Gruselle, Bart Spiessens, Frédéric F. Lehmann, Stefan Suciu, Wim H.J. Kruit, Alexander M.M. Eggermont, Johan Vansteenkiste, and Vincent G. Brichard

J Clin Oncol 2013;31:2388-2395
Conclusions

• Epigenetic plays important role in lung cancer development, differentiation and prognosis, and specific aberrations may constitute therapeutic targets

• Gene expression signatures may define additional subtypes of major lung cancer histological types

• Expression signatures can define prognosis and predict benefit from adjuvant chemotherapy, yet routine clinical application still awaits further prospective validation

• Tissue or Pathway specific gene expression signatures may provide important biological insights into complexity of targeted therapies