CTD2: Functional Cancer Genomics
Characterization of cancer genomes is essential but not sufficient

- Hundreds to thousands of candidates in each tumor
- Distinguishing Driver vs. Passenger mutations
- Drivers: Tumor initiation or maintenance
- Context-specific actions of particular genetic elements

Prioritization must be based on both genomic and biological weight of evidence
Functional interrogation of cancer genomes

Gain-of-function: ORFs

Connect genotype to function

Loss-of-function: RNAi

Identify potential Achilles’ Heels

Experimental cancer models

Cancer Genome Annotation

The Cancer Genome Atlas

COSMIC
Aim 1: Genome-scale LOF Screens
RNAi screen for viability in 20 cell lines/tumor type

Essential Genes

Aim 2: Targeted in vitro GOF Screens
Genomically Altered Genes Defined by TCGA

Amplified GEOIs (2000 ORFs)

Mutated GEOIs (200 ORFs/tumor type)

Genomically altered and Transforming Genes

Aim 3: in vivo Context-Specific Screens
Genomically Altered, Essential and transforming genes

In vivo Orthotopic Context

Validated Hits
Genome scale barcoded shRNA screens

Pooled shRNA plasmid library

45,000 distinct shRNA plasmids

Packaged into lentivirus

Infect

CANCER CELLS

4-week culture

EARLY-INFECTION SAMPLES

ENDPOINT SAMPLES

Harvest genomic DNA

Amplify hairpin region by PCR

Cut with Xho I

Hybridize to microarray

Identify lethal hairpins

Identify essential genes

Biao Luo
Tony Cheung
Aravind Subramanian
David Root
Identification of genes essential in ovarian cancer
Integrating functional and structural genomics in ovarian cancer

270 amplified genes in ovarian tumors

1350 shRNAs targeting amplified genes in ovarian cell lines

Essential and amplified genes

KRAS (-2.6, -2.5)
MDS1 (-2.3)
ID4 (-2.1)
(+35 others)

Most lethal shRNAs

(shRNA enrichment (median of ovarian cell lines))
Transformation of immortalized ovarian surface epithelial cells

**Immortalized**

SV40 LT/ST, hTERT

**Transformed**

<table>
<thead>
<tr>
<th>Cell line</th>
<th># tumors/# injection sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector</td>
<td>0/9</td>
</tr>
<tr>
<td>ID4</td>
<td>0/9</td>
</tr>
<tr>
<td>MEK$^{DD}$ + lacZ</td>
<td>4/21</td>
</tr>
<tr>
<td>MEK$^{DD}$ + ID4</td>
<td>21/27</td>
</tr>
<tr>
<td>MEK$^{DD}$ + ID4_DM</td>
<td>2/18</td>
</tr>
</tbody>
</table>
Identification of ID4 as an ovarian cancer oncogene

**Loss-of-Function**
Genes essential for ovarian cancer proliferation

**Gain-of-Function**
Genes that induce ovarian tumor formation

Cancer Genome Annotation
Cross reference with genes in amplified regions in OvCa (TCGA)

**Control**  **Anti-ID4**

Yin Ren, Sangeeta Bhatia
Tony Cheung, Jesse Boehm, Glenn Cowley
Context Specific Functional Genomic Screening Platform

ORF Library of GEOI

Genetically defined Context specific TARGET cell

Orthotopic injection

Phenotype = tumorigenicity

Biological Validation

Control shRNA

Responder ID

GEOI shRNA

Context specific Tumor cell

GEOI “HIT”
Integrated genomic pipeline

Genome-scale LOF Screens
- Identify genes essential to ovarian cancer and GBM viability.

Targeted GOF in vitro Screens

Context-specific GOF screens
- Define cell- and genetic contexts in which GEOI is functionally relevant
- Identify GEOIs with in vivo activity in specific context

Novel validated cancer drivers that merit consideration for drug discovery efforts
Integrate cancer genome computational analysis, mouse models, and in vivo screening to identify and validate new cancer genes, pathways, and tumor dependencies / therapeutic targets
1. Computational Analysis of Cancer Genomes

2. Construction of oncogenomically focused shRNA and cDNA libraries

3. Screen for oncogenicity with a transplantable mouse model

4. Test for tumor dependency with mouse models and human cancer cell lines

Under construction
Summary of findings

• Discovery and validation of 20 novel TSGs and oncogenes
• Unexpected number of identified tumor suppressors encode secreted proteins
• Discovered FGF19 oncogene dependency in human HCC cell lines containing the FGF19 amplicon
• This pinpoints for the first time a candidate cancer drug that selectively targets a genetic abnormality in HCC.
Objective: to employ parallel phenotypic screening of genome-wide siRNA libraries and a diverse chemical compound file to return authentic drug lead/target relationships
mRNA Expression Profiles Identify 6 Major Subtypes (Clades) of Non-Small Cell Lung Cancer

Multidimensional Scaling Plot

6 HBECs
56 NSCLCs
mRNA Defined Clades from the NSCLC Lines Are also Found in Primary NSCLCs

Probability (using PAM, prediction analysis of microarray method) of each primary tumor sample belonging to a particular NSCLC Line Defined Clade

Low probability of belonging to a Clade

High probability of belonging to a Clade

(N = 111)

(NSCLC Data from Bild Nature 2006 (439), 353-357)
mRNA Defined Clades Identify Different NSCLC Drug Response Phenotypes

Drug Sensitivity Frequency in Clades

High probability of sensitivity

High probability of Resistance
Genome Wide siRNA Library Screens Reveal Clade-Selective Vulnerabilities
Cancer Target Discovery and Development (CTD\(^2\)) Network

**Network interactions and synergy**

*State of the art technological platforms*

*Data sharing*

*Model sharing*

*Development of new informatics*

**Deliverables to cancer research community**

*Reagents and Informatic tools*

*Large scale functional datasets (in vitro and in vivo)*

*Experimental models*

*Integrative data to inform investigator initiated research*