Defining the functional cancer genome using RNAi analysis and screening

Natasha J. Caplen, Ph.D.
Gene Silencing Section
Genetics Branch, CCR

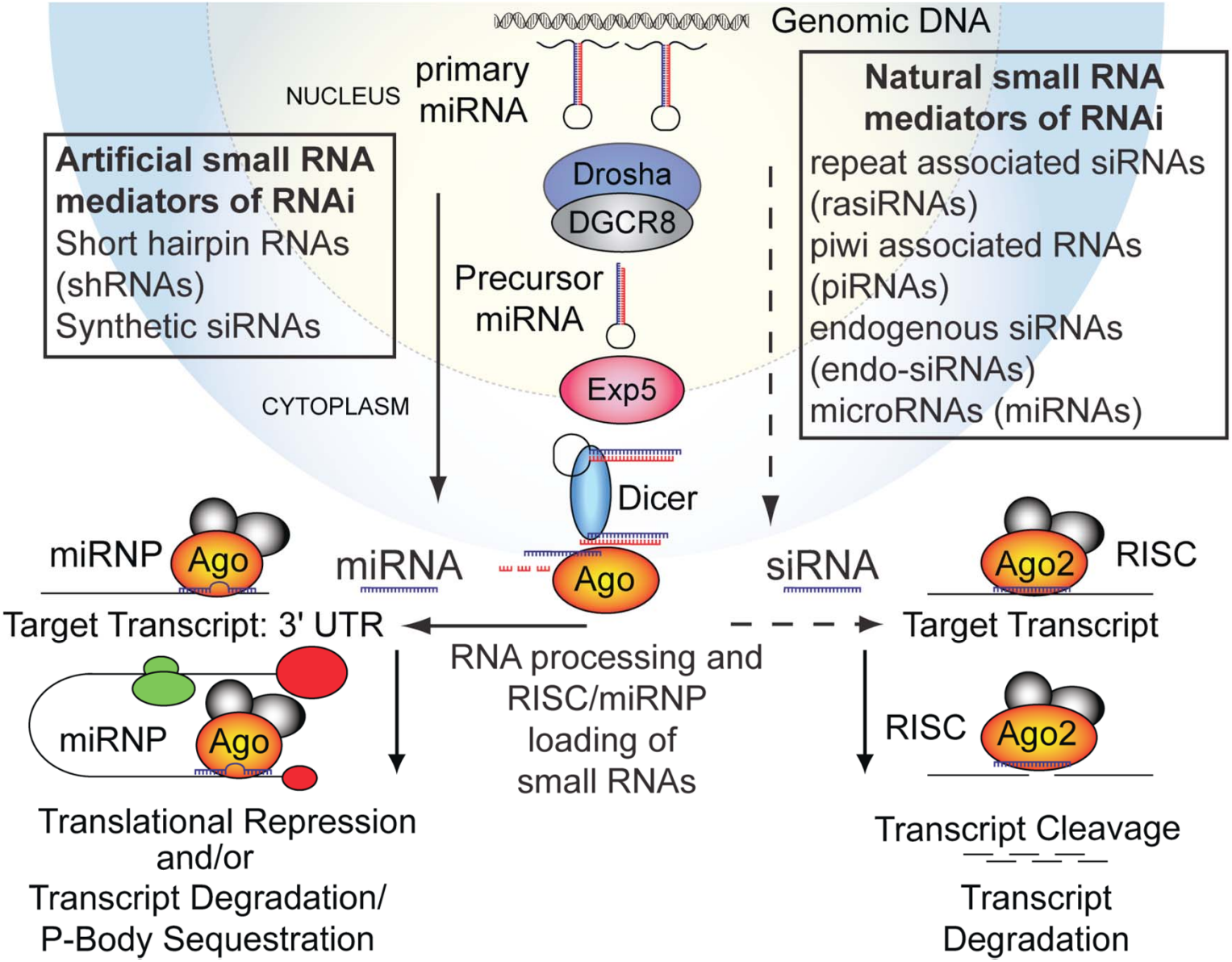
NCAB December 9, 2008

Center of Excellence in Chromosome Biology

CCR Center for Cancer Research

Center of Excellence in Integrative Cancer Biology and Genomics

RNAi gene silencing pathways



Caplen, Genetics Branch, CCR, NCI: NCAB, December 2008 (2/21)

Goals

Programmatic goal

To build a program that will guide and assist CCR investigators, at all career levels, interested in investigating and/or applying RNAi-mediated gene silencing to their research.

Scientific goals

1. To use RNAi-based technologies to discover and interrogate the function of cancer genes, including those that impact anti-cancer drug activity.
2. To investigate the role that RNAi plays in the dysregulated gene expression that is the hallmark of cancer.

Research program

1: The induction of gene-specific RNAi for the study of cancer biology.

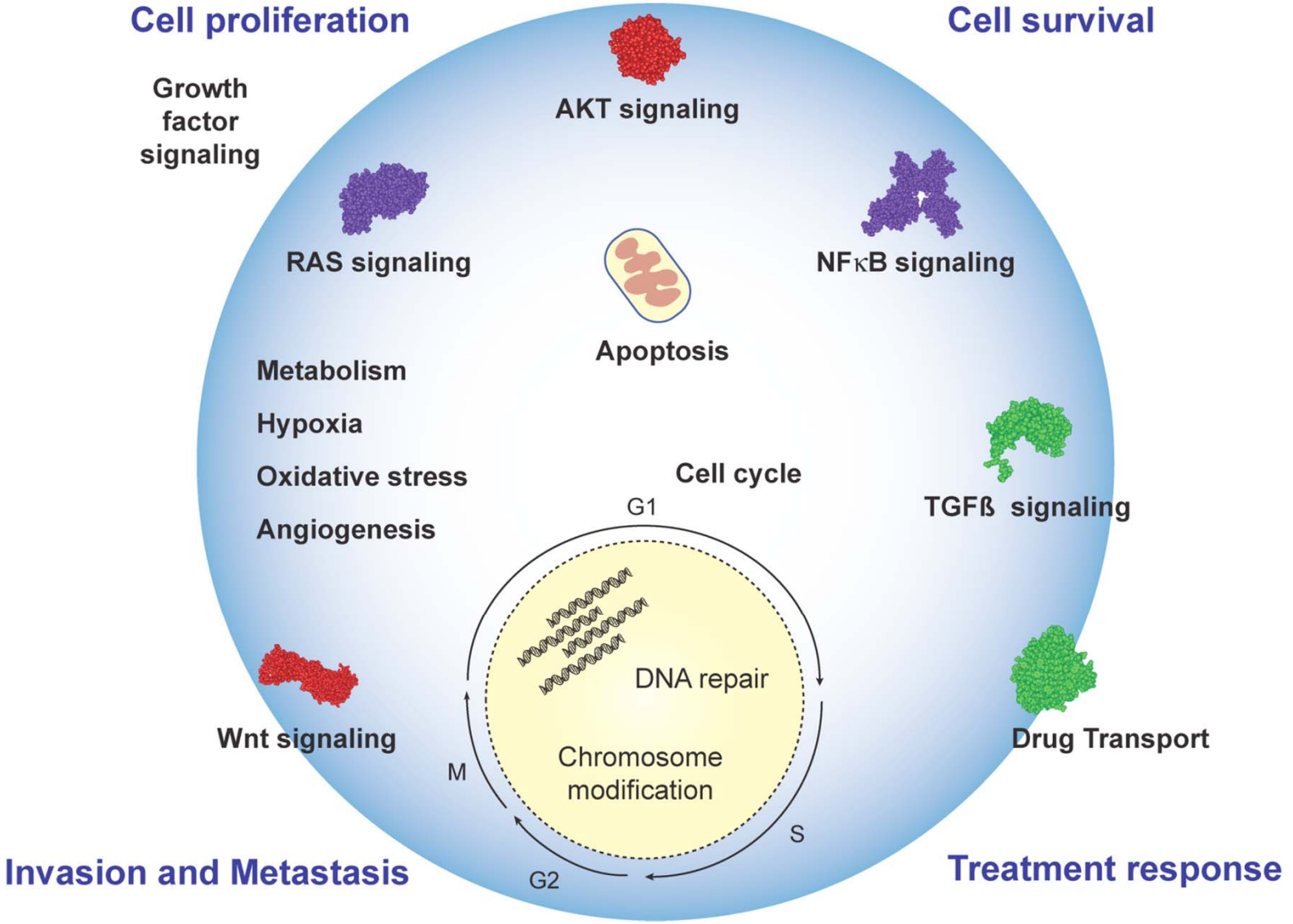
2: The application of RNAi analysis for the study of gene:drug interactions relevant to anti-cancer therapeutic approaches.

3: The role of miRNA-mediated RNAi in the biology of cancer.

Gene-specific RNAi analysis and screening for the study of cancer biology and gene:drug interactions relevant to anti-cancer therapeutic approaches.

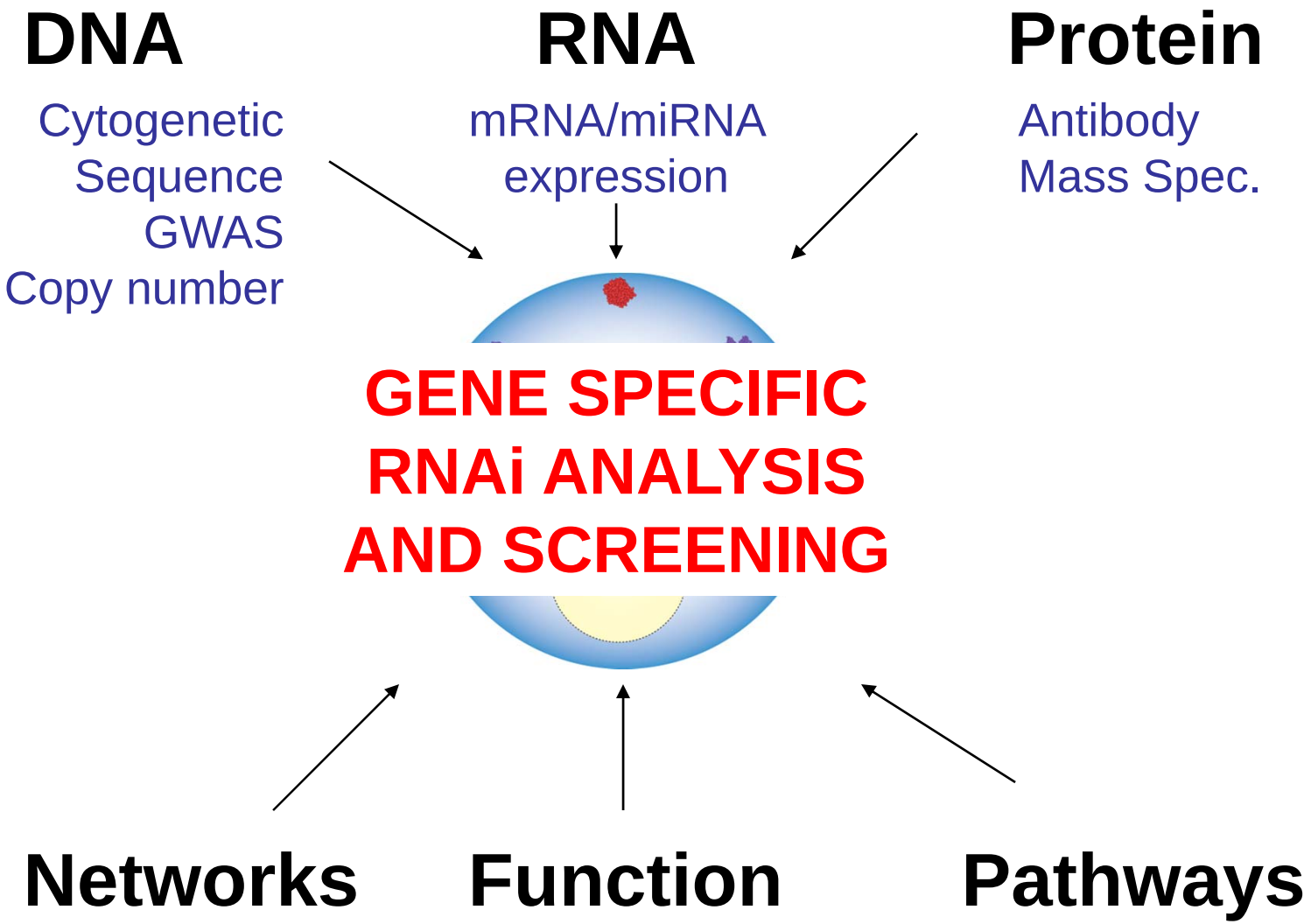
- The development, optimization and application of RNAi analysis and RNAi screening strategies for analysis of normal and cancer related gene function.
- The application of RNAi analysis to study correlative relationships between gene expression and drug activity.
- The identification of TAK1 as a modulator of the activity of a class of topoisomerase 1 inhibitors using chemosensitization RNAi screening.
- Future major program efforts - large scale RNAi screening.

Defining the functional cancer genome using RNAi analysis and screening



Caplen, Genetics Branch, CCR, NCI: NCAB, December 2008 (6/21)

Defining the functional cancer genome using RNAi analysis and screening



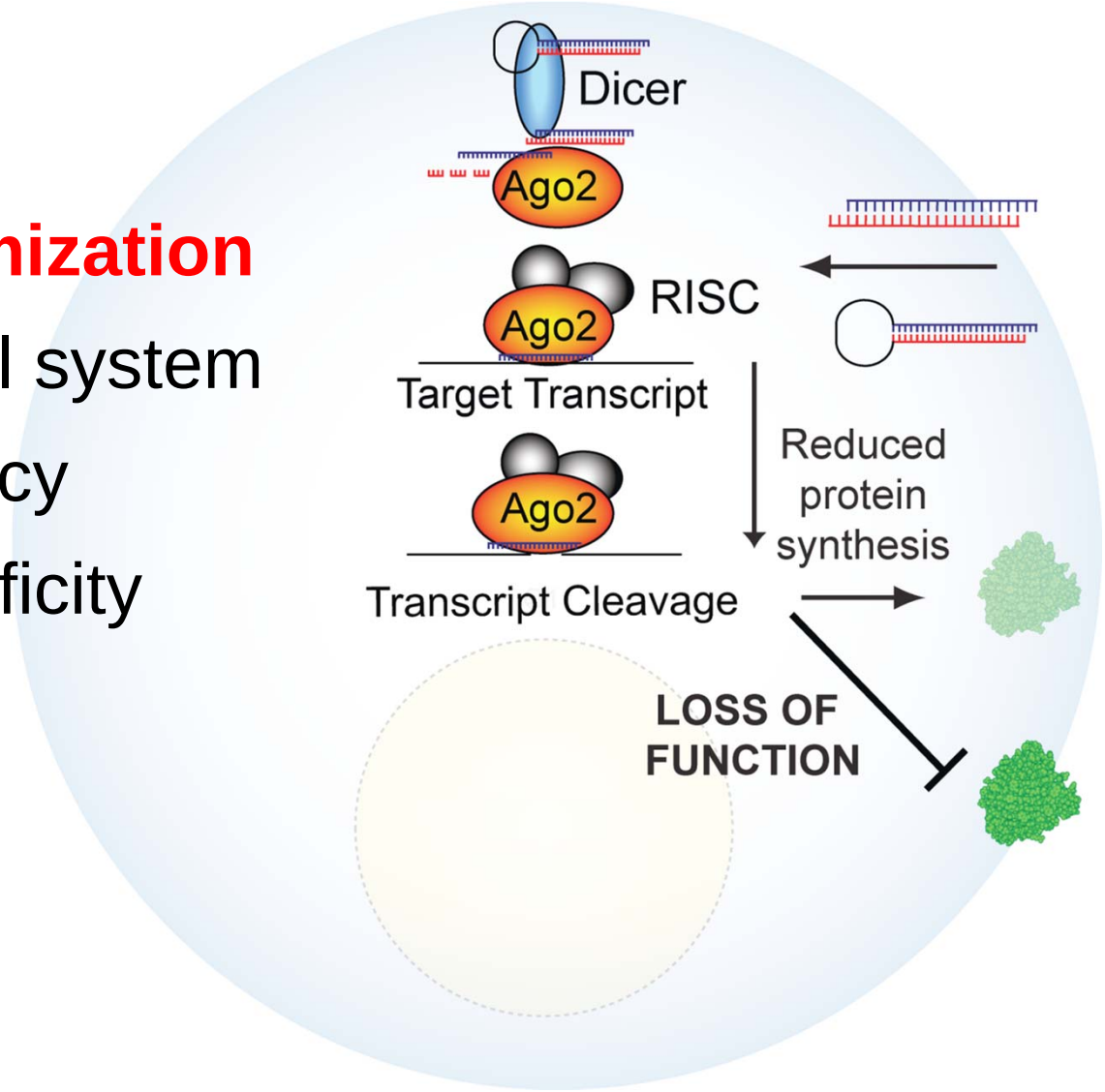
The induction of gene-specific RNAi against cancer-associated genes

Optimization

Model system

Efficacy

Specificity



Delivery

siRNA

shRNA

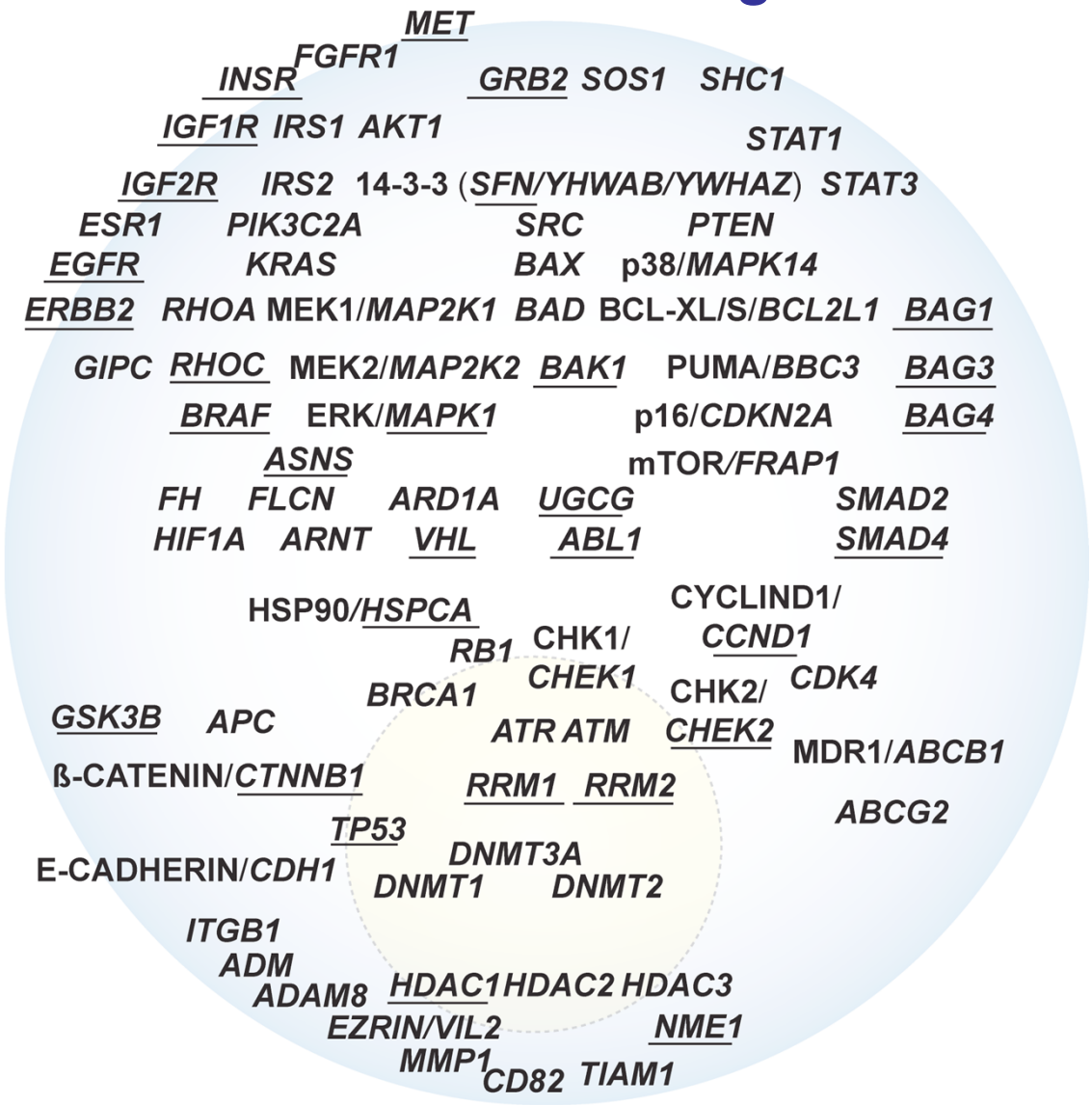
Assays

mRNA

Protein

Function

The induction of gene-specific RNAi against cancer-associated genes



Martin *et al*., Nuc. Acids. Res. (2007) **35** e57

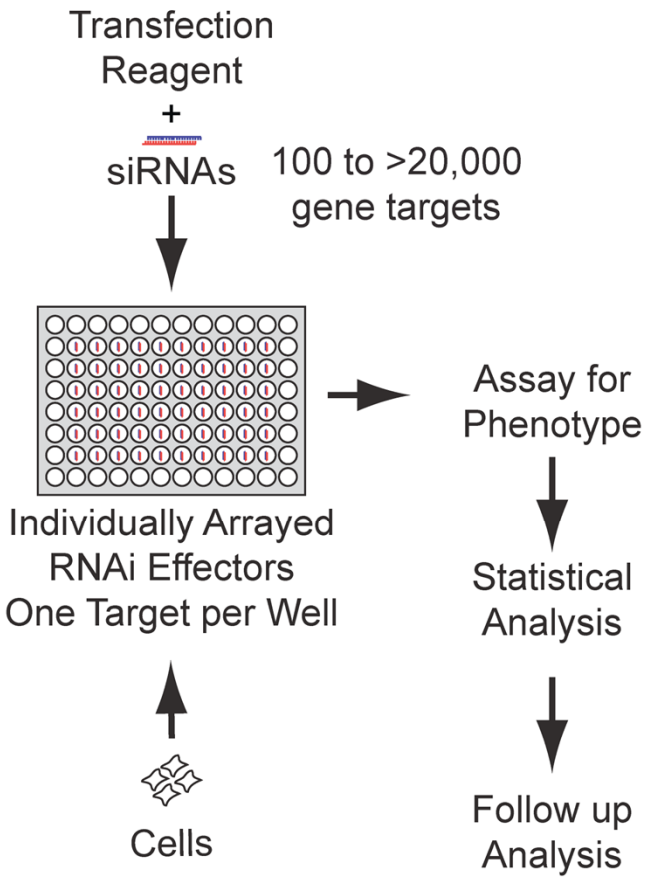
The application of gene-specific RNAi

Examples of on-going studies applying RNAi based loss of function (LOF) analysis for the study of cancer associated genes

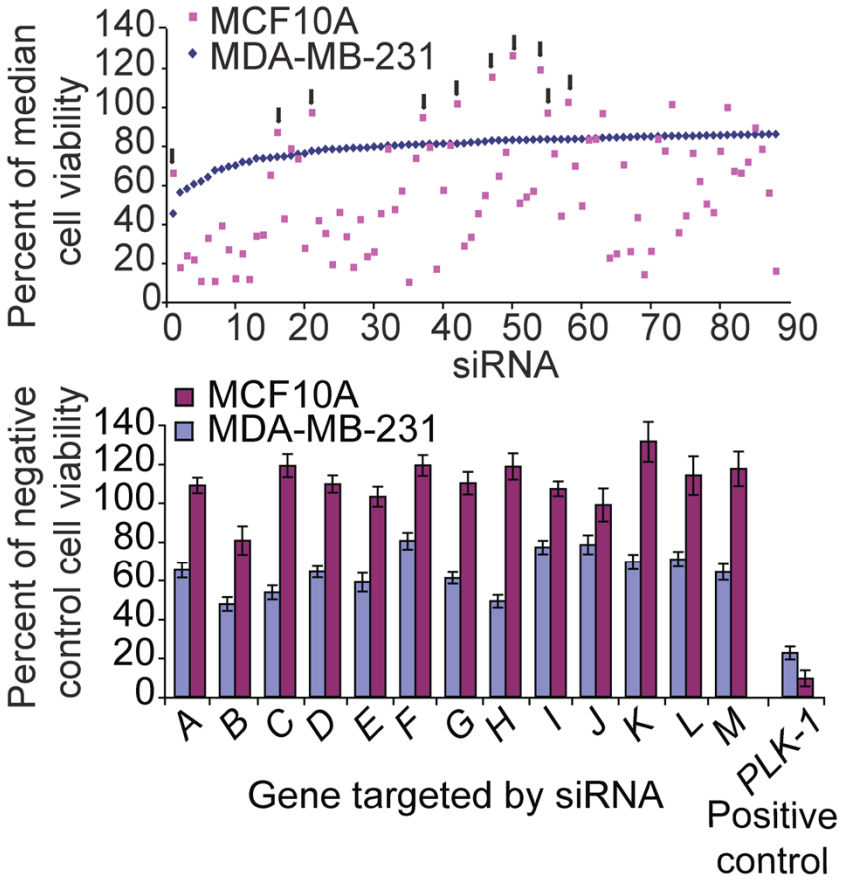
- Investigation of putative or established gene function (normal and cancer related).
- Investigation of genes present within regions of DNA amplification and/or over-expressed in specific cancers.
- Allele (mutation) or transcript variant (isoform) specific analysis.
- Investigation of protein encoding genes with no known function including those identified through RNAi screening.

RNAi screening

Arrayed synthetic siRNA RNAi screens

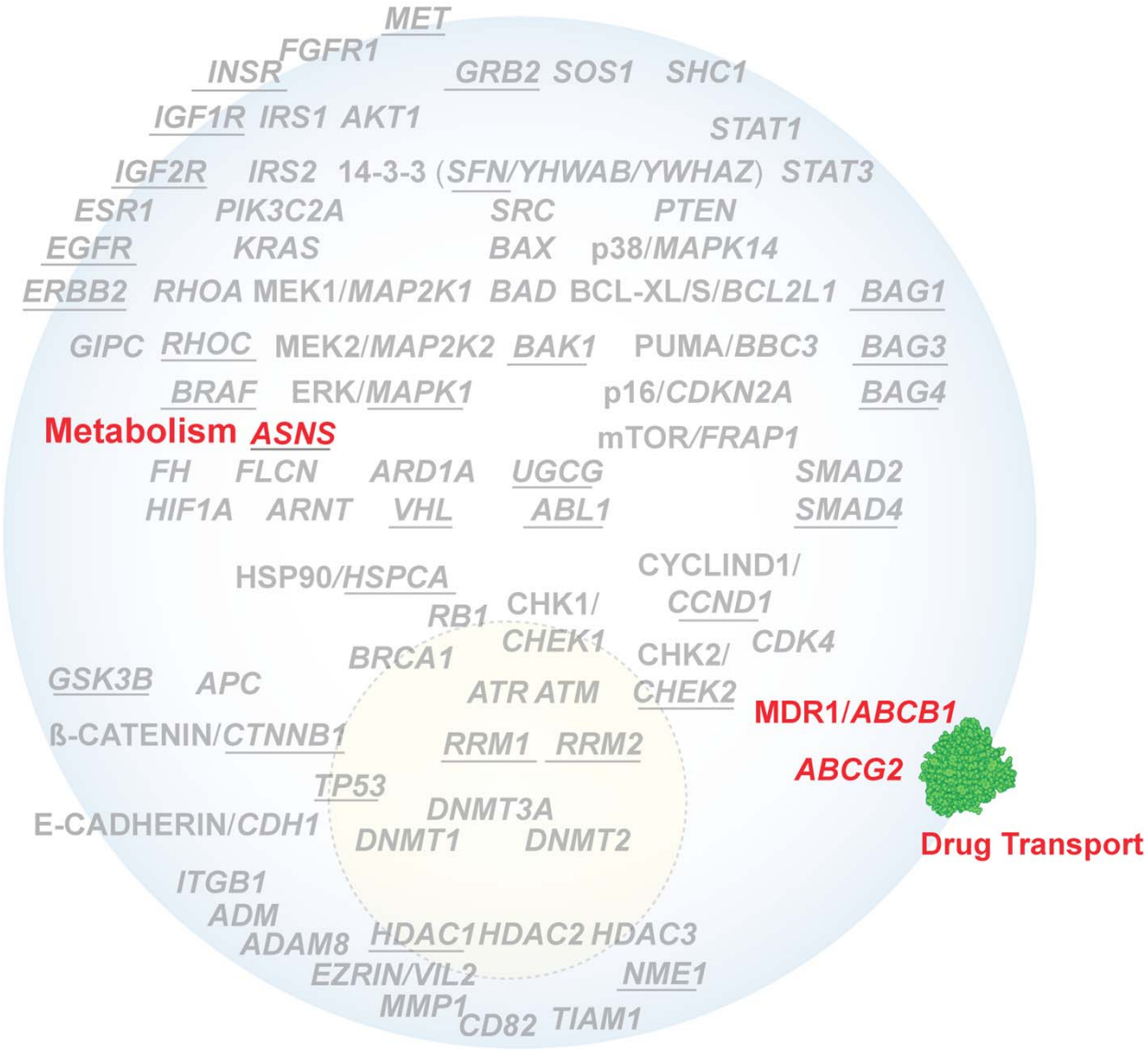


Comparative synthetic siRNA RNAi screens



S.E. Martin & N.J. Caplen, unpublished data

The functional validation of gene-drug interactions using RNAi analysis

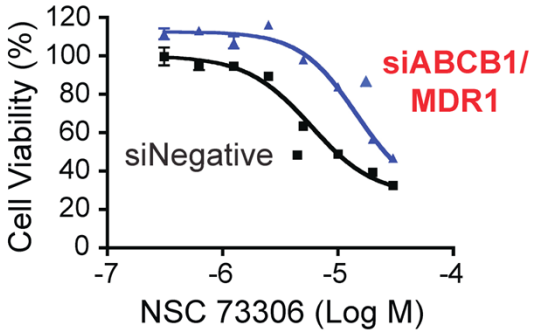


The functional validation of gene-drug interactions using RNAi analysis

1

siRNA mediated silencing of ABCB1 and NSC73306 cytotoxicity (NCI/ADR-RES cells)

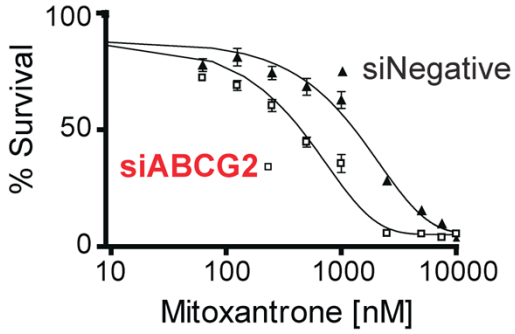
Ludwig *et al.*, Cancer Research (2006) **66** 4808.



2

siRNA mediated silencing of ABCG2 and mitoxantrone cytotoxicity (Low dose Doxorubicin selected MCF7 cells)

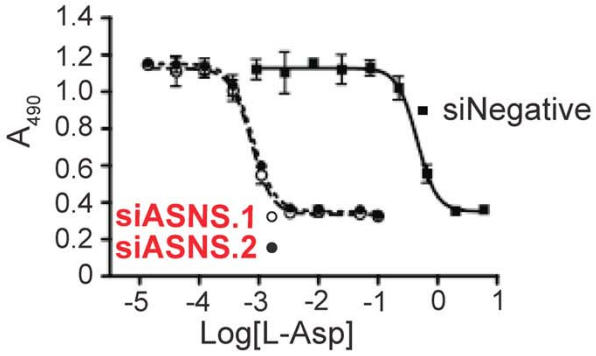
Calcagno *et al.*, Brit. Jour. Cancer (2008) **98** 1515.



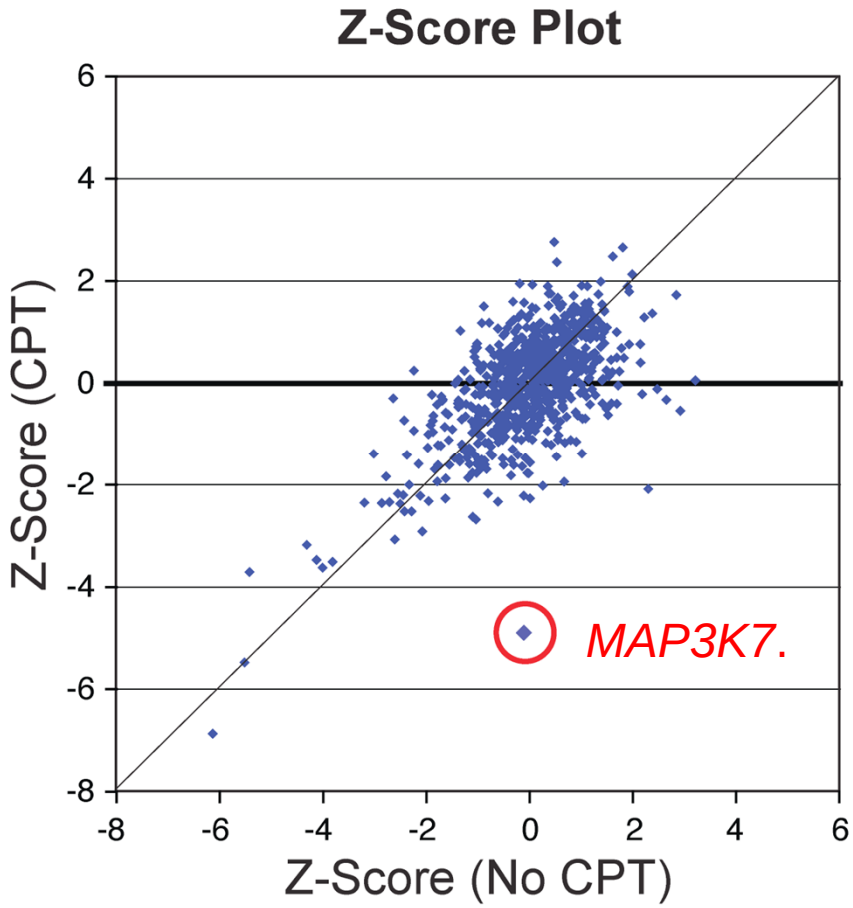
3

siRNA mediated silencing of ASNS and L-Asparaginase cytotoxicity (OVCAR8 cells)

Lorenzi *et al.*, Mol. Cancer Ther. (2006) **5** 2613



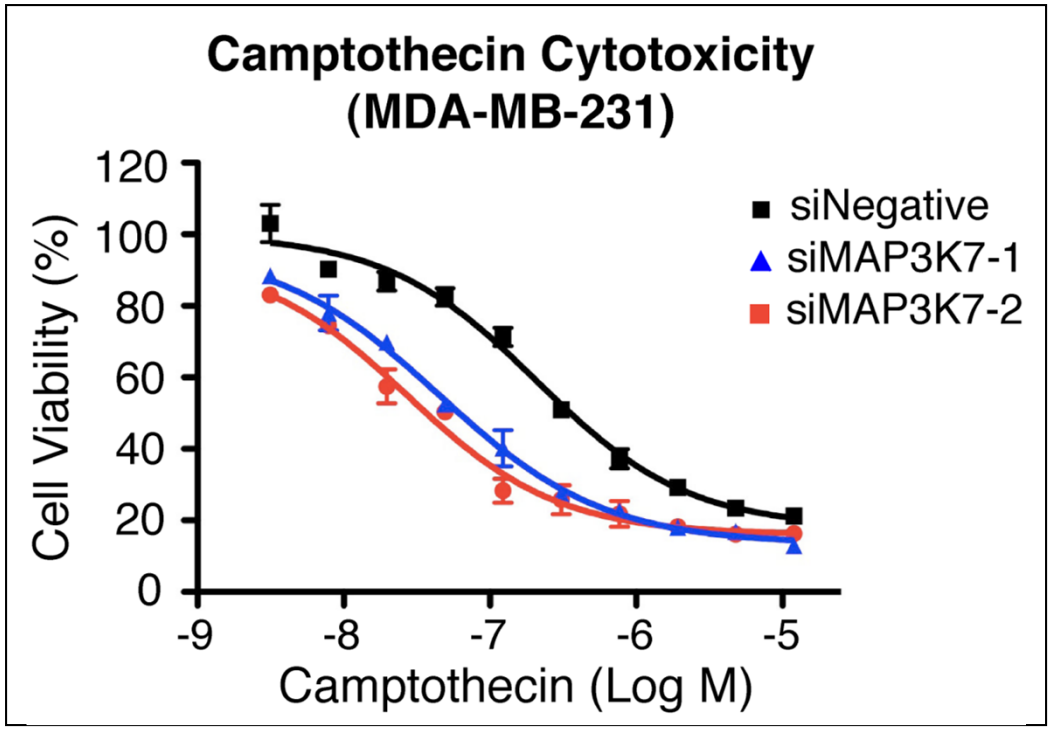
A chemosensitization siRNA-based RNAi screen of camptothecin



MDA-MB-231 cells
418 genes
2 siRNAs per gene
Library focus
- Apoptosis

S.E. Martin & N.J. Caplen, unpublished data

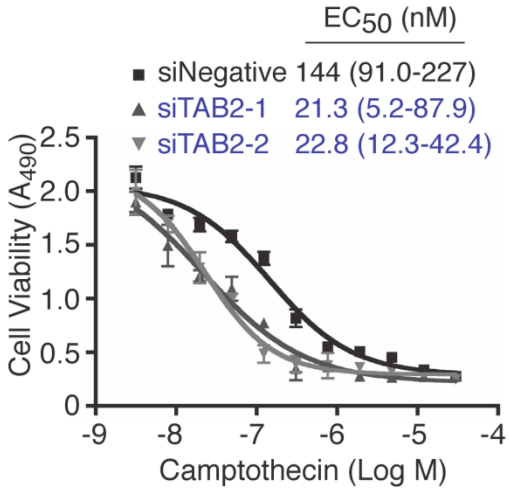
The silencing of *MAP3K7* potentiates camptothecin activity

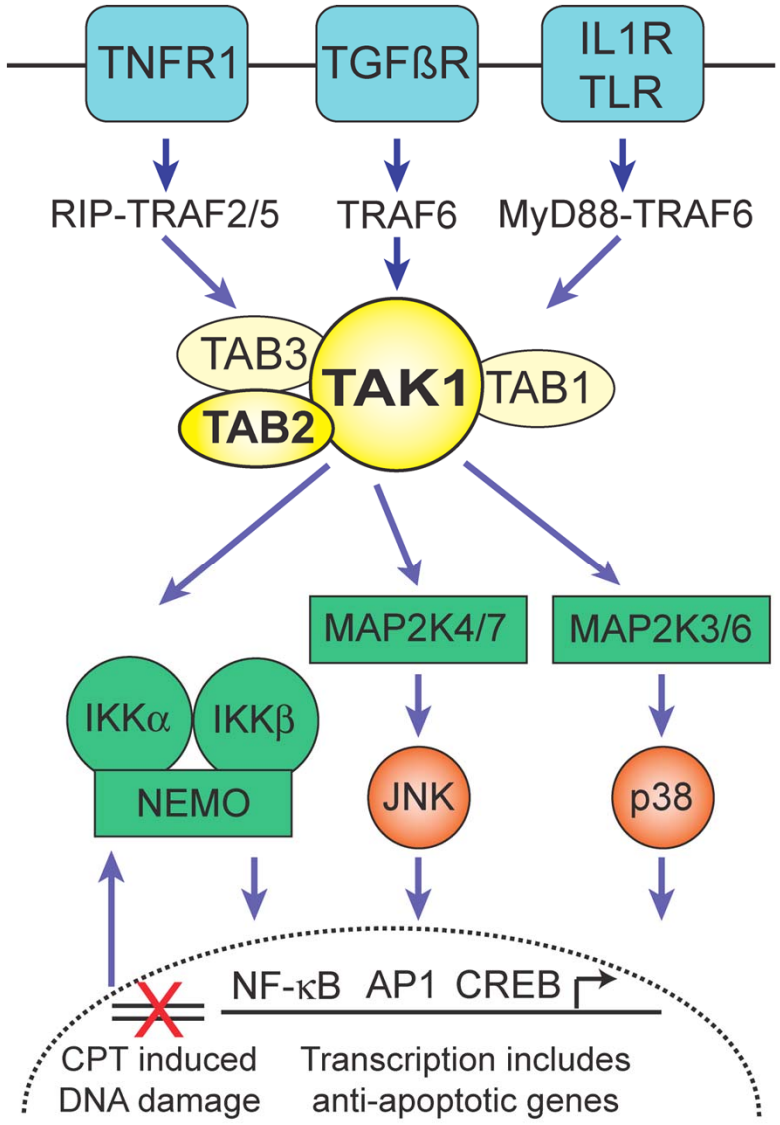


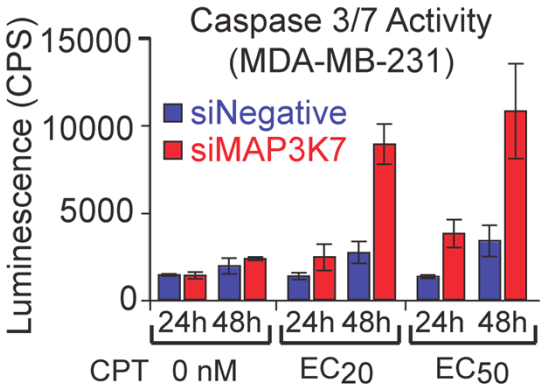
~7 fold shift in camptothecin EC_{50} value corresponding to a 65-80% reduction in *MAP3K7* mRNA levels.

S.E. Martin & N.J. Caplen, unpublished data

MAP3K7 encodes TAK1 MAP 3-kinase







S.E. Martin & N.J. Caplen, unpublished data

Overview

In the last four years we have:

Interacted with scientists from nearly half the Branches and Laboratories within CCR.

Helped expedite the further development of two anti-cancer therapeutic approaches, the use of NSC77306 for the treatment of MDR and the use of L-Asparaginase for the treatment of solid tumors.

Defining the functional cancer genome using RNAi analysis and screening

- **The integrated analysis of the downstream molecular effects of RNAi perturbations.**
  - Expression profiling including analysis of transcript variants.
  - Use of multiplexed RNA and protein (proteomic) assays.
- **The application of emerging technologies and research approaches.**
  - Next generation sequencing.
  - Systems biology.

Defining the functional cancer genome using RNAi analysis and screening

A Trans-NIH program with NCI as lead Institute for establishment of large-scale RNAi screening.

Based within the NIH Chemical Genomic Center - NCGC (Director, Dr. Chris Austin).

The expansion of RNAi screening capacity (up to whole genome).

Application of state of the art assay end-points.

Defining the functional cancer genome using RNAi analysis and screening

Aims:

- A collaborative environment for
  - development of genome-wide compatible synthetic siRNA screens,
  - performance of large (up to genome scale) synthetic siRNA screens,
  - performance of sophisticated downstream statistical and bioinformatic analysis,
  - assisting the collaborator Investigator with follow-up experiments to confirm and extend the screening findings, so as to result in high-profile publications.
- Application of a broad range of quantitative high throughput screening (qHTS) and high content screening (HCS) assays.
- Improve and extend capabilities to remain both cutting-edge scientifically and world-leading in efficiency and quality.

Gene Silencing Section
Genetics Branch

Current Members

Konrad Huppi

Kristen Gehlhaus

Tamara Jones

Jenny Llamas

Mark Mackiewicz

Scott Martin

Past Members

Dac Nguyen

Tim Runfola

Cheryl Thomas

Brady Wahlberg

CCR outreach

David Goldstein - OSTP, CCR
Shoshana Segal - OSTP, CCR

Bioinformatics

***Micheal Ryan** - Contract: LMP, CCR
***John Weinstein** - CCR/MD Anderson

*Published study

#Manuscript submitted or in preparation

RNAi analysis of gene function

Collaborations

***Eric Lader** - Qiagen Inc.

***Michele Gunisor** - APPU, OSTP, CCR
***Paul Goldsmith** - APPU, OSTP, CCR

***Marjan Huizing** - NHGRI

***John Weinstein** - CCR/MD Anderson

Paul Meltzer - GB - CCR

Technology Transfer (CCR, NCI)

S. Ambudkar
L. Anderson
O. Aprelikova
M. Barasi
D. Bottaro
M. Gottesman
C. Khanna
E. Kohn
S. Lipkowitz
F. Mushinski
Y. Pommier
N. Popescu
S. Rane
P. Steeg
*C. Thiele
S. Thorgeirsson
J. Vogel
A. Weissman
J. Weinstein
H. Young

***Other* - Panomics Inc.** - CA

RNAi-based profiling and screening

Collaborations

Lyndsay Murrow - LCMB - CCR
Stanley Lipkowitz - LCMB - CCR

Amanda Hummon - GB - CCR
Thomas Ried - GB - CCR
Marian Grade - GB - CCR/
Uni. Medicine, Göttingen, Germany

Christopher Austin - NCGC, NIH

Technology Transfer

Patricia Tsang - POB - CCR
Javed Khan - POB - CCR

Barry O'Keefe - MTDP - CCR
James McMahon - MTDP -CCR

RNAi analysis of gene-drug interactions

Collaborations

***Anna Calagano** - LMP -CCR
***Suresh Ambudkar** - LMP -CCR

Laurent Ozbun - CCBB, CCR
Micheal Birrer - CCBB, CCR/Harvard

***Gergely Szakacs** - LCB - CCR
***Joseph Ludwig** - LCB - CCR
***Micheal Gottesman** - LCB - CCR

*#**Philip Lorenzi** - LMP, CCR
*#**John Weinstein** - LMP, CCR/MD Anderson

#**Yong-Wei Zhang** LMP, CCR
#**Yves Pommier** - LMP - CCR

#**Zhao-Hui Wu** - University of Wisconsin
#**Shigeki Miyamoto** - University of Wisconsin

miRNA analysis

Collaborations / Technology Transfer (CCR, NCI)

***Nozomu Yanaihara** - LHC - CCR
***Curtis Harris** - LHC - CCR

***Frederic Mushinski** - LCBG - CCR
***Natalia Volfovsky** - SAIC, NCI-Frederick
***Robert Stephens** - SAIC, NCI-Frederick

***Matthias Wabl - UCSF, CA**

Joe Gray - Lawrence Berkeley Nat. Lab, CA
Eric Collisson - UCSF, CA

Michael Emmert-Buck - LP - CCR

Yun-Xing Wang - SBL - CCR

#**Robert Cornelison** - GB - CCR
Paul Meltzer - GB - CCR