Exploiting science for engineering: BRCA2 targeted therapies

Orsi Kiraly
Engelward lab
Homologous recombination is important

- No HR
  - chromosomal aberrations
  - cell death

- Faulty HR
  - premature aging
  - cancer

Sonoda 1998

WS patient age 15 yrs  WS patient age 48 yrs
Homologous recombination is critical for repair of broken replication forks
Homologous recombination is critical for repair of broken replication forks.
Understanding biology leads to new technologies
Recombinant DNA toolbox is from nature

Plasmids

Heat stable DNA polymerases

Restriction endonucleases

Radloff 1967 PNAS

Photo: Agriculture and Agri-Food, Canada

Lydia Dorner and Ira Schildkraut, New England Biolabs
OBSERVATION
Understanding science leads to new technologies: BRCA2 targeted therapy

Roitt 1956

PARP Inhibitors Represent New Direction in Cancer Treatment
Metabolism is suppressed by alkylating agent

In tumor cells

\[ \text{CO}_2 \text{ from catabolism} \]

![Graph showing CO2 from catabolism over time with and without alkylating agent treatment.]

Roitt 1956
NAD levels are lowered by alkylating agent

In tumor cells

![Graph showing CO$_2$ from catabolism](null)

alkylating agent

no treatment

Roitt 1956
NAD levels are lowered by alkylating agent

Why?
Less NAD is made? No
More NAD is degraded? No
Used up to build something?? Yes!

Poly(ADP-ribose) polymerase (PARP)

Whish 1975
NAD is a precursor for poly(ADP-ribose)
What is the role of poly(ADP-ribosylation)?

Inhibit it, see what happens:

Inhibition of PARP affects survival after treatment with DNA damaging agent

PARP has a role in DNA repair

Durkacz 1980
Mouse leukemia cells
What is the role of poly(ADP-ribosylation)?

Inhibit it, see what happens:

- More HR (Waldman 1991)
- More single strand breaks (Boulton 1999)

In the absence of PARP, HR is needed to fix broken fork.

What about cancer cells that can’t do HR?
What about cancer cells that can’t do HR?

BRCA2-/-

No PARP

Potentially TOXIC

NHEJ
BRCA2 null cells are extremely sensitive to PARP inhibitors

In cells

Bryant 2005

PARP inhibitor

Surviving fraction

BRCA2 null

wild type

BRCA2 null

PARP inhibitor (µM)

Bryant 2005
BRCA2 null cells are extremely sensitive to PARP inhibitors targeted to tumor by virtue of the tumor’s own mutation.

Farmer 2005

TARGETED to tumor by virtue of the tumor’s own mutation!
Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from BRCA Mutation Carriers

Peter C. Fong, M.D., David S. Boss, M.Sc., Timothy A. Yap, M.D., Andrew Tutt, M.D., Ph.D., Peijun Wu, Ph.D., Marja Mergui-Roelvink, M.D., Peter Mortimer, Ph.D., Helen Swaisland, B.Sc., Alan Lau, Ph.D., Mark J. O’Connor, Ph.D., Alan Ashworth, Ph.D., James Carmichael, M.D., Stan B. Kaye, M.D., Jan H.M. Schellens, M.D., Ph.D., and Johann S. de Bono, M.D., Ph.D.

advanced ovarian cancer
Synthetic lethality and an assay to find it

Each decrease expression of a single gene

Chemical or RNAi library

Wild type

TSG⁻/⁻
Lecture 1: Intro to importance of HR

Lecture 2: How HR works

Lecture 3: Why understanding matters: BRCA2 and HR

Lecture 4: Exploiting scientific understanding for engineering: BRCA2 targeted therapies

Lecture 5: Measuring HR in genotoxicity testing, using HR in genome engineering of mice

Lecture 6: Journal article discussion

Lecture 7: Statistics

Lecture 8: Flow Cytometry: How it works and how to do it
Mod 1: DNA Engineering

Engineering in vitro recombination assay

Day 4
A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells
1. PCR
2. Purif.
3. Digest
4. Gel Purif.
5. Gel Anal.
6. Plan Lig.
7. Ligate
8. Transform

Steps:
1. PCR
2. Purification
3. Digestion
4. Gel Purification
5. Gel Analysis
6. Plan and Ligate
7. Transform into E. coli
Ligase from T4 bacteriophage is used in recombinant DNA technology
Your ligation reaction

- Ligase
- ATP
- Buffer
  - Water, salts, buffer system
  - DTT

Ligase has NO activity w/o DTT
DTT is unstable
Make single use aliquots
Vortex to suspend
SMELL

- Vector
- Insert

How much?
In what ratio?
Your ligation reaction

- Vector
- Insert

How much?
In what ratio?

Reaction conditions

Optimal temperature for T4 ligase is 25°C
Your ligation reaction: possible outcomes

Population of different products

Need to separate and amplify individual products to analyze and select correct one
Transforming bacteria with ligation reaction

- **MIX**
- **SHOCK**
- **PLASMID TAKEN UP**
- **RECOVER**
- **SEPARATE**
- **SELECT**
- **AMPLIFY**

1. **SHOCK**
   - **PLASMID TAKEN UP**
   - **electric** (10-20 kV/cm)
   - **temperature** (42°C → ice)

2. **grow in rich media**

3. **separate by spreading**
Proceed to grow up individual colonies and analyze ligation products

SEPARATE
SELECT
AMPLIFY

GROW UP
INDIVIDUAL
COLONIES

ISOLATE
PLASMID DNA

ANALYZE