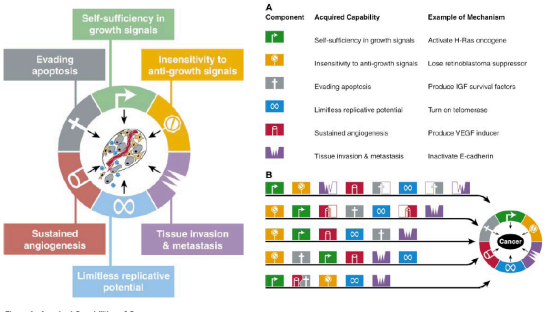
BRCA2, DNA Repair, & Cancer

K.D. Wittrup
20.109 ½ lecture

Hallmarks of Cancer

Hanahan & Weinberg, Cell 100:57'00



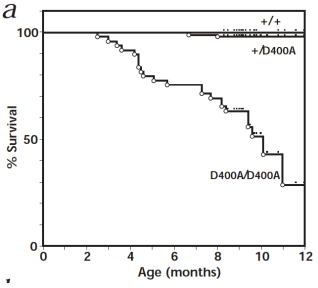
Sources of chromosomal error

- Mistakes in replication
  - Hence proliferation is mutagenic
- Spontaneous nucleotide reactions
  - Depurination & depyrimidination (>10^4/d/cell)
  - Deamination of 5-methylcytosine
- Reaction with mutagens
  - Exogenous
    - Chemical
    - Physical (UV, X-ray, γ-ray)
  - Endogenous – metabolic byproducts
    - Reactive oxygen species (ROS)

Defects in proofreading polymerase lead to early cancer deaths in mice

Defect in exonucleolytic proofreading activity of Pol-δ

12 thymic lymphomas,
2 diffuse lymphomas
2 sarcomas
6 tail-skin squamous-cell carcinomas
1 lung adenocarcinoma



Nat. Med 7:638, '01

Defective DNA repair is associated with cancer

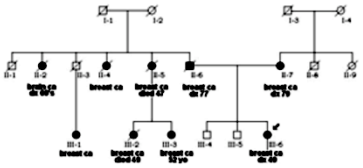
Name of syndrome	Name of gene	Cancer phenotype	Enzyme or process affected
HNPCC	(4–5 genes)[a]	colonic polyposis	mismatch repair enzymes
XP[b]	(8 genes)[b]	UV-induced skin cancers	nucleotide-excision repair
AT[c]	*ATM*	leukemia, lymphoma	response to dsDNA breaks
AT-like disorder[c]	*MRE11*	not yet determined	dsDNA repair by NHEJ
Familial breast, ovarian cancer	*BRCA1, BRCA2*[d]	breast and ovarian carcinomas	homology-directed repair of dsDNA breaks
Werner	*WRN*	several cancers	exonuclease and DNA helicase[e], replication
Bloom	*BLM*	solid tumors	DNA helicase, replication
Fanconi anemia	(9 genes)[f]	AML, HNSCC	repair of DNA cross-links and ds breaks
Nijmegen break[g]	*NBS*	mostly lymphomas	processing of dsDNA breaks, NHEJ
Li-Fraumeni	*TP53*	multiple cancers	DNA damage alarm protein
Li-Fraumeni	*CHK2*	colon, breast	kinase signaling DNA damage

The Biology of Cancer, R.A. Weinberg

Cloning BRCA2 – the history

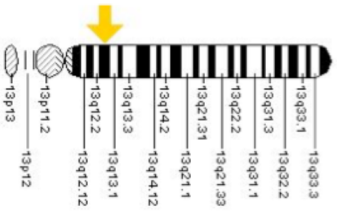
- Identified potential carriers of a gene for familial breast cancer
  - Early onset breast cancer (before menopause) in several relatives over different generations
  - Relatives with breast cancer in both breasts
  - Male relatives with breast cancer

Create pedigrees



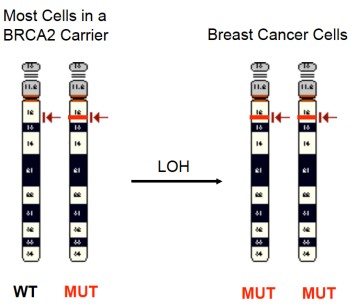
- Obtain DNA samples

Trace a common chromosomal locus



- From physical location, identify sequences
- A bit medieval – this can now be done via whole-genome sequencing

Loss of heterozygosity in cancer in BRCA2 carriers



Defects in BRCA1 lead to high breast and ovarian cancer incidence

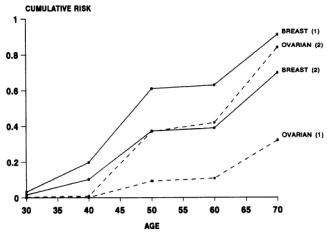


Figure 1 Cumulative risks of breast and ovarian cancer in BRCA1 gene carriers, allowing for allelic heterogeneity with two susceptibility alleles (1) and (2).

Am J. Hum. Genet. 56:265,'95

BRCA2 cloning

Identification of the breast cancer susceptibility gene *BRCA2*

Richard Wooster*, Graham Bignell*, Jonathan Lancaster†, Sally Swift†, Sheila Seal*, Jonathan Mangion*, Nadine Collins*, Simon Gregory‡, Curtis Gumbs‖, Gos Micklem§, Rita Barfoot*, Rifat Hamoudi¶, Sandeep Patel¶, Catherine Rice‡, Patrick Biggs*, Yasmin Hashim*, Amanda Smith‡, Frances Connor†, Adalgeir Arason#, Julius Gudmundsson#, David Ficenec*, David Kelsell††, Deborah Ford‡, Patricia Tonin**, D. Timothy Bishop††, Nigel K. Spurr‡‡, Bruce A. J. Ponder‡‡, Rosalind Eeles*, Julian Peto‡, Peter Devilee§§, Cees Cornelisse§§, Henry Lynch‖‖, Steven Narod**, Gilbert Lenoir*¶, Valdgardur Egilsson#, Rosa Bjork Barkadottir#, Douglas F. Easton‡‡, David R. Bentley‡, P. Andrew Futreal‖, Alan Ashworth† & Michael R. Stratton***

disease. The breast cancer susceptibility gene, *BRCA2*, was recently localized to chromosome 13q12-q13. Here we report the identification of a gene in which we have detected six different germline mutations in breast cancer families that are likely to be due to *BRCA2*. Each mutation causes serious disruption to the open reading frame of the transcriptional unit. The results indicate that this is the *BRCA2* gene.

sequence. The known sequence of 2,329 amino acids encoded by the *BRCA2* gene does not show strong homology to sequences in the publicly available DNA or protein databases, and therefore we have no clues to its functions. However, some weak matches

This part still often happens!

Nature 378:789, '95

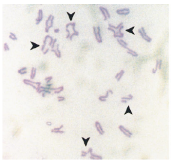
Common tactic: break it and see what happens

Molecular Cell, Vol. 1, 347–357, February, 1998, Copyright ©1998 by Cell Press

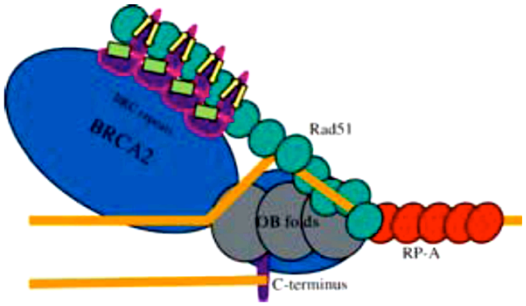
Involvement of Brca2 in DNA Repair

Ketan J. Patel,[1,4] Veronica P. C. C. Yu,[1,4] Hyunsook Lee,[1] Anne Corcoran,[1] Fiona C. Thistlethwaite,[2,7] Martin J. Evans,[2] William H. Colledge,[3] Lori S. Friedman[4,7] Bruce A. J. Ponder,[4] and Ashok R. Venkitaraman[1,5]

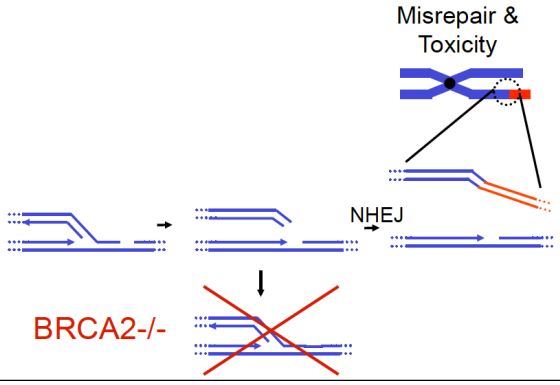
Abnormalities precipitated by a targeted truncation in the murine gene *Brca2* define its involvement in DNA repair. In culture, cells harboring truncated *Brca2* exhibit a proliferative impediment that worsens with successive passages. Arrest in the G1 and G2/M phases is accompanied by elevated p53 and p21 expression. Increased sensitivity to genotoxic agents, particularly ultraviolet light and methylmethanesulfonate, shows that Brca2 function is essential for the ability to survive DNA damage. But checkpoint activation and apoptotic mechanisms are largely unaffected, thereby implicating Brca2 in repair. This is substantiated by the spontaneous accumulation of chromosomal abnormalities, including breaks and aberrant chromatid exchanges. These findings define a function of Brca2 in DNA repair, whose loss precipitates replicative failure, mutagen sensitivity, and genetic instability reminiscent of Bloom syndrome and Fanconi anemia.



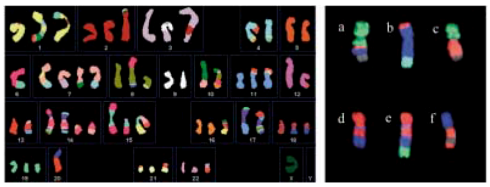
BRCA2 Loads Rad51 to create a nucleoprotein filament



BRCA2 is critical for repair of broken forks



BRCA2- tumor cell lines have aberrant chromosomes



BRCA2-mutant cell line CAPAN-1
Cytogenet. Gen. Res. 104:333, '04