Germline risk and early intervention in cancer

Ashok Venkitaraman

Zoellner Professor of Cancer Research & Director,
Medical Research Council Cancer Unit

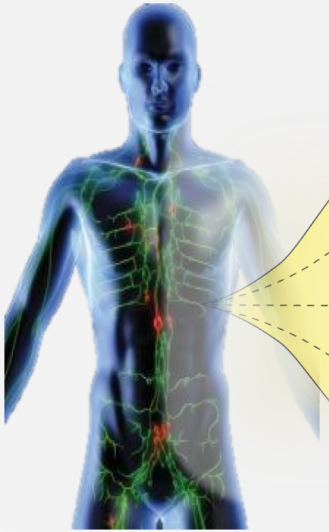
MRC Cancer Unit

UNIVERSITY OF CAMBRIDGE

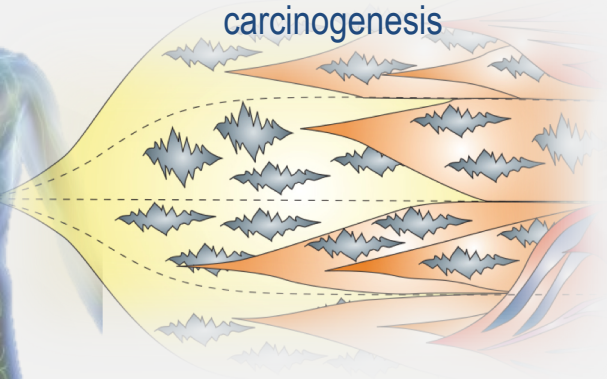
Intervention & interception in cancer

- **Which individuals are most susceptible to cancer?**
- **Which early lesions are most likely to progress?**
- **Can progression be delayed or prevented?**
- **Can progressive lesions be eliminated?**

Genetic & environmental risk

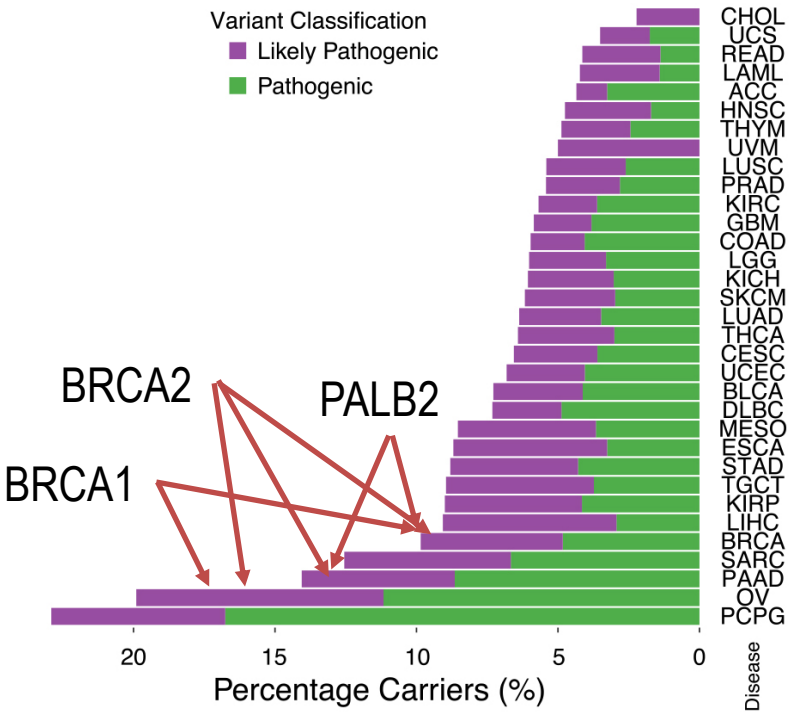


Molecular & cellular events during early steps in carcinogenesis



Enabling technologies

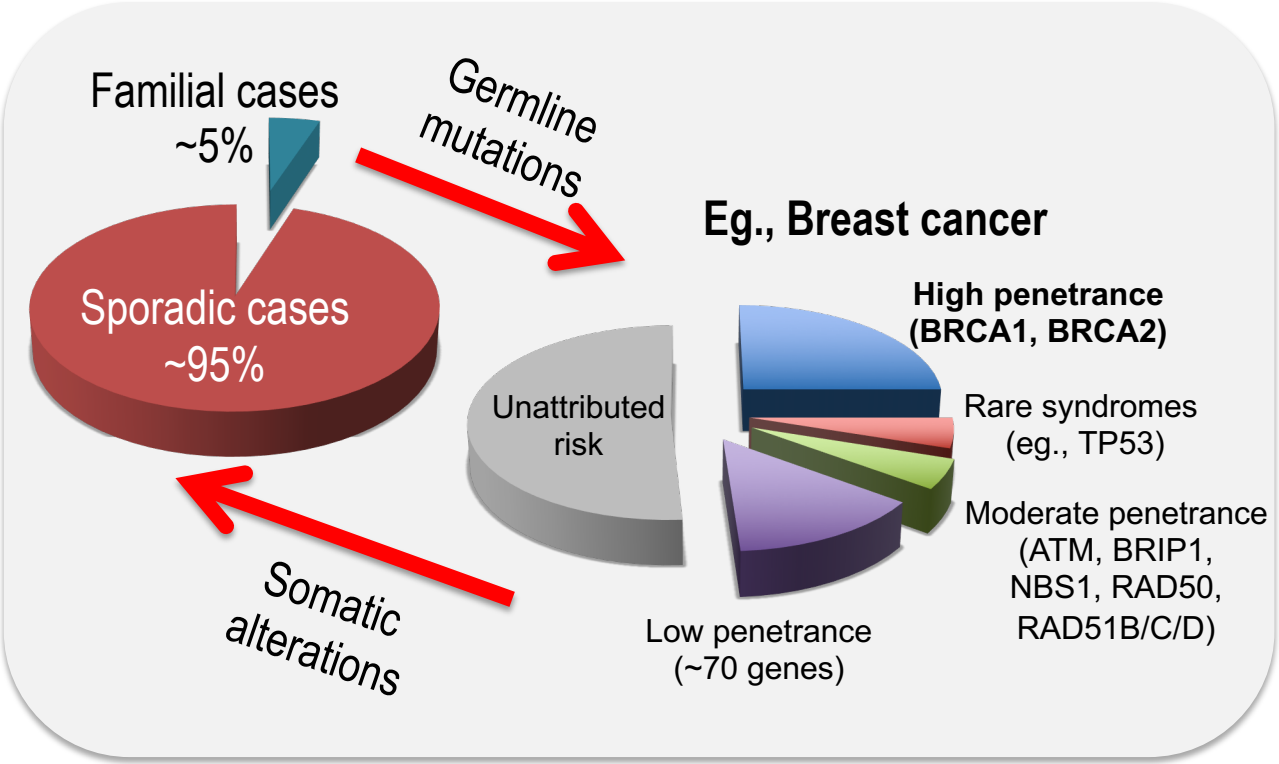
Germline variants in cancer susceptibility genes underlie a significant fraction of human cancer



Huang et al *Cell* (2018)

- Pathogenic or likely pathogenic germline variants in cancer susceptibility genes occur in 8% of 10,389 cancers of 33 different types
- Such variants occur at a relatively high frequency in certain cancer types (eg., ovarian cancers ~20%; pancreatic cancers ~15%)
- Variants affecting a small cluster of genes (*BRCA2*, *PALB2*, *ATM*, *BRCA1*) are relatively common in several cancer types

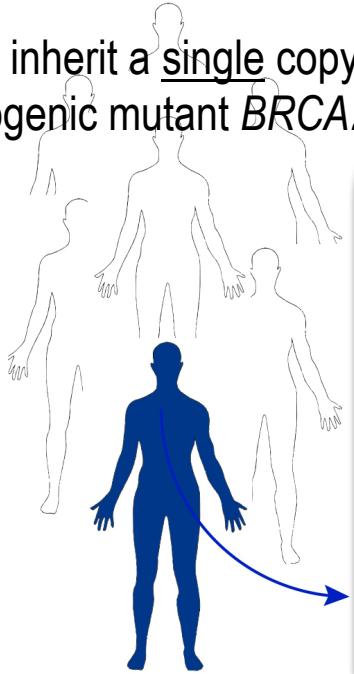
Germline mutations affecting genome stability increase early-onset cancer risk



- Germline *BRCA2* mutations affecting a single allele predispose to breast, ovarian, pancreatic, prostatic and other cancers
- Carriers exhibit a cumulative risk of ~70% for breast cancer, and ~25% for ovarian cancer, by age 80 yrs. (ie., ***highly penetrant***)
- Cancer risk increases rapidly until ~45 yrs. (ie., ***early onset***)

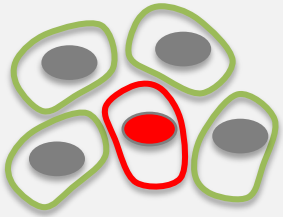
Carcinogenesis in *BRCA2* mutation carriers

Carriers inherit a single copy of pathogenic mutant *BRCA2*



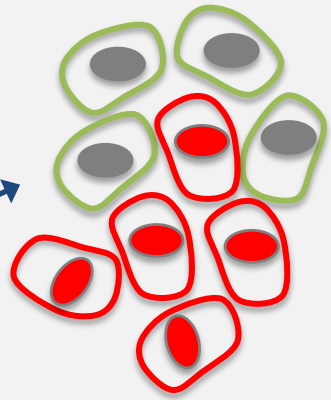
Knudson "two hit" paradigm

The second *BRCA2* copy is lost in certain cells



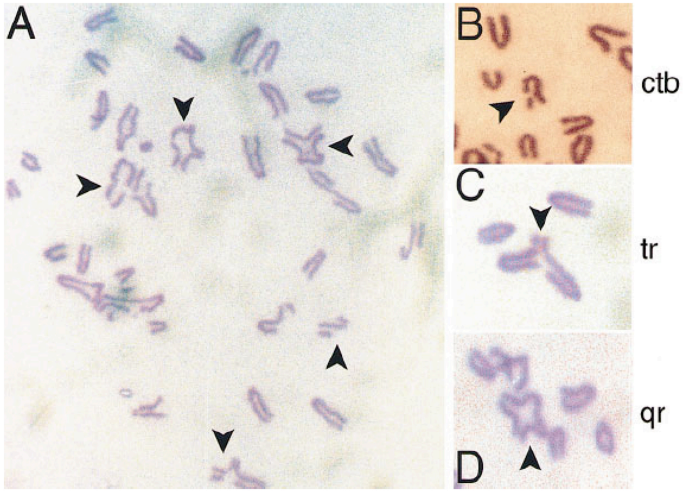
"LOH"

BRCA2 inactivation leads to cancer



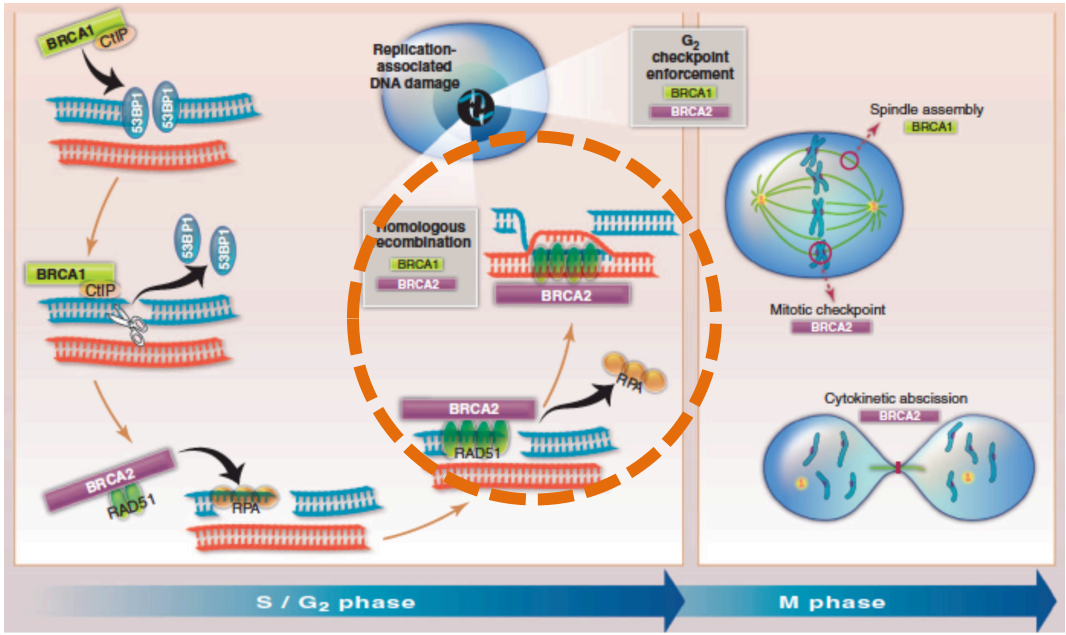
Loss of the second *BRCA2* copy (LOH) is believed to be essential for carcinogenesis

BRCA2 inactivation by LOH causes chromosomal instability…..



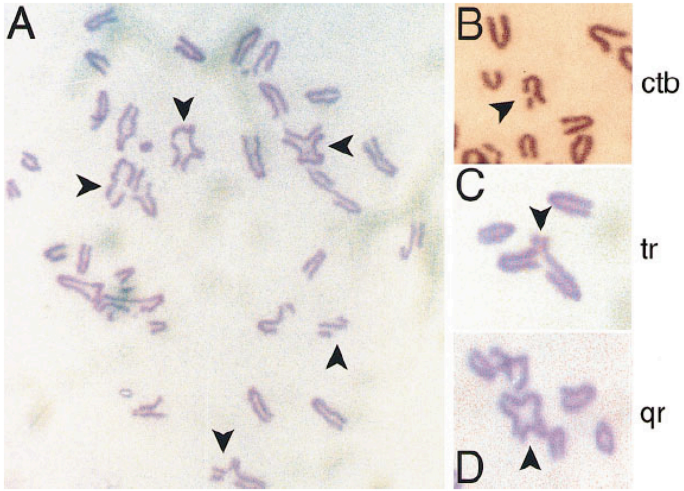
Patel, Yu et al. *Mol Cell* (1998)

…..by disrupting DNA repair & replication



Venkitaraman *Science* (2014)

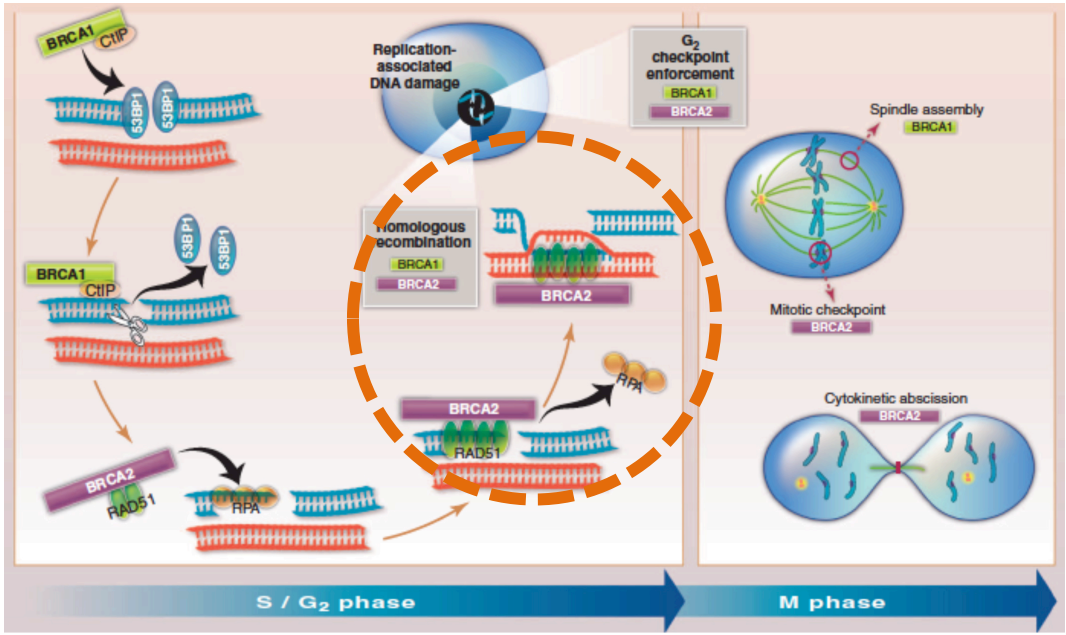
BRCA2 inactivation by LOH causes chromosomal instability…..



Patel, Yu et al. *Mol Cell* (1998)

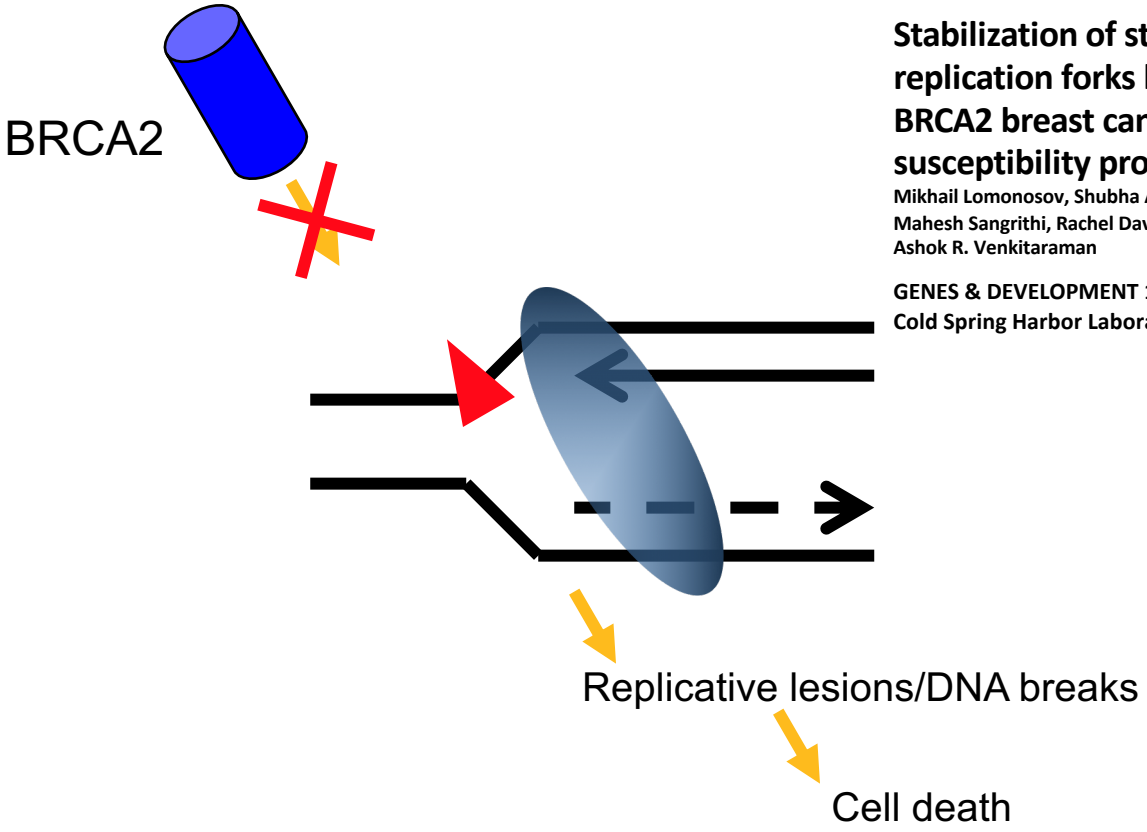
What is the source of the DNA damage that drives spontaneous chromosomal instability?

…..by disrupting DNA repair & replication



Venkitaraman *Science* (2014)

DNA replication as a source of spontaneous DNA damage

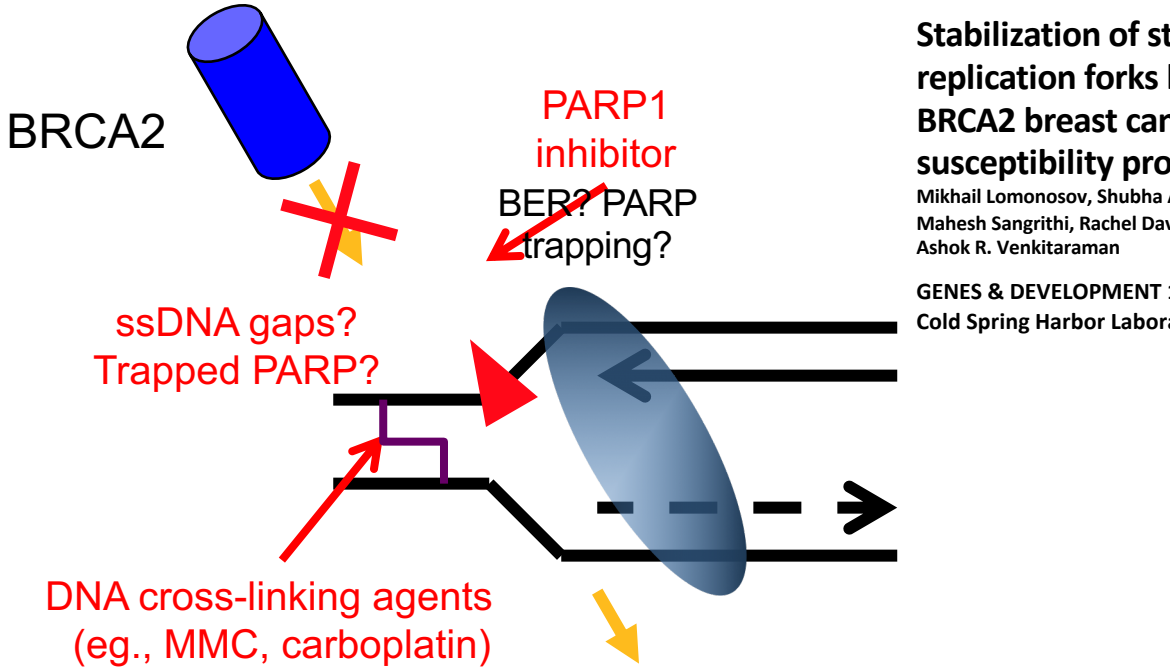


Stabilization of stalled DNA replication forks by the BRCA2 breast cancer susceptibility protein

Mikhail Lomonosov, Shubha Anand, Mahesh Sangrithi, Rachel Davies, and Ashok R. Venkitaraman

GENES & DEVELOPMENT 17:3017–3022 © 2003 by Cold Spring Harbor Laboratory Press

Homozygous but not heterozygous *BRCA2* inactivation sensitizes cells to agents that stall DNA replication



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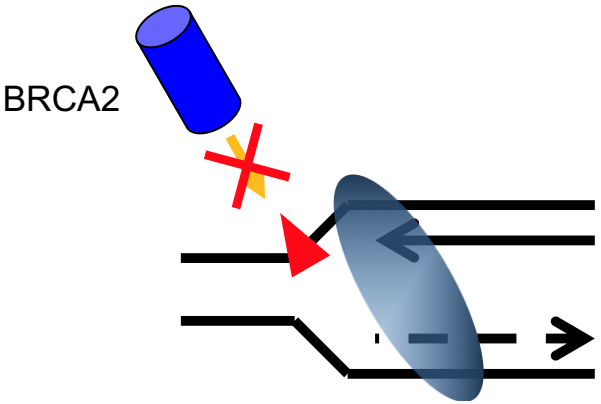
Yu et al., *Genes Dev* 2000; Lomonosov et al., *Genes Dev* 2003

Bryant et al., 2005, Farmer et al., 2005

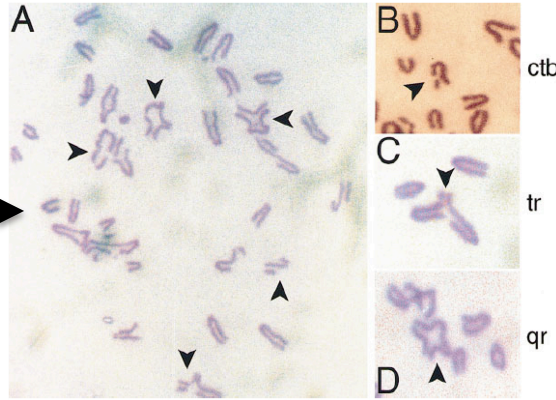
How does bi-allelic *BRCA2* inactivation by LOH cause cancer susceptibility?



Replication stalling

DNA breakage

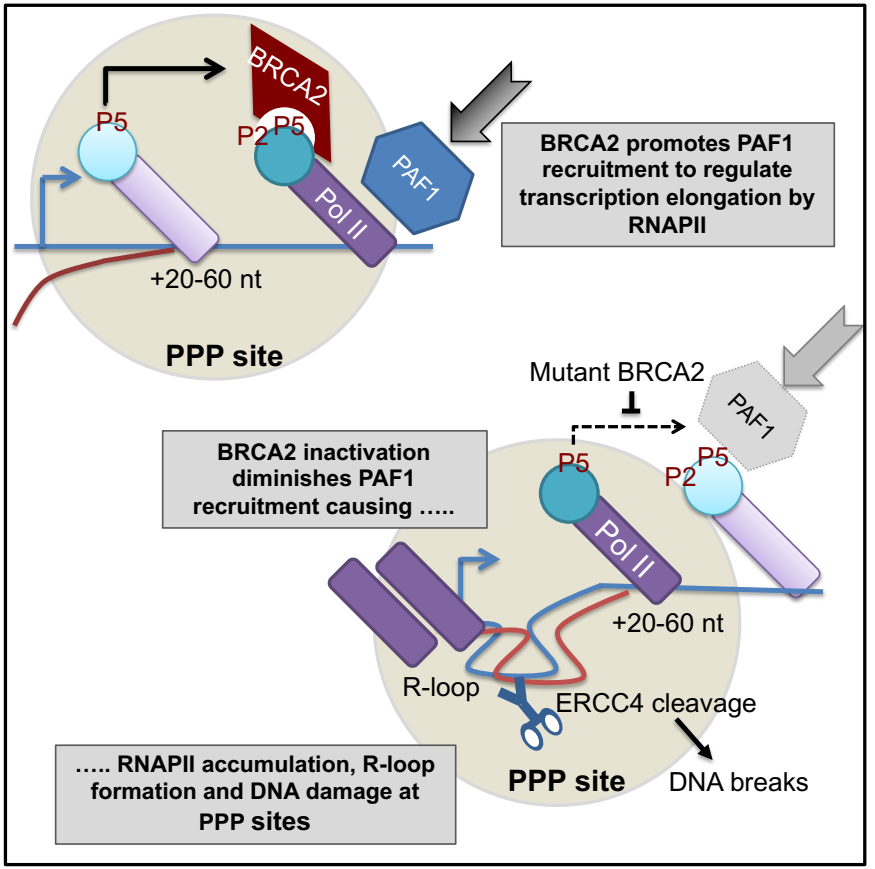
Defective HDR



Genome instability

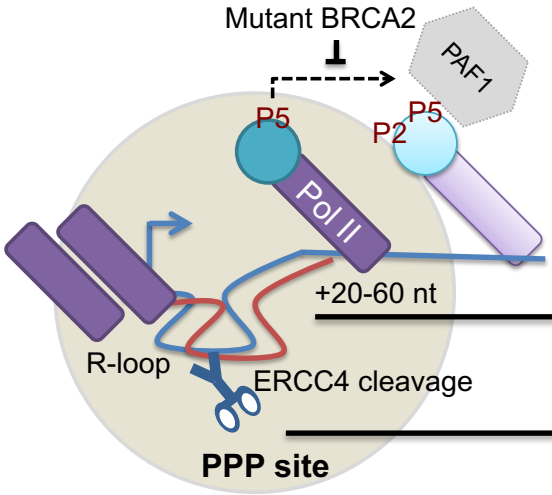
BRCA2 regulates transcription elongation by RNA polymerase II to prevent R-loop accumulation & DNA breakage

- BRCA2 interacts with RNA polymerase II (RNAPII)
- This interaction promotes recruitment of PAF1 to RNAPII, allowing the polymerase to "switch" from promoter-proximal pausing to elongation
- *BRCA2* inactivation inhibits this PAF1-mediated switch, triggering unscheduled RNA-DNA hybrids (R-loops) at promoter-proximal pausing sites
- ERCC4/5 endonuclease cleaves unscheduled R-loops into DNA breaks



Mahmud Shivji, Xavier Renaudin, Cigdem Williams

Are unscheduled R-loops an ”endogenous mutagen” driving genome instability in *BRCA2*-deficient cells?

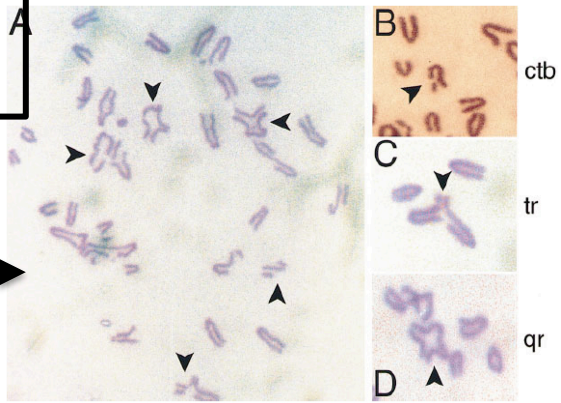


R-loop digestion with RNAse H1 *ameliorates chromosomal aberrations* in *BRCA2*-deficient cells
Tan et al., *Cell* (2017)

Replication stress

DNA breaks

Defective HDR

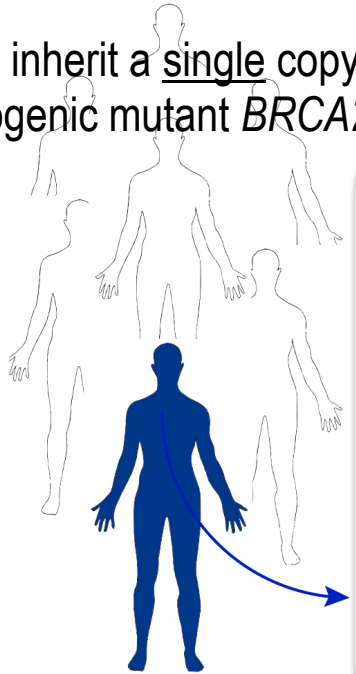


Shivji, Renaudin et al., *Cell Rep* (2018)

Remarkably, Lurbinectedin – a modulator of R-loop mediated cytotoxicity - shows strong activity in BRCA2-deficient cancers (ORR ~60% in a Phase II trial)
(Cruz et al., *J Clin Oncol* (2018))

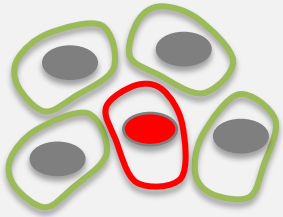
Carcinogenesis in *BRCA2* mutation carriers

Carriers inherit a single copy of pathogenic mutant *BRCA2*



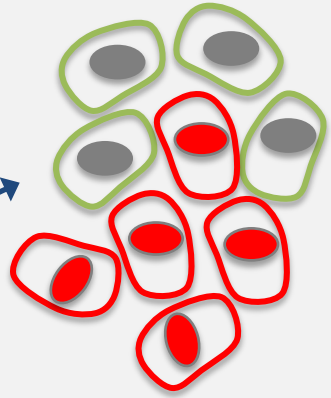
Knudson "two hit" paradigm

The second *BRCA2* copy is lost in certain cells



"LOH"

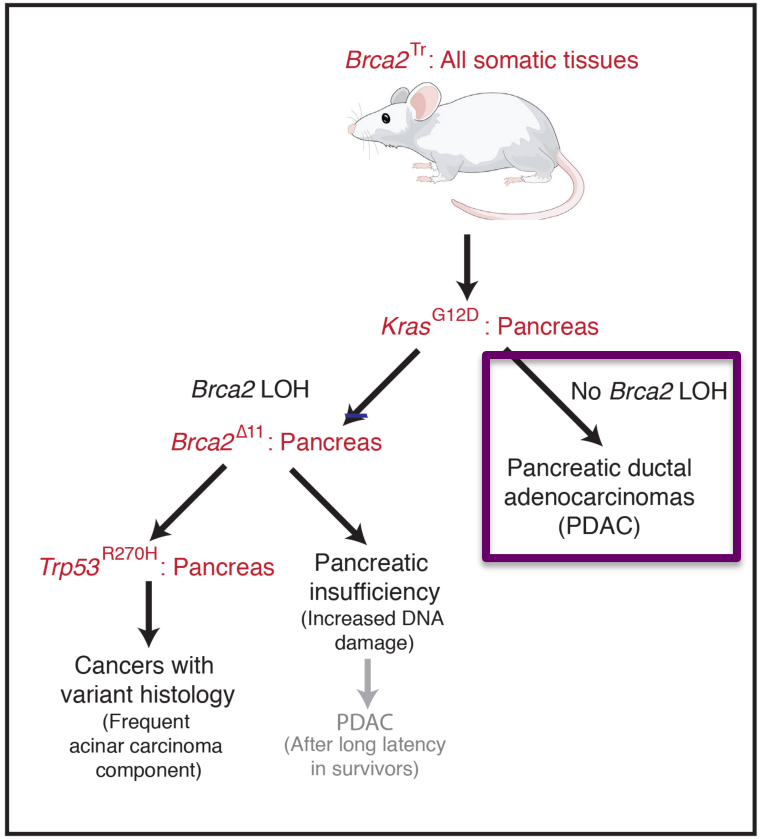
BRCA2 inactivation vitiates HDR & induces replicative DNA damage

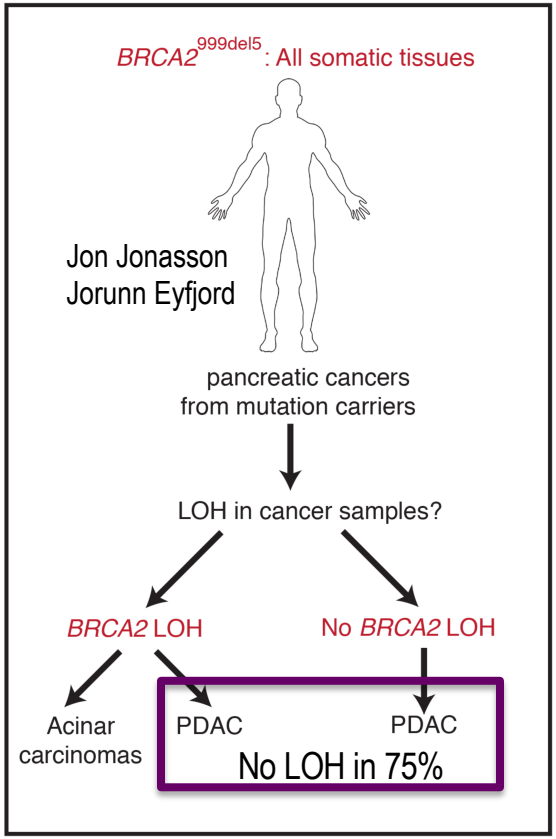


Loss of the second *BRCA2* copy (LOH) provokes genome instability & cancer

LOH confers the sensitivity of these cancers to targeted agents like carboplatin or PARP1i

BRCA2 can violate the Knudson "two-hit" paradigm for tumour suppression

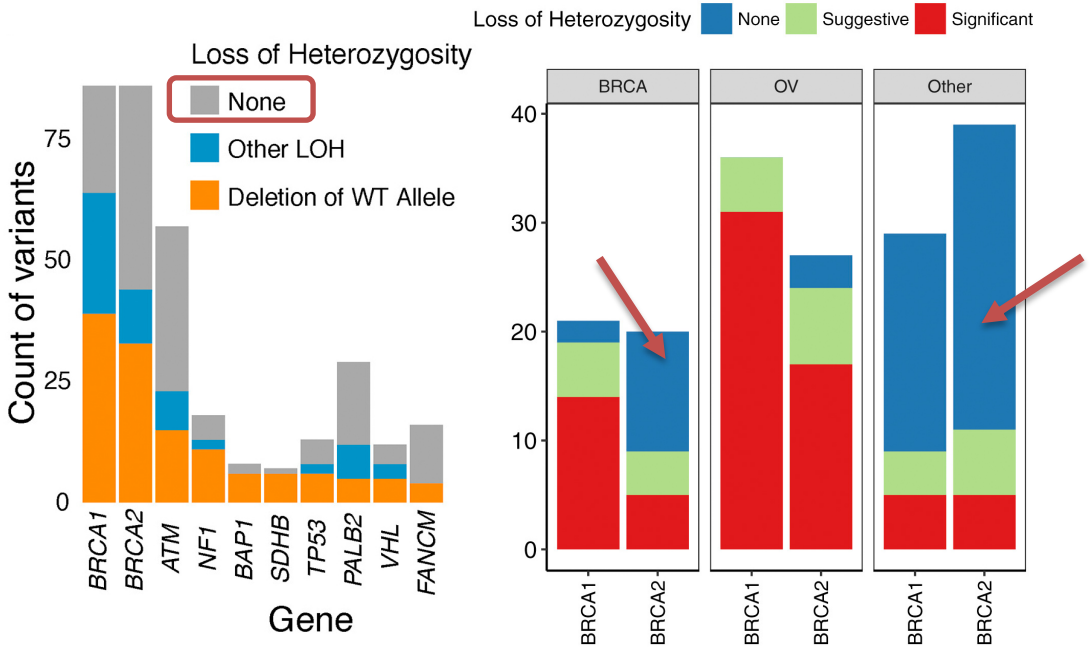




- Heterozygosity for *BRCA2* truncations suffices for carcinogenesis in mice and men
- Cancers in which the second allele persists exhibit primary resistance to targeted therapies like carboplatin or PARP1i

Skoulidis et al., *Cancer Cell* (2010)

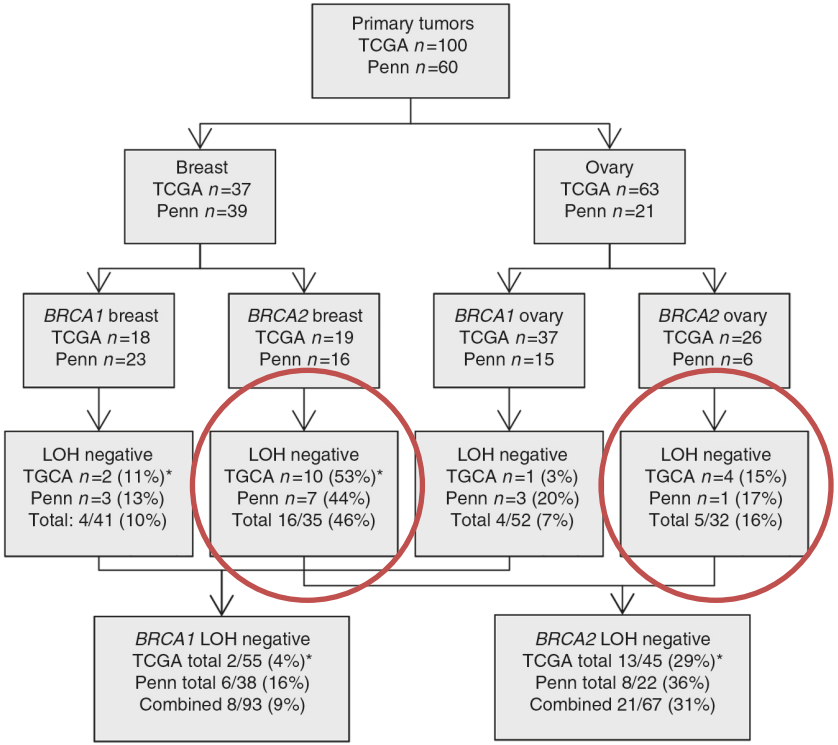
BRCA2 can violate the Knudson "two-hit" paradigm for tumour suppression



- Over half of cancers bearing a pathogenic *BRCA2* mutation retain the wild-type allele (ie., there is no LOH)
- Absence of *BRCA2* LOH is different in cancers arising in different tissues (eg., breast >> ovary)
- Varying fractions of cancers mutant in *BRCA1*, *ATM* and related genes also fail to undergo LOH in different tissues

Huang et al *Cell* (2018)

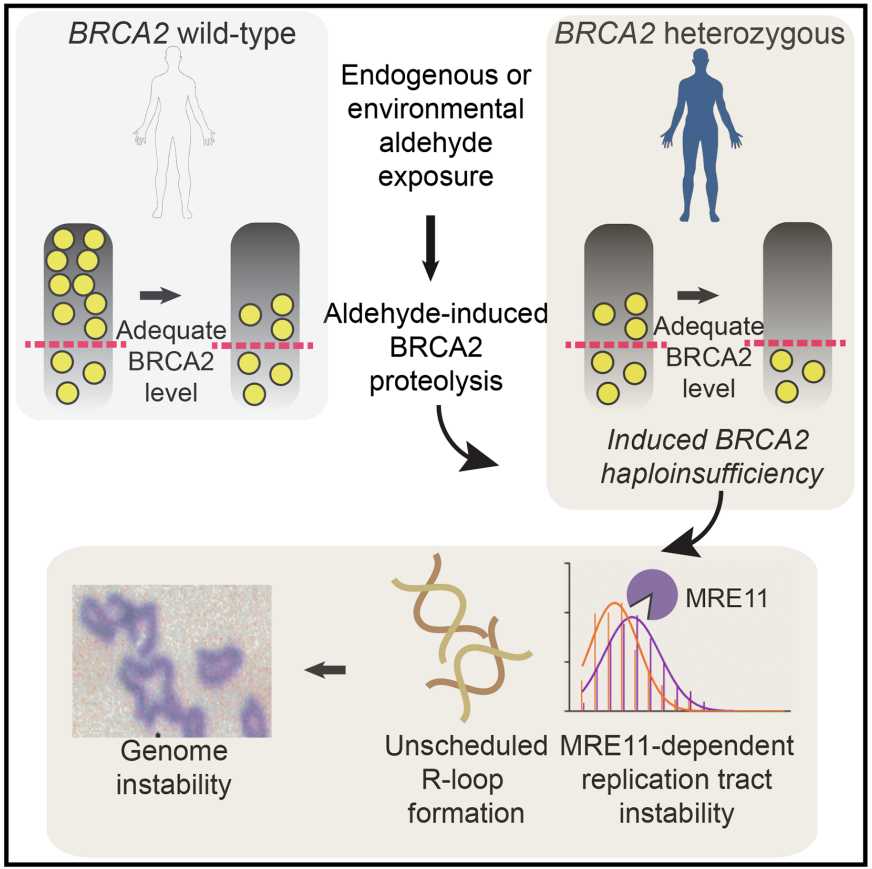
BRCA2 can violate the Knudson "two-hit" paradigm for tumour suppression



- As many as 46% of breast cancers and 17% of ovarian cancers arising in *BRCA2* mutation carriers retain the wild-type allele (ie., there is no LOH)
- These cancers are expected to be insensitive to targeted therapies like carboplatin or PARP1i
- NGS analyses of the tumours suggests the absence of genomic signatures typical of defects in homologous recombination

Susan Domchek, Kate Nathanson *Nature Comm* (2017)

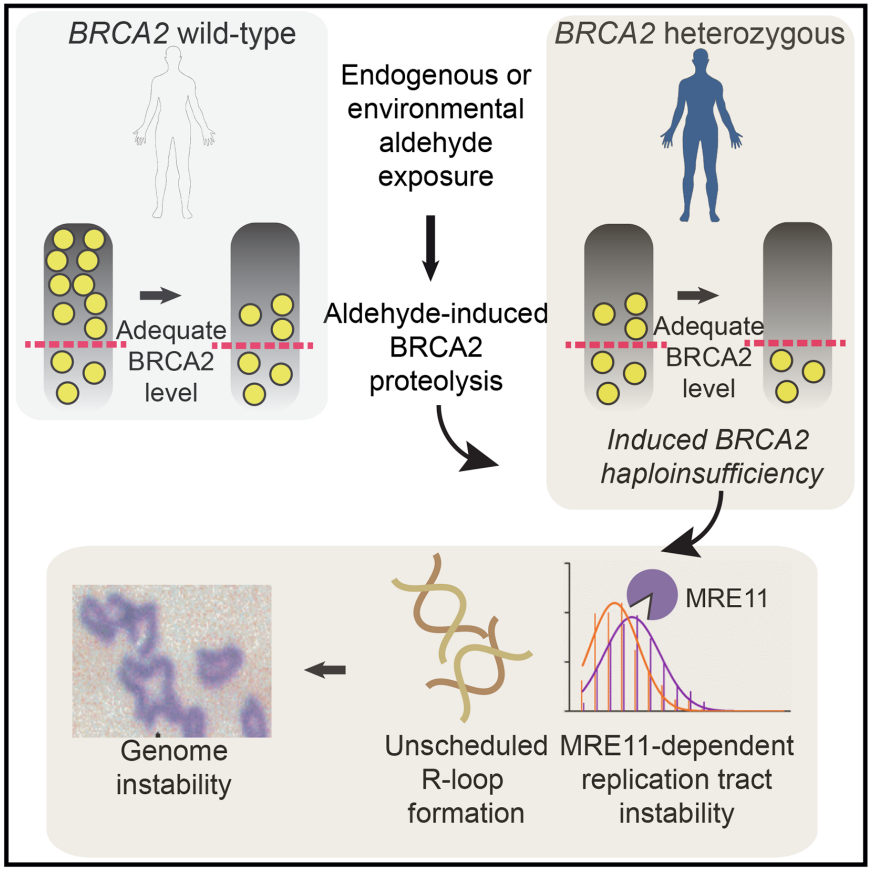
“Induced haploinsufficiency” as a mechanism underlying cancer predisposition



- Aldehydes selectively deplete BRCA2 protein by a proteasome-dependent, ubiquitin-independent pathway
- Aldehyde exposure sensitises cells from carriers of heterozygous *BRCA2* truncating mutations to genome instability via “induced haploinsufficiency”
- Does *exogenous* aldehyde exposure modify carcinogenesis in *BRCA2* mutation carriers?
- Do differences in *endogenous* aldehyde accumulation in different cell types help explain the tissue-specificity of carcinogenesis in *BRCA2* mutation carriers?

Tan et al., *Cell* (2017)

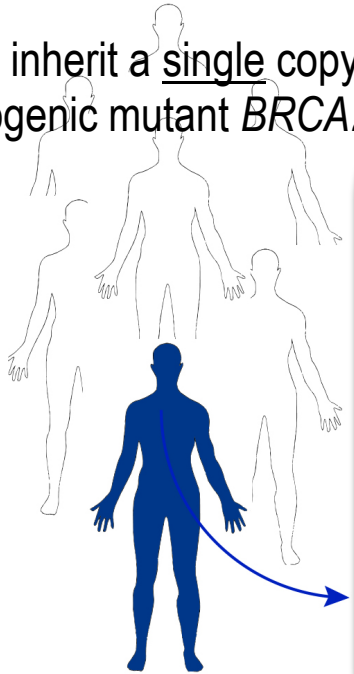
“Induced haploinsufficiency” as a mechanism underlying cancer predisposition



Tan et al., *Cell* (2017)

Carcinogenesis in *BRCA2* mutation carriers without LOH

Carriers inherit a single copy of pathogenic mutant *BRCA2*



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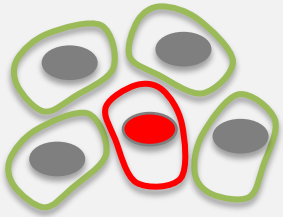
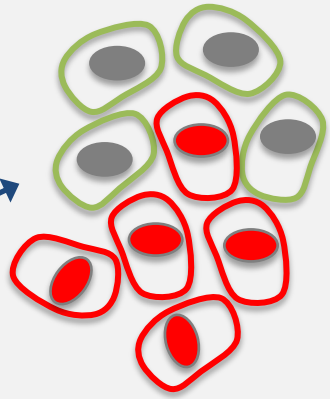


"LOH"

BRCA2 LOH not necessary for cancer



- Truncating BRCA2 mutations can predispose to cancer without LOH
- These cancers retain the capacity for HR and may exhibit **primary resistance** to PARP1i
- Allele-specific mechanisms may need to be incorporated into risk stratification and management strategies

The Medical Research Council Cancer Unit

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The mission of the MRC Cancer Unit is to discover the fundamental mechanisms underlying early steps in carcinogenesis, and to exploit this knowledge for early intervention in the clinic, using innovative enabling technologies.