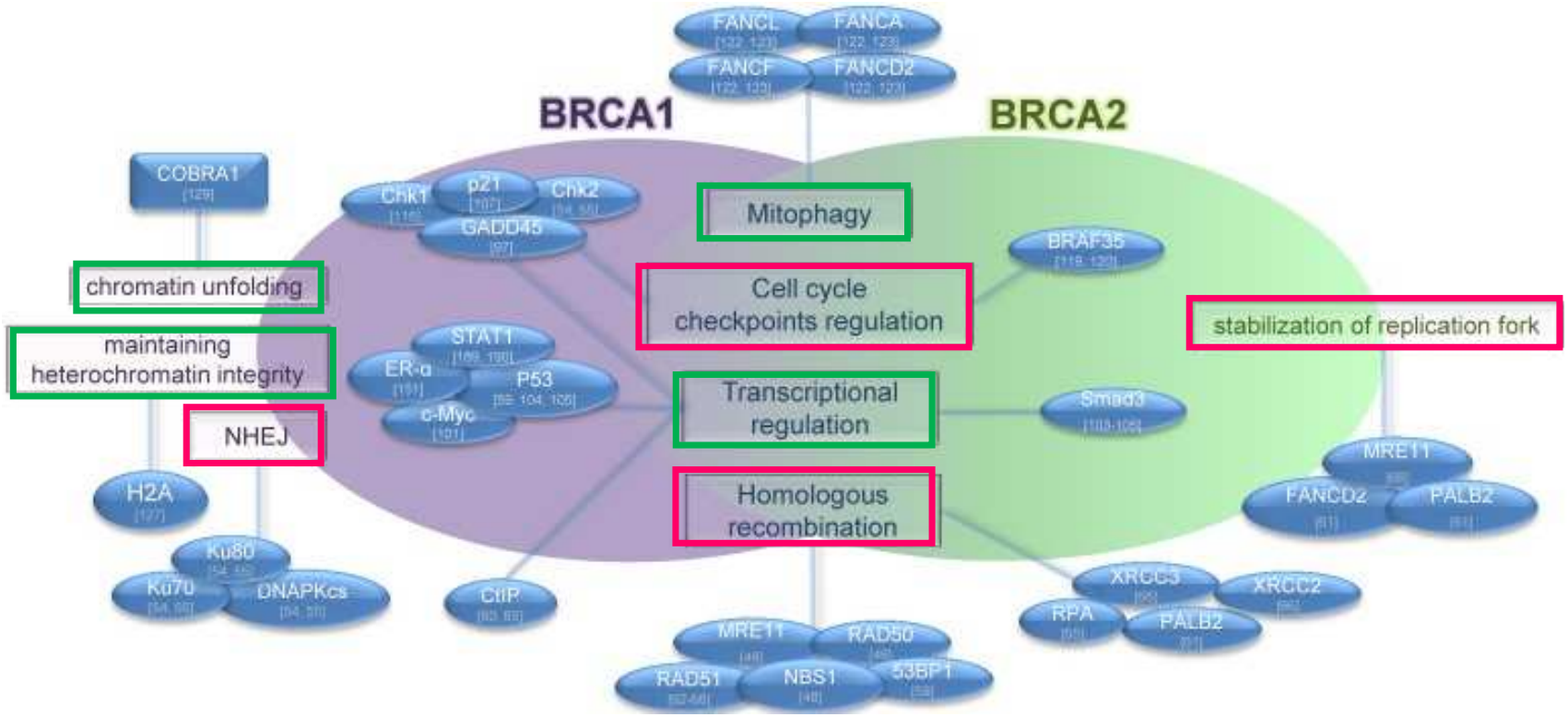
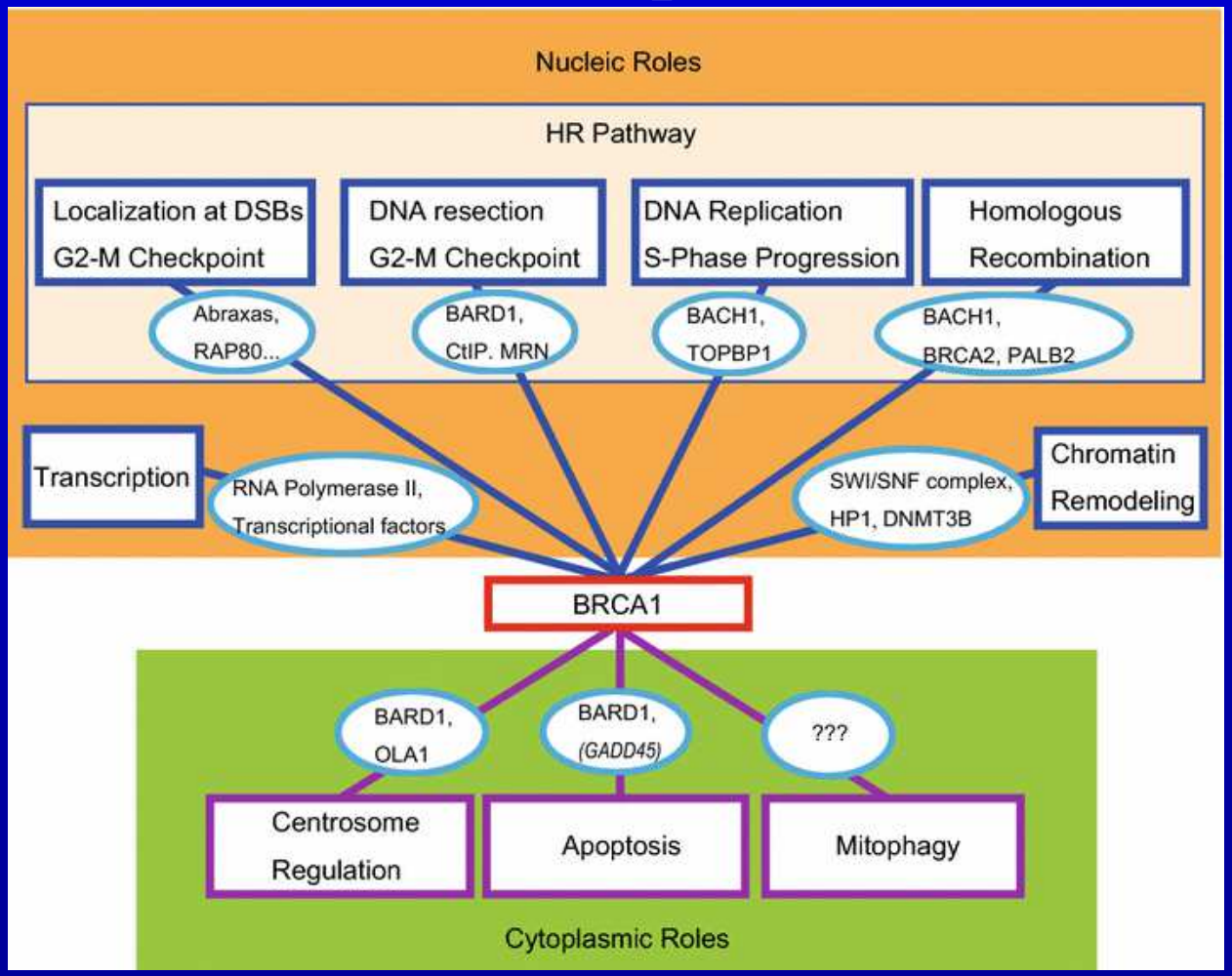
Functional features of BRCA proteins



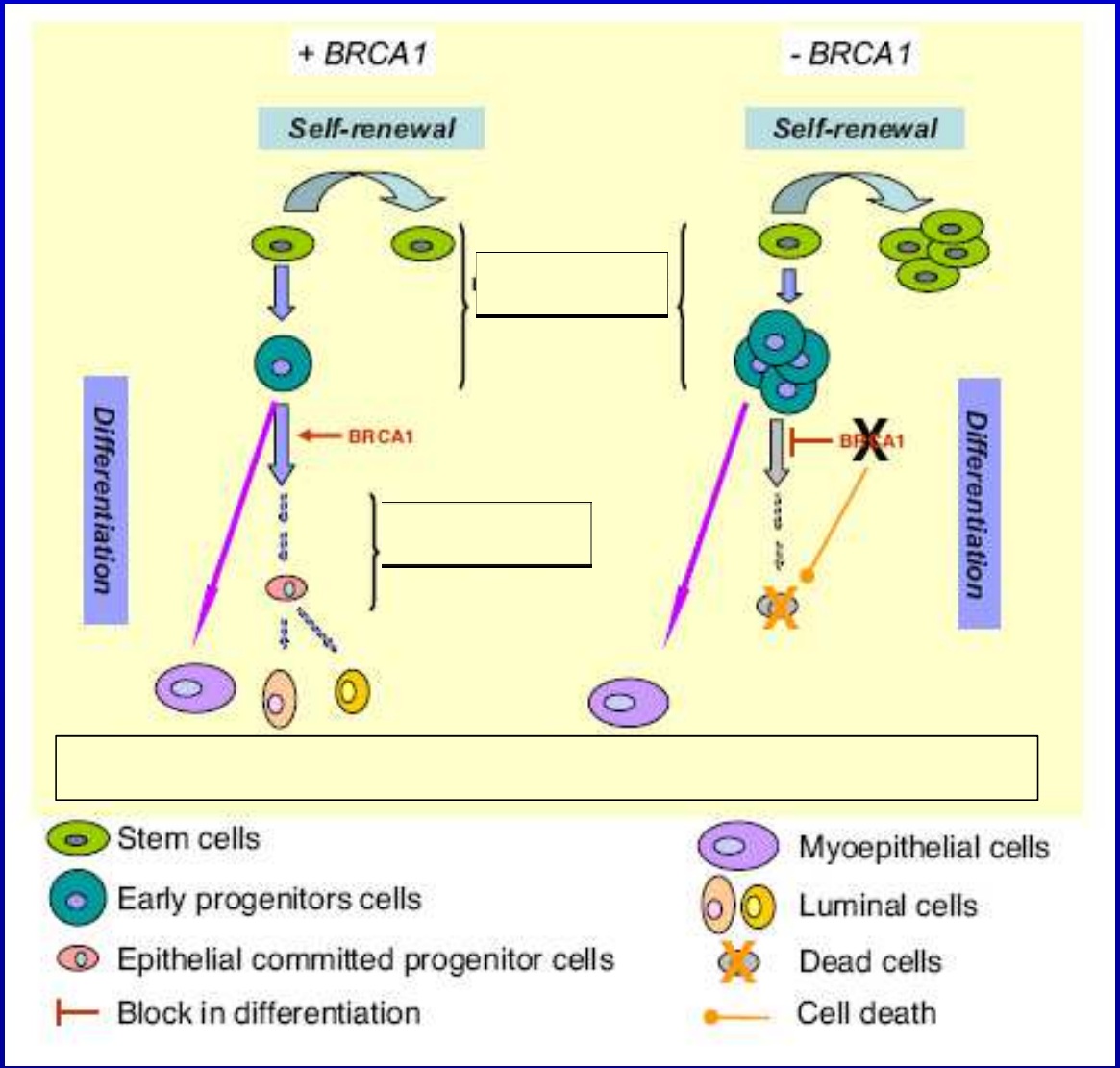
Multifunctional protein BRCA1



BRCA1

Differenziamento cellulare

BRCA1 regulates human mammary stem/progenitor cell fate



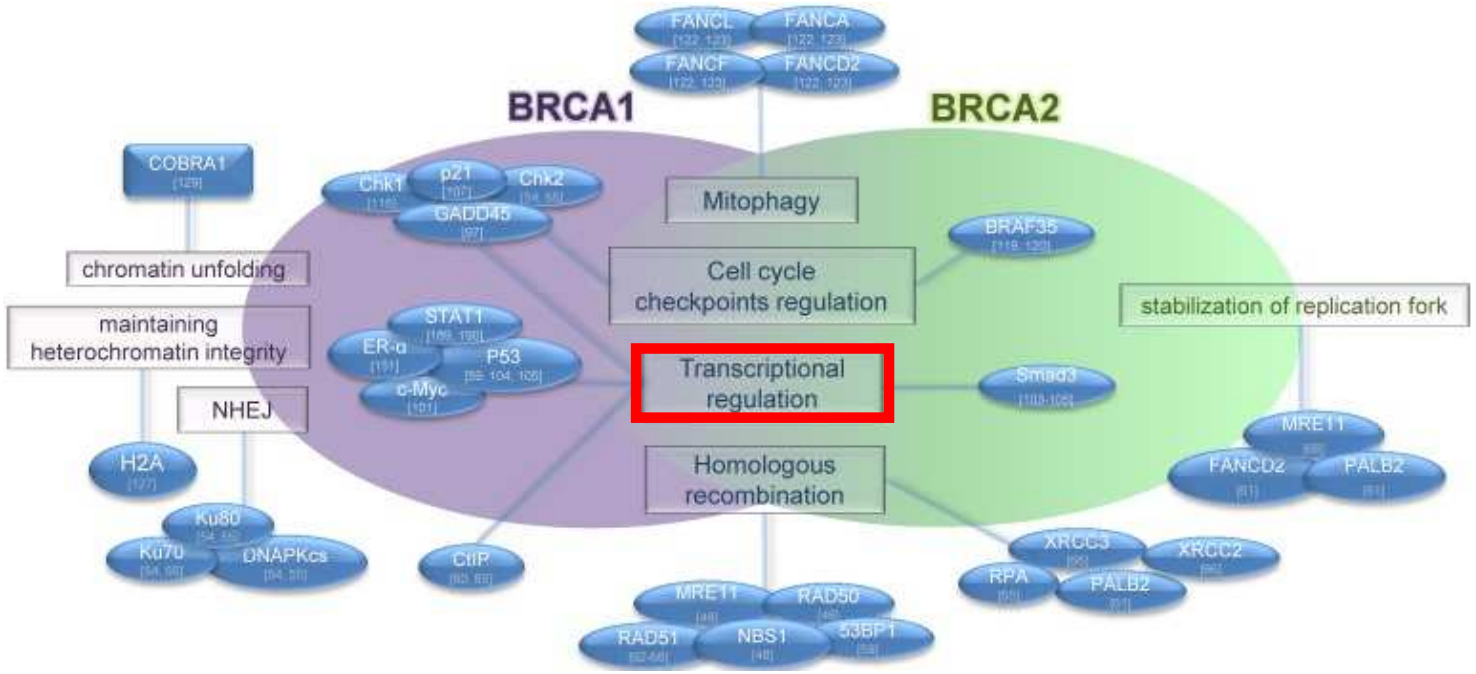
PNAS
February 5, 2008

BRCA1 is necessary for the maintenance of mammary epithelial cell differentiation

- Interstrand crosslink repair stabilizes mammary epithelial cell differentiation

Depleting BRCA1 caused aberrant differentiation

Functional features of BRCA proteins



myoepithelial cell differentiation in BRCA mutation carriers

2019

BRCA in cellular differentiation

- Transcription Factors play key roles in cellular differentiation.
- Transcription Factors are differentially expressed in WT and BRCA mutated cells

Among these Transcriprion Factors

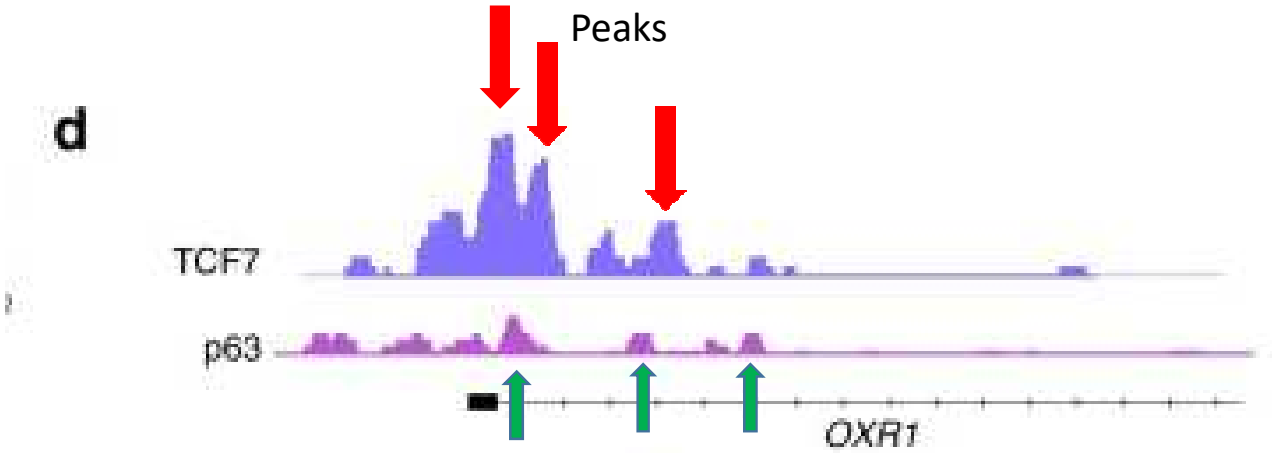
p63 plays key roles in epithelial progenitors

TCF7 deletion in mice leads to mammary gland adenomas

In BRCA1 mutation carriers the expression of p63 and TCF7 decreased

Chromatin Immuno Precipitation-seq (Method)

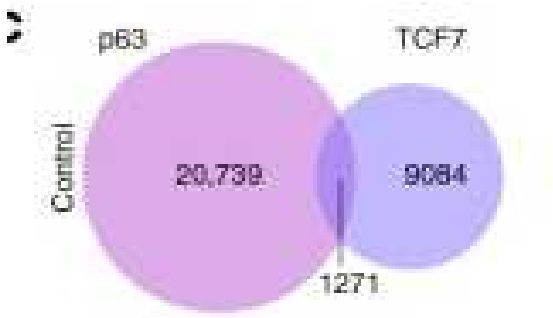
- genomic targets of p63 and TCF7



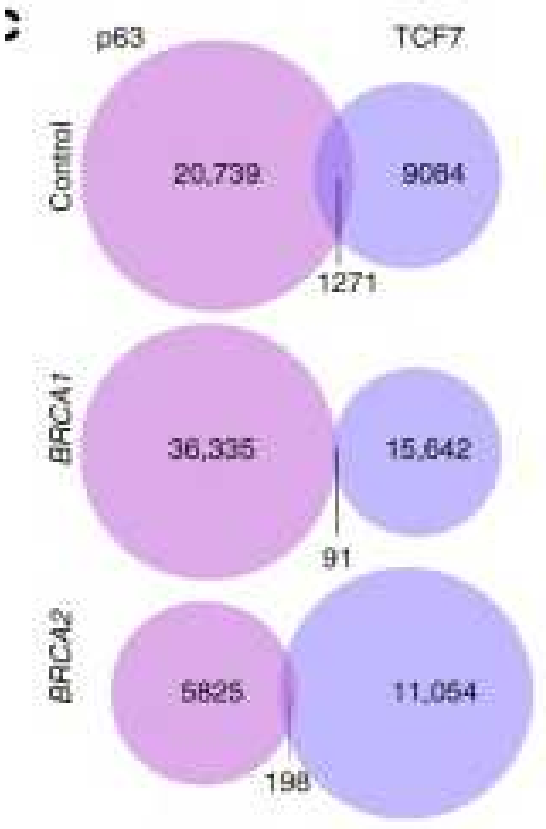
Gene tracks depicting p63 and TCF7 signal at genomic locus OXR1 (protection from oxidative stress

differences due to BRCA mutation in normal myoepithelial cells

Overlap between p63 peaks and TCF7 peaks in control women



Overlap between p63 peaks and TCF7 peaks in control women, BRCA1 and BRCA2 mutation carriers.



- we investigated the genomic targets of p63 and TCF7 and potential differences due to BRCA mutation status in normal myoepithelial cells by Chromatin Immuno Precipitation-seq.

- We identified significant differences in both p63 and TCF7 genomic binding between control and BRCA mutation carriers

- We detected significant overlap between p63 and TCF7 peaks only in non-carrier tissues and not in BRCA1/2 mutation carriers

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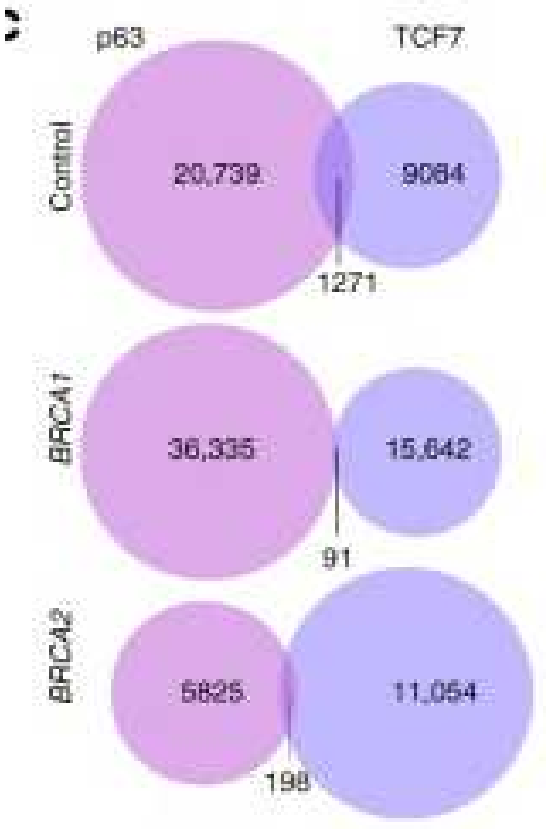
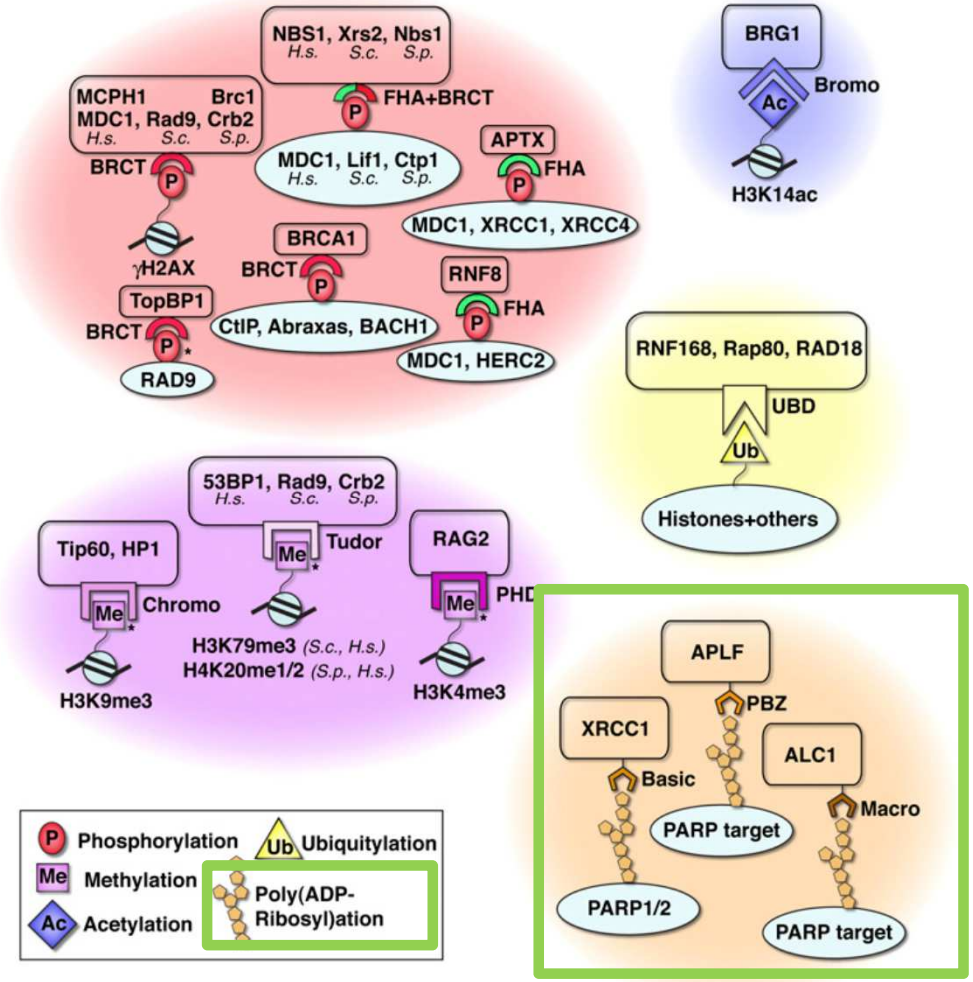


- These myoepithelial perturbations in normal breast tissues of BRCA1 germline mutation carriers may play a role in their higher risk of breast cancer

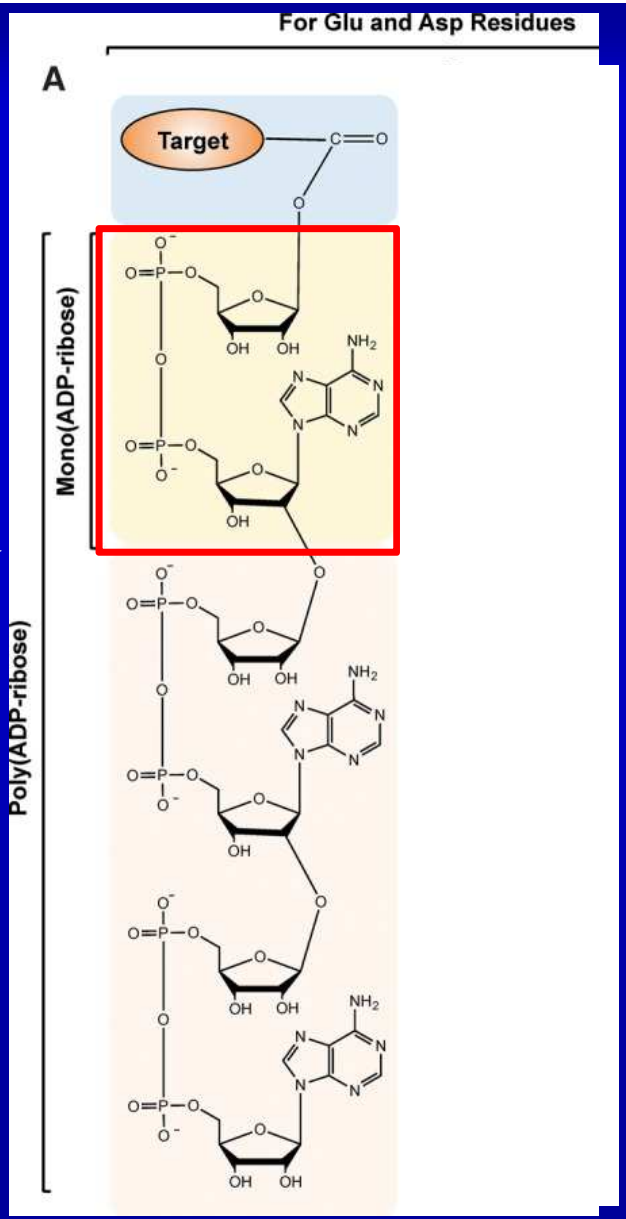
- The fraction of p63+TCF7+ myoepithelial cells is also significantly decreased in ductal carcinoma in situ , which may be associated with invasive progression

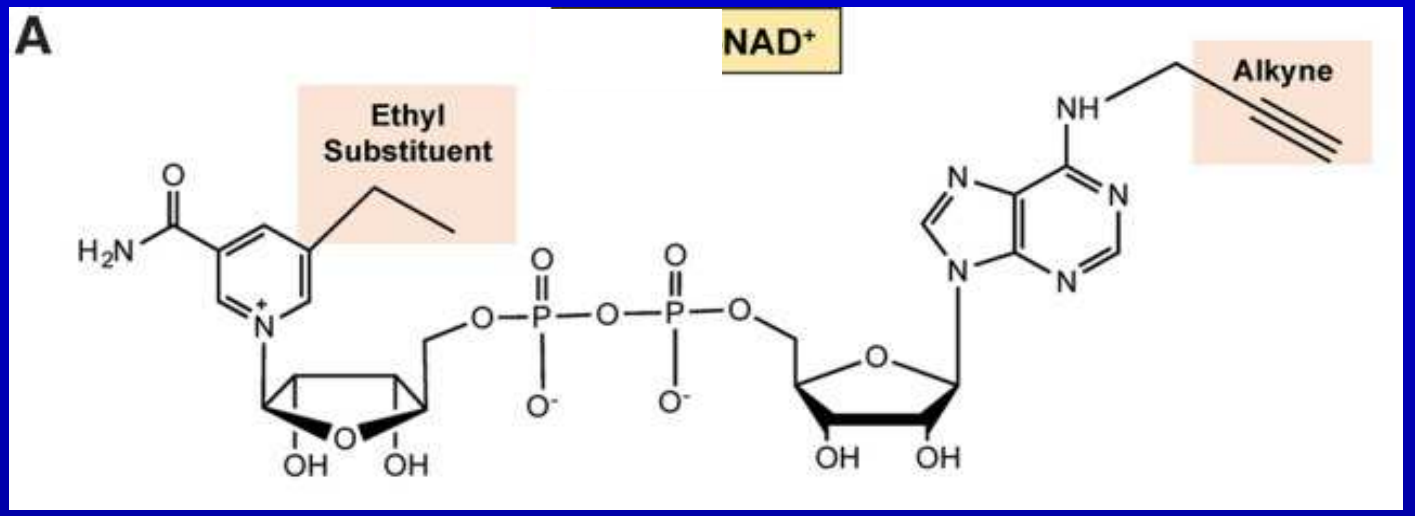
Post-translational modifications (PTMs) at DNA breaks:

Poly-ADP-Ribosylation



the negatively charged polymer of polyADP-ribose





PARPs use NAD+ as substrate to form the negatively charged polymer of poly(ADP-ribose) (PAR)

PolyADP-ribosylation

- PolyADP-ribosylation of proteins is a posttranslational modification mediated by **poly(ADP-ribose) polymerases PARPs**

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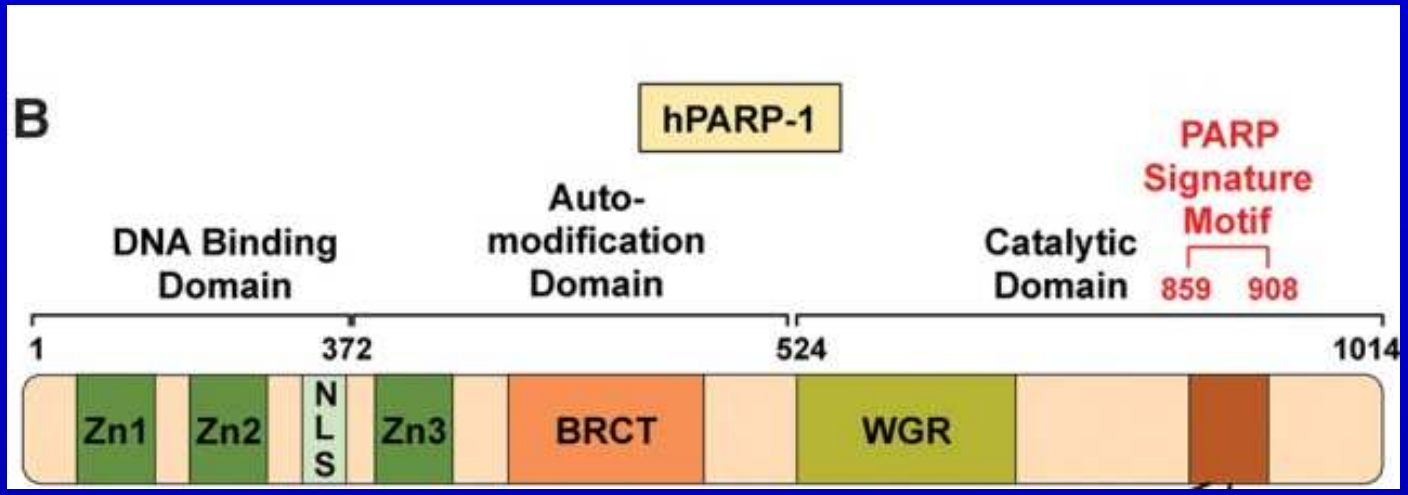

After DNA damage, PARP-1 is responsible for approximately 90% of the total cellular PARylation activity.

PARP1 and DNA lesions

1) PARP1 recognizes and binds to DNA lesions leading to a dramatic increase of its catalytic activity

2) Activated PARP1 catalyzes the formation of PAR chains on its target proteins, PARP1 itself and histones, in the vicinity of the breaks.

PARP1 and DNA lesions

……

3) **These PAR chains act as a binding platform for PAR-binding effector proteins including chromatin remodelers**

4) The action of the remodelers combined with electrostatic repulsion between DNA and PAR chains decorating the chromatin fiber contribute to the rapid relaxation of the chromatin structure at DNA damage sites

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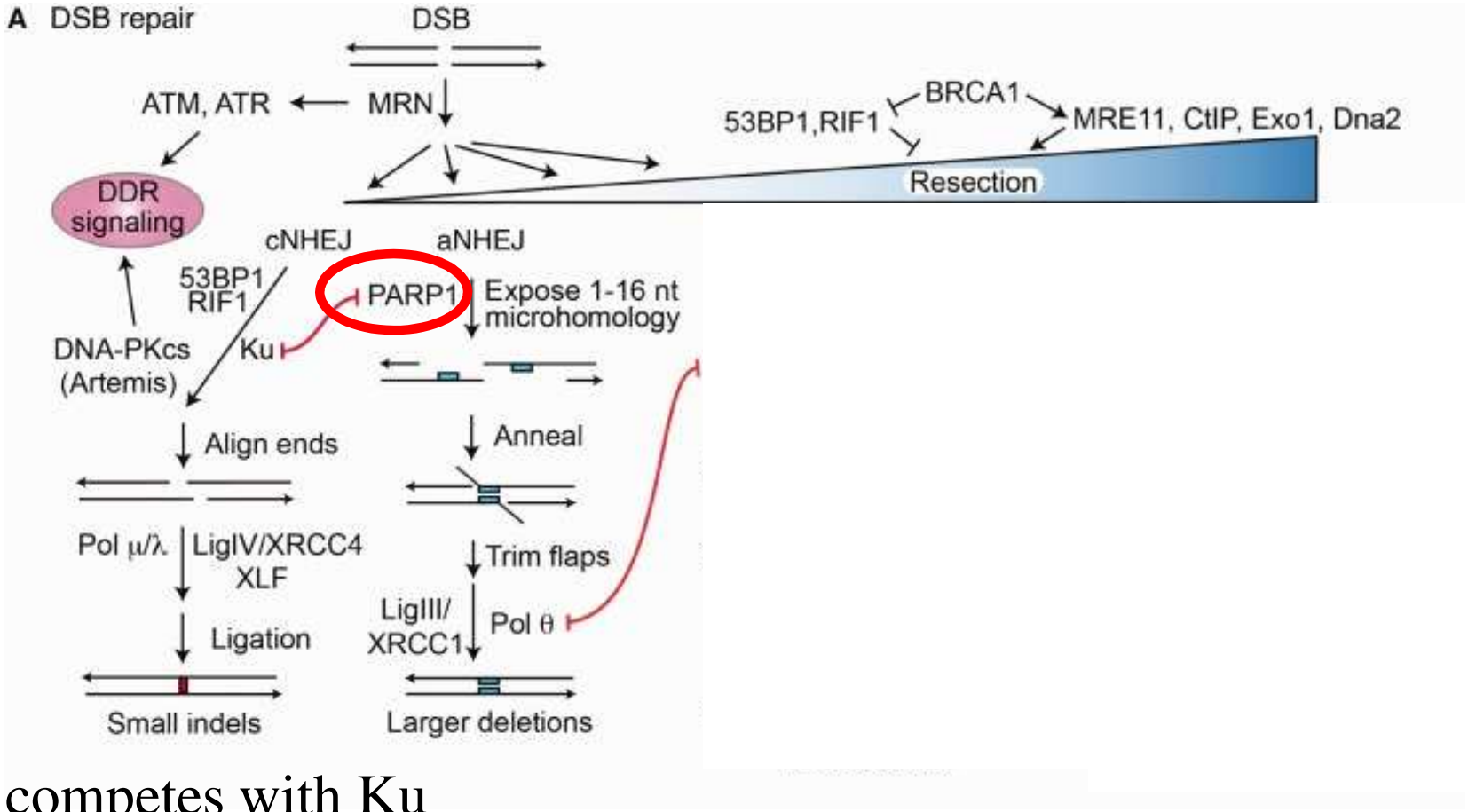
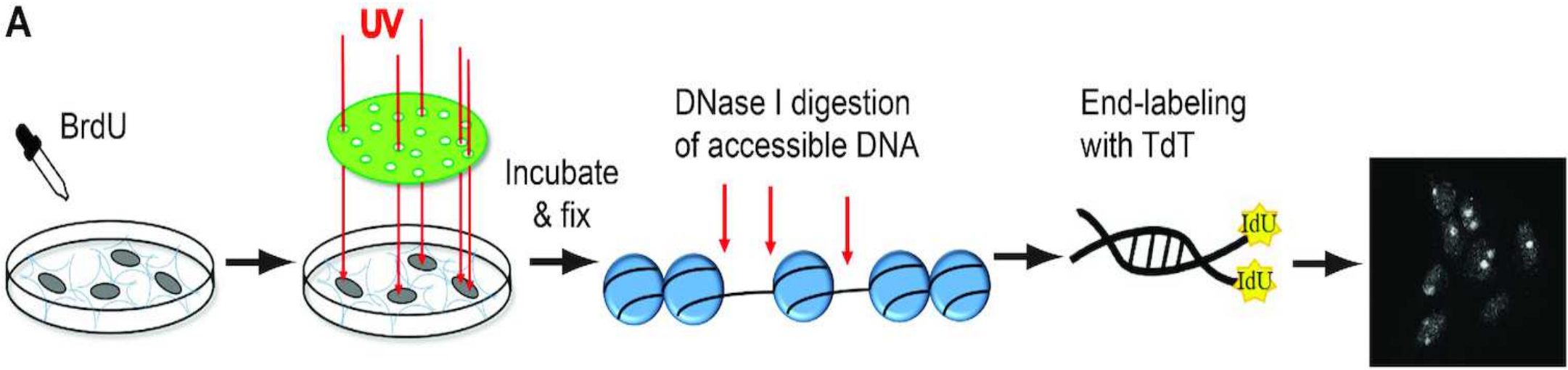
The addition of poly(ADP-ribose) (PAR) chains along the chromatin fiber due to PARP1 activity regulates the recruitment of multiple factors to sites of DNA damage

DNA repair pathways in mammalian cells



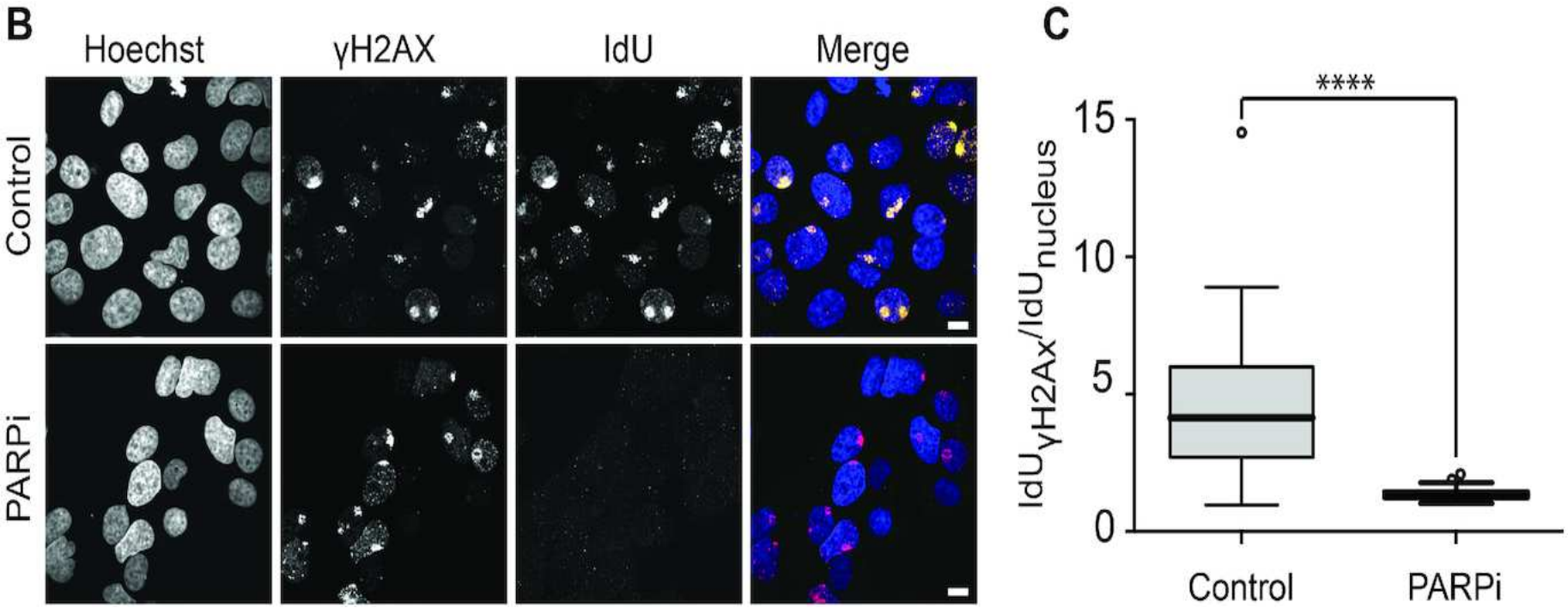
PARP1 competes with Ku
promotes limited end resection for alternative nonhomologous end joining (aNHEJ).

The accessibility of DNase I at sites of irradiation is regulated by PAR-signaling



1) Cells are labeled with BrdU to photosensitize the DNA
2) After irradiation (UVC light) and fixation, chromatin is digested by DNase I.
3) Free DNA-ends are labeled with 5-iodo-2′-deoxyuridine (IdU) by Terminal Deoxynucleotidyl Transferase (TdT)
4) Visualized by immunofluorescence with anti-IdU antibodies

The accessibility of DNase I at sites of micropore irradiation is regulated by PAR-signaling (2)



PARPi= 10 μM Olaparib

Nucleic Acids Research, gkz820, https://doi.org/10.1093/nar/gkz820

Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation

N Engl J Med. 2017

:

- Olaparib is an oral poly(adenosine diphosphate-ribose) polymerase inhibitor that has antitumor activity in patients **with metastatic breast cancer and a germline BRCA mutation.**

- CONCLUSIONS

Among patients with metastatic breast cancer and a germline BRCA mutation,

olaparib monotherapy provided a significant benefit over standard therapy;

median progression-free survival was **2.8 months longer** and the risk of disease progression or death was 42% lower with olaparib monotherapy than with standard therapy (Funded by AstraZeneca)