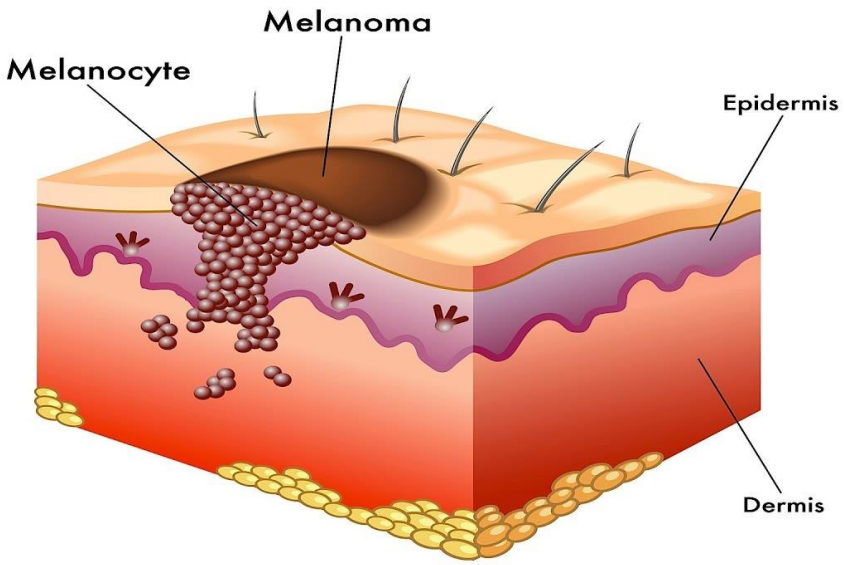
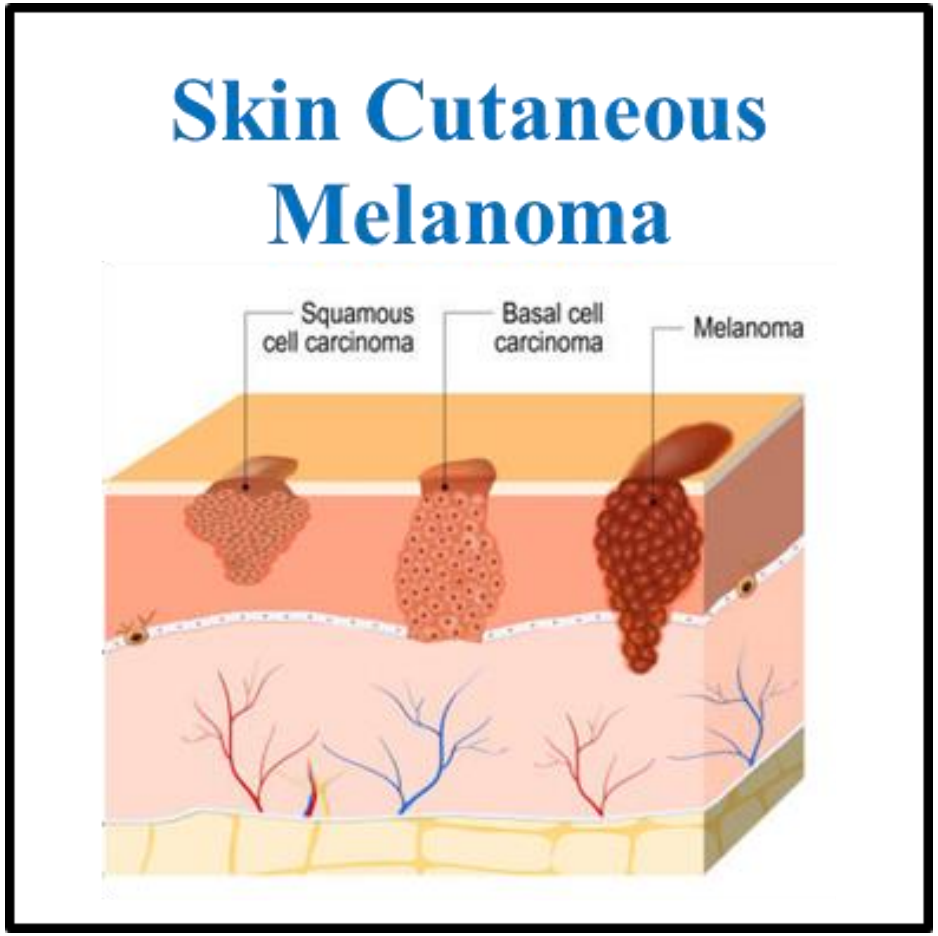
FUNCTION OF PARP1 IN DNA REPAIR

By Elaine Bai



Introduction

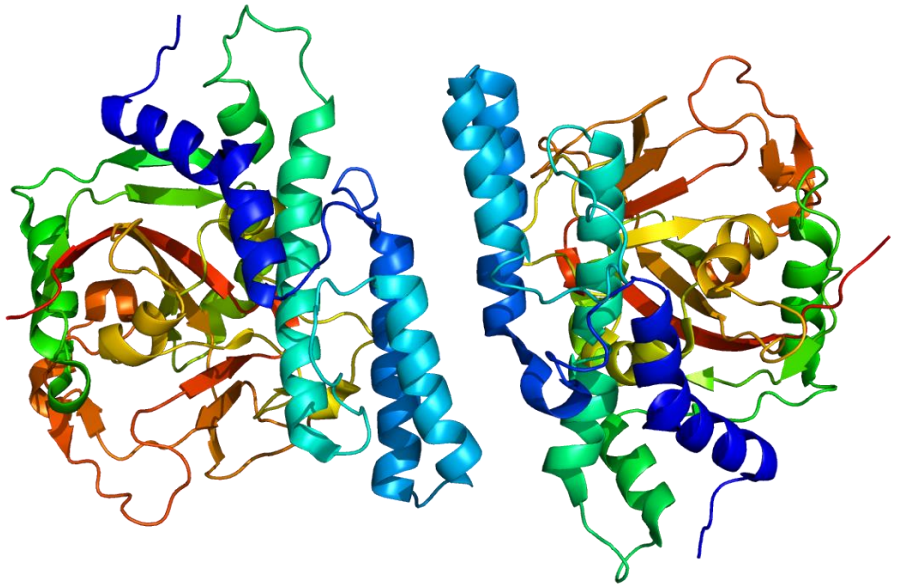
- **Melanoma**: Cancer controls cell and controls the pigmentation in skin
  - Develops when melanocytes spread rapidly to different areas of the skin
  - More dangerous than other skin cancers
  - Metastasis likely if left undetected



https://oncovar.org/

Introduction

- Cutaneous Melanoma
  - Caused by
  - Exposure to UV rays
- Begins in the epidermis
  - Can become invasive

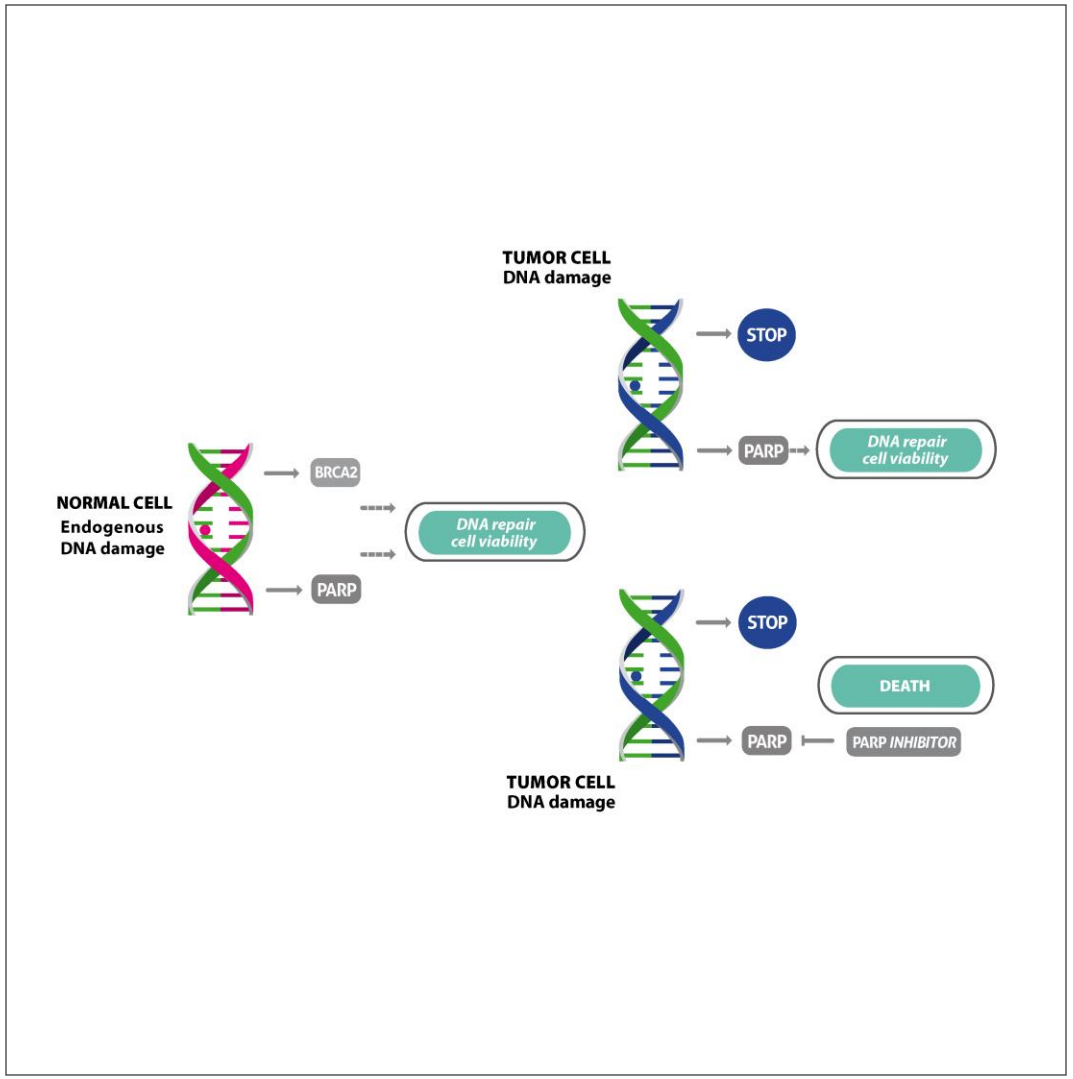


https://en.wikipedia.org/wiki/PARP1

Introduction

Poly-Adenosine Diphosphate-ribose polymerase (PARP):

- Enzymes
  - **18** types of PARP
  - Critical for DNA repair

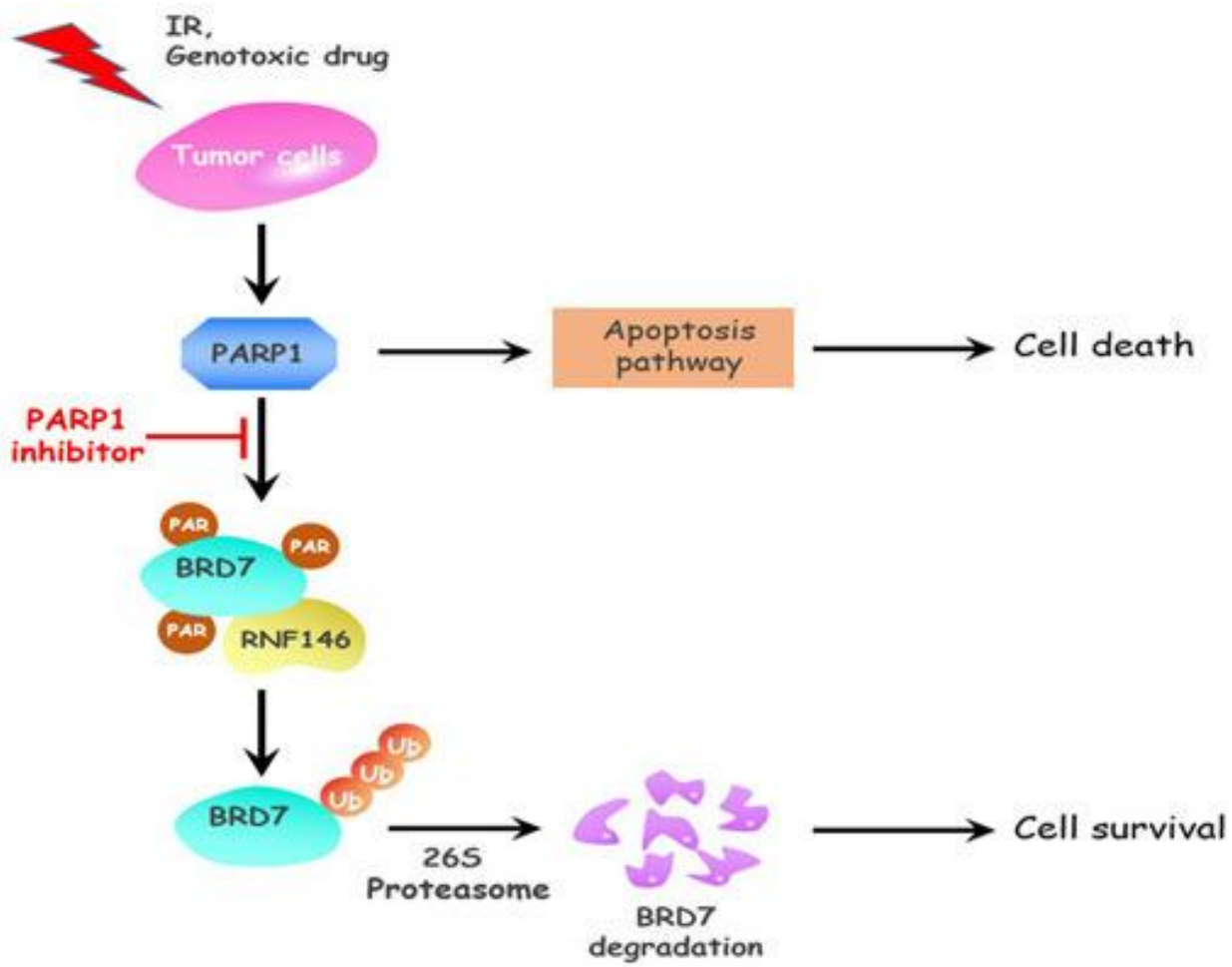


https://www.tebu-bio.com/blog/tag/parp/

PARP Enymes

- Catalyzes transfer of **ADP-ribose** to target proteins

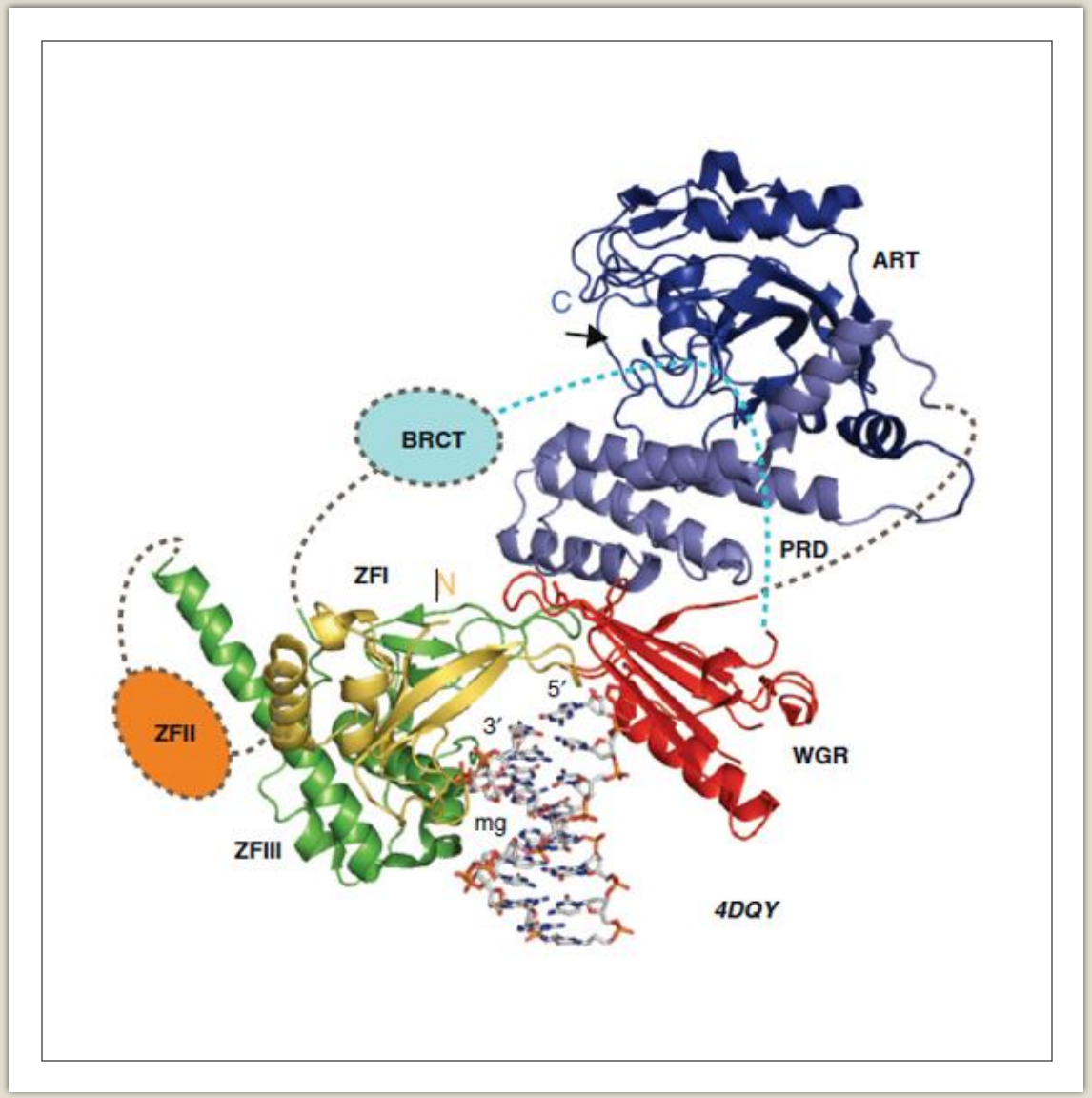
- **PARP1** is **most studied**
- PARP2 is most closely related to PARP1
  - 69% similarity in catalytic domain



https://www.embopress.org/doi/full/10.15252/embr.201846166

Introduction

- PARP1 & PARP2
  - Both regulate DNA polymerase beta (Polβ)
    - Base-excision repair
- Mechanism
  - Binds to DNA single-strand and double-strand breaks

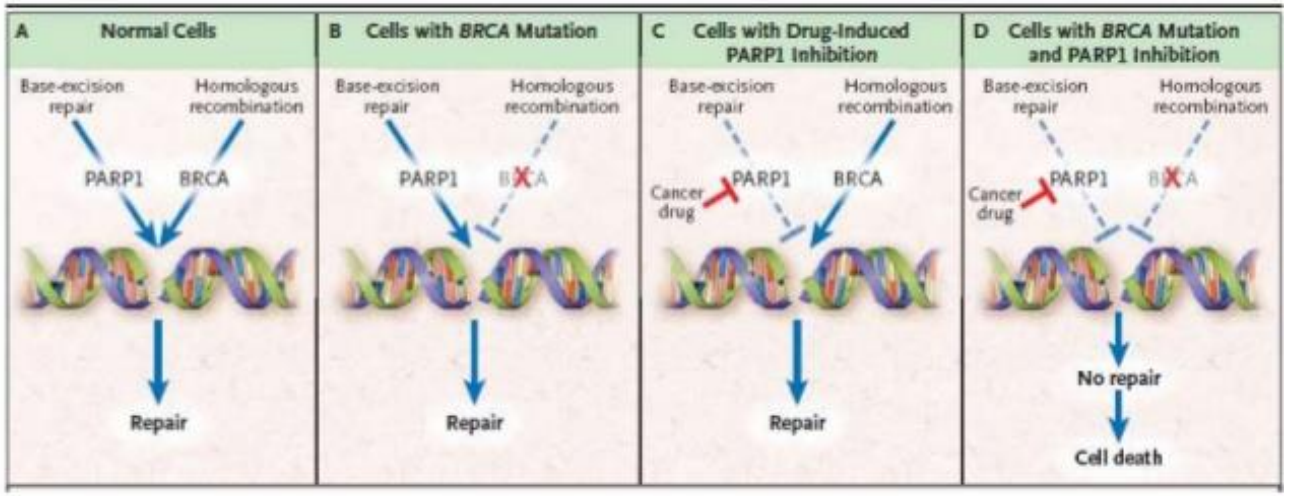


Introduction

- Cells exposed to:
  - Endogenous
  - Exogenous
- Causes skin damage

- PARP1
  - Activation caused by damaging agents and radiation-induced DNA damage

PARP Inhibitors

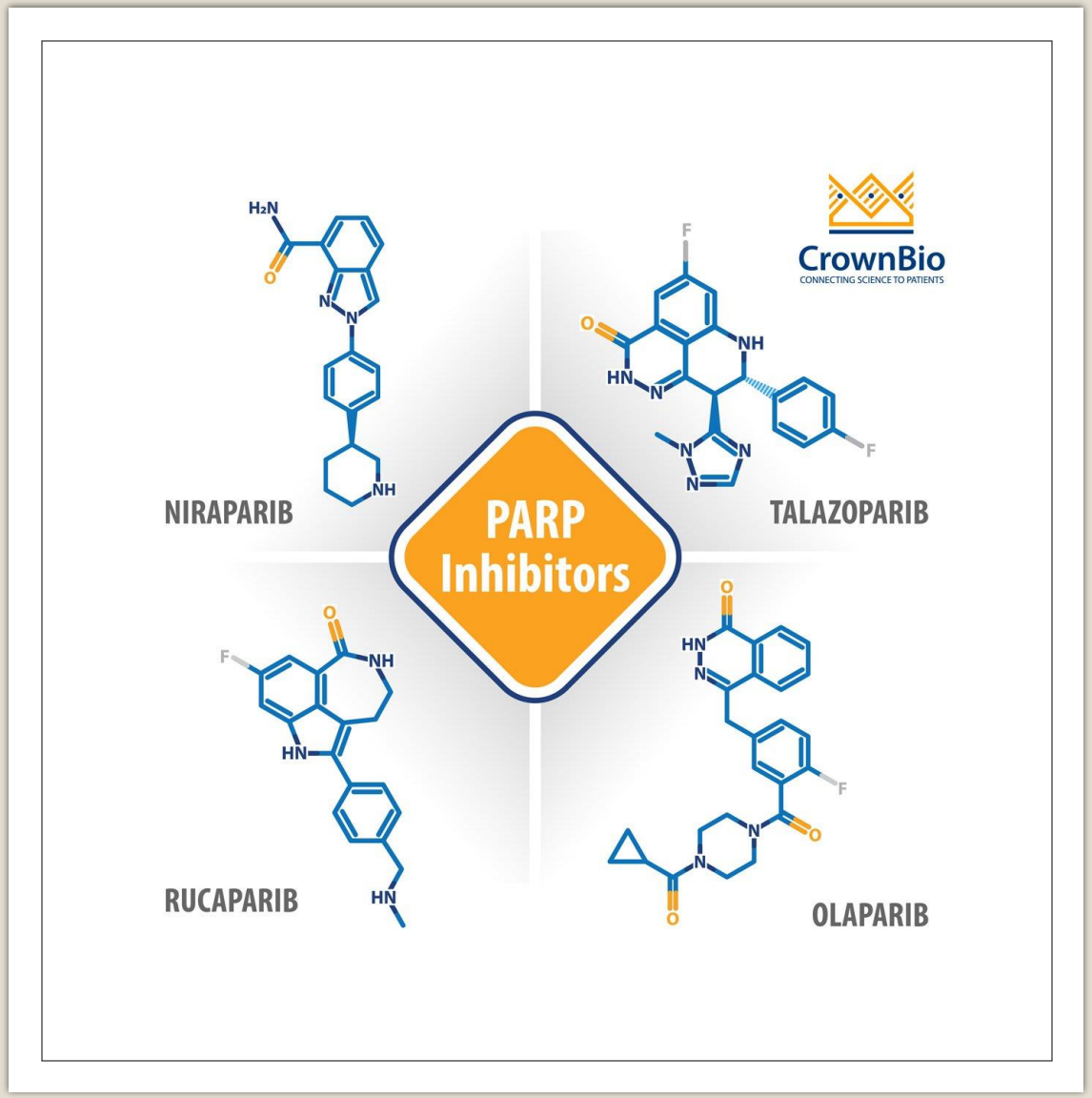


Igelhart JD and Silver DP. NEJM 2009

https://yrf.ipsf.org/d/21-parp-inhibitors

Introduction

- PARP Inhibition
  - Cancer drug
- Prevents cancer cells from repairing
  - Causes cell death
- Targets cancer cells, avoids healthy cells



https://blog.crownbio.com/parp-inhibitors-2020

PARP Inhibition Interacting with Cancer Cells

- Interfere with certain enzymes that help cancer cells replicate
- Less harmful effect than traditional chemotherapy

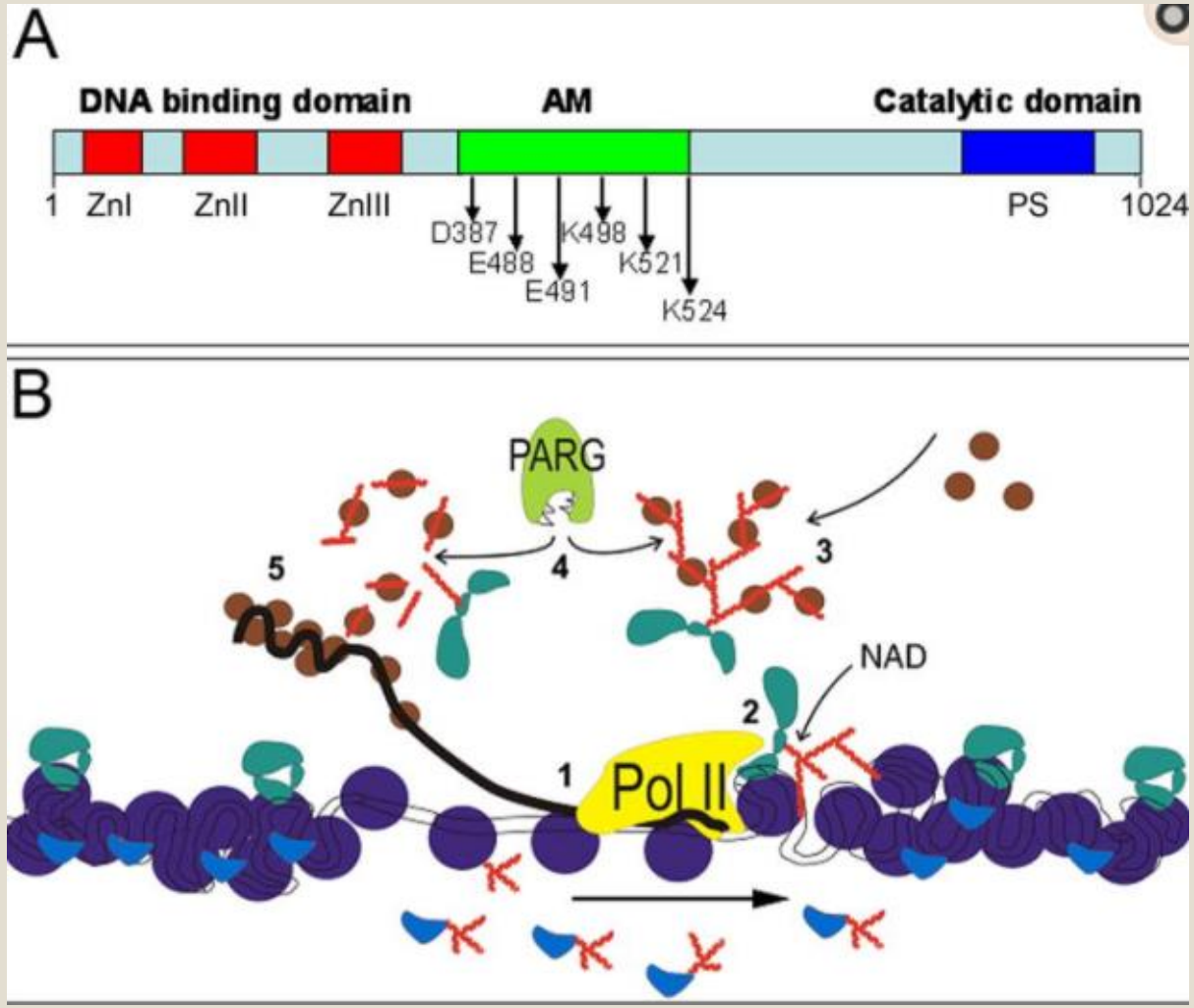
Literature Review:
The roles of PARP1 in gene control and cell differentiation

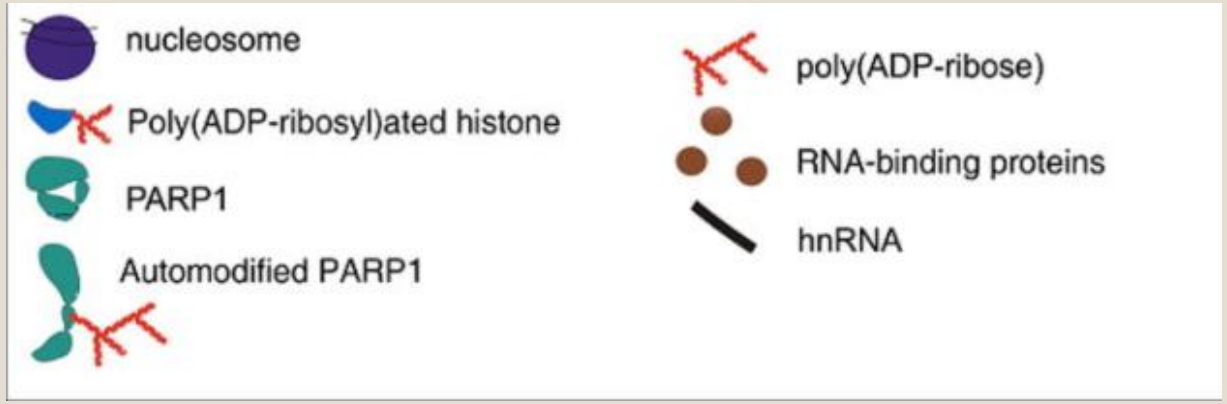
Ji, Y., & Tulin, A. V. (2010)

? Roles of PARP1 in transcriptional control during development

- PARP1 prevents transcription both locally and globally in euchromatin and heterochromatin
- Automodified PARP1 interacts with histone H3 and H4 and their variants after activated by developmental cues

Ji, Y., & Tulin, A. V. (2010)





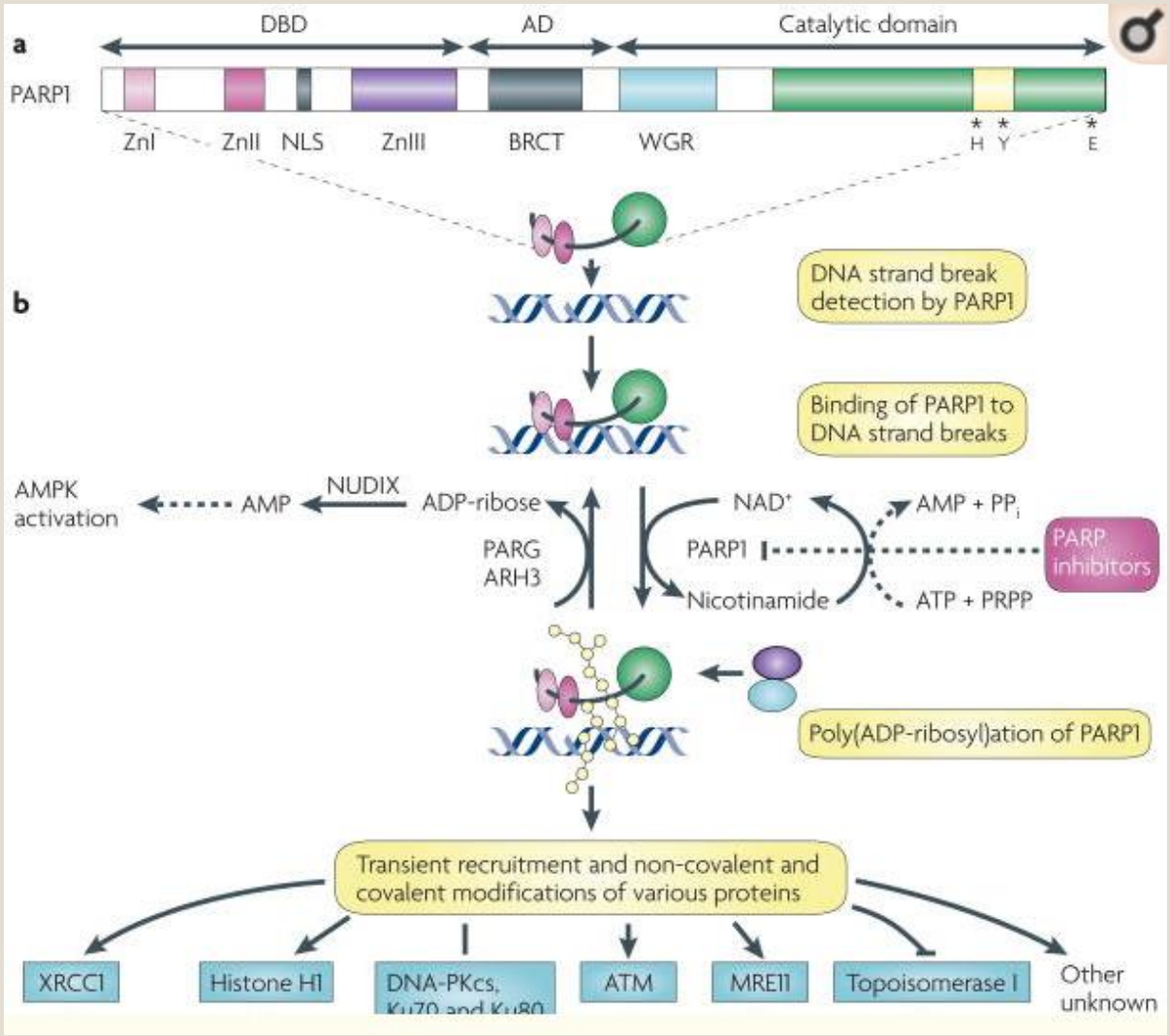
Domain structure of PARP1 protein

Literature Review:
PARP inhibition: PARP and beyond

Rouleau, M., Patel, A., Hendzel, M. J., Kaufmann, S. H., & Poirier, G. G. (2010)

? Investigate functions and structure of enzymes in PARP family

- PARP inhibitors are active anticancer agents in *BRCA1*- and *BRCA2*-mutant tumors
- PARP1 is activated by DNA strand breaks



Rouleau, M., Patel, A., Hendzel, M. J., Kaufmann, S. H., & Poirier, G. G. (2010)

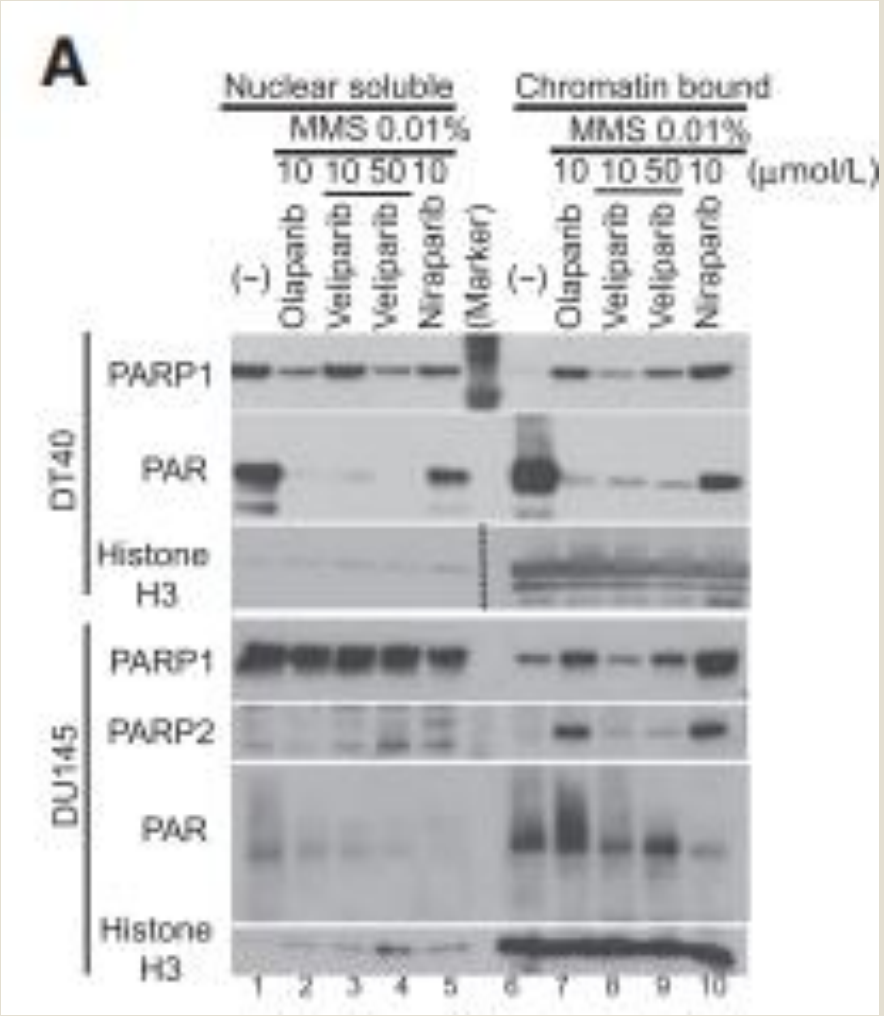
PARP1 is shown with its DNA-binding (DBD), automodification (AD) and catalytic domains

Literature Review:
Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors

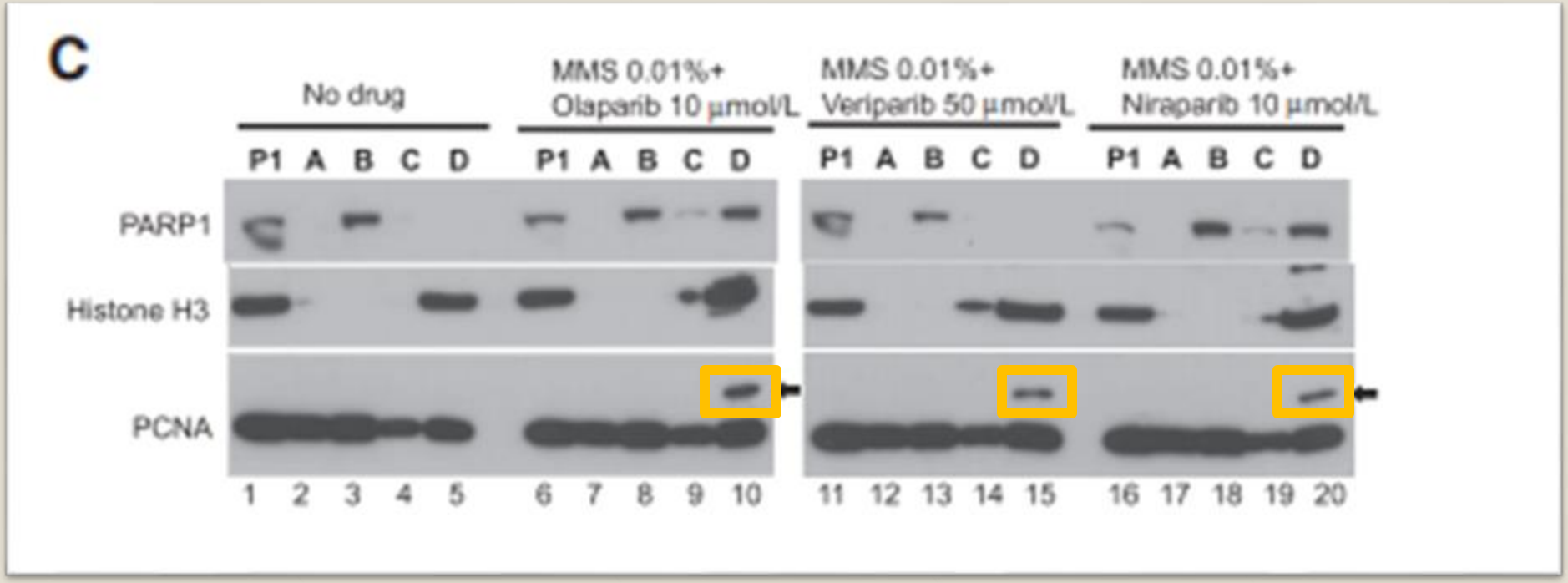
Murai, J., Shar-yin, N. H., Das, B. B., Renaud, A., Zhang, Y., Doroshow, J. H., ... & Pommier, Y. (2012).

? PARP inhibitors trap the PARP1 and PARP2 enzymes at damaged DNA

- The toxic PARP– DNA complex by PARP inhibitors
- Different potency to poison PARP among clinical PARP inhibitors



Differential cellular trapping of PARP1 and PARP2 by clinical PARP inhibitors



Differential cellular trapping of PARP1 and PARP2 by clinical PARP inhibitors

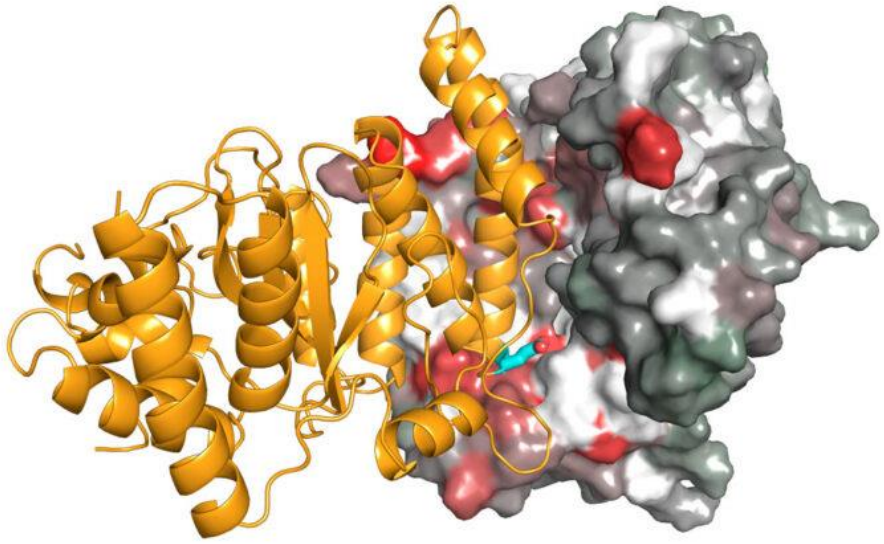
Gap in research

- PARP1 inhibitor based therapy
  - Observed to play essential role in cutaneous melanoma
- Lack of research
  - Invitro
  - Clinical

https://stke.sciencemag.org/content/4/174/mr5/F3

Purpose Statement

- Function of PARP1 inhibition
  - How its involved in cutaneous melanoma invitro
  - Full length structure of PARP1
  - Comparing roles of other PARPs



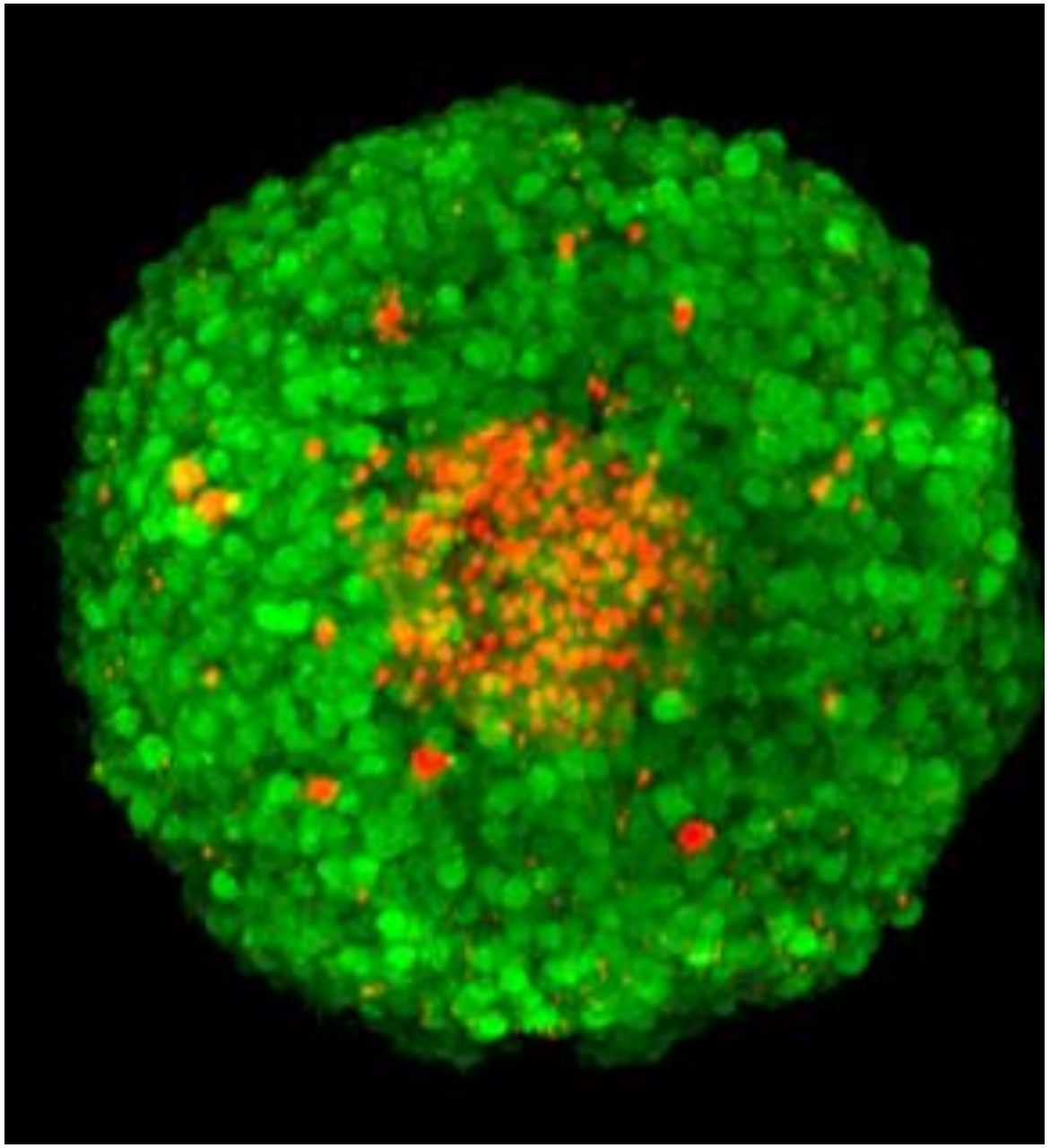
https://www2.mrc-lmb.cam.ac.uk/how-two-proteins-work-together-in-dna-damage-detection/

Hypothesis

- PARP1 participates in DNA repair
- Provided the first evidence for the existence of PARP2

Methodology

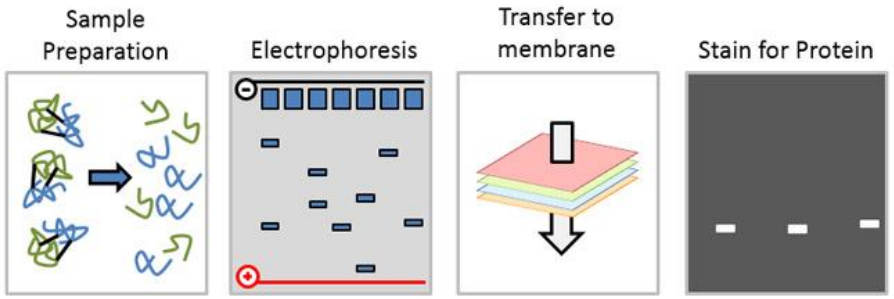
- Cell Lines: Cultures of animal **cells** can be propagated repeatedly and sometimes indefinitely
- Immunoblotting: use antibodies to identify target proteins



https://ibidi.com/content/216-confocal-microscopy

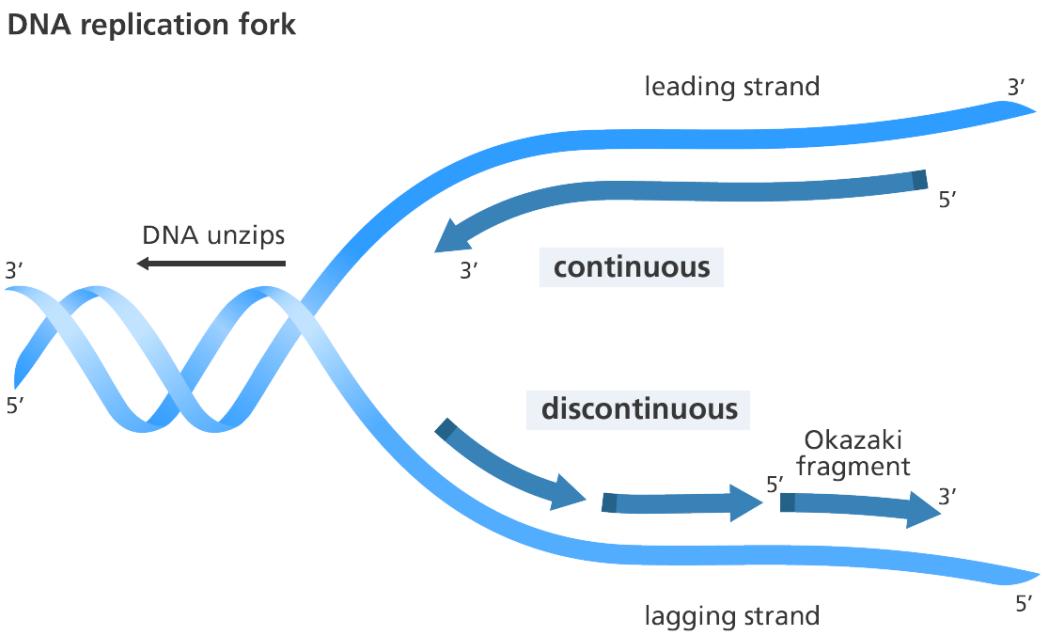
Methodology

- Cell culturing: Cells grown under controlled conditions
  - Cultured in M-254 growth medium supplemented
  - Confocal Microscopy and Image Analysis: imaging technique for increasing optical resolution
    - Performed in two independent cultures, five randomly selected areas from culture plate wells were further analyzed



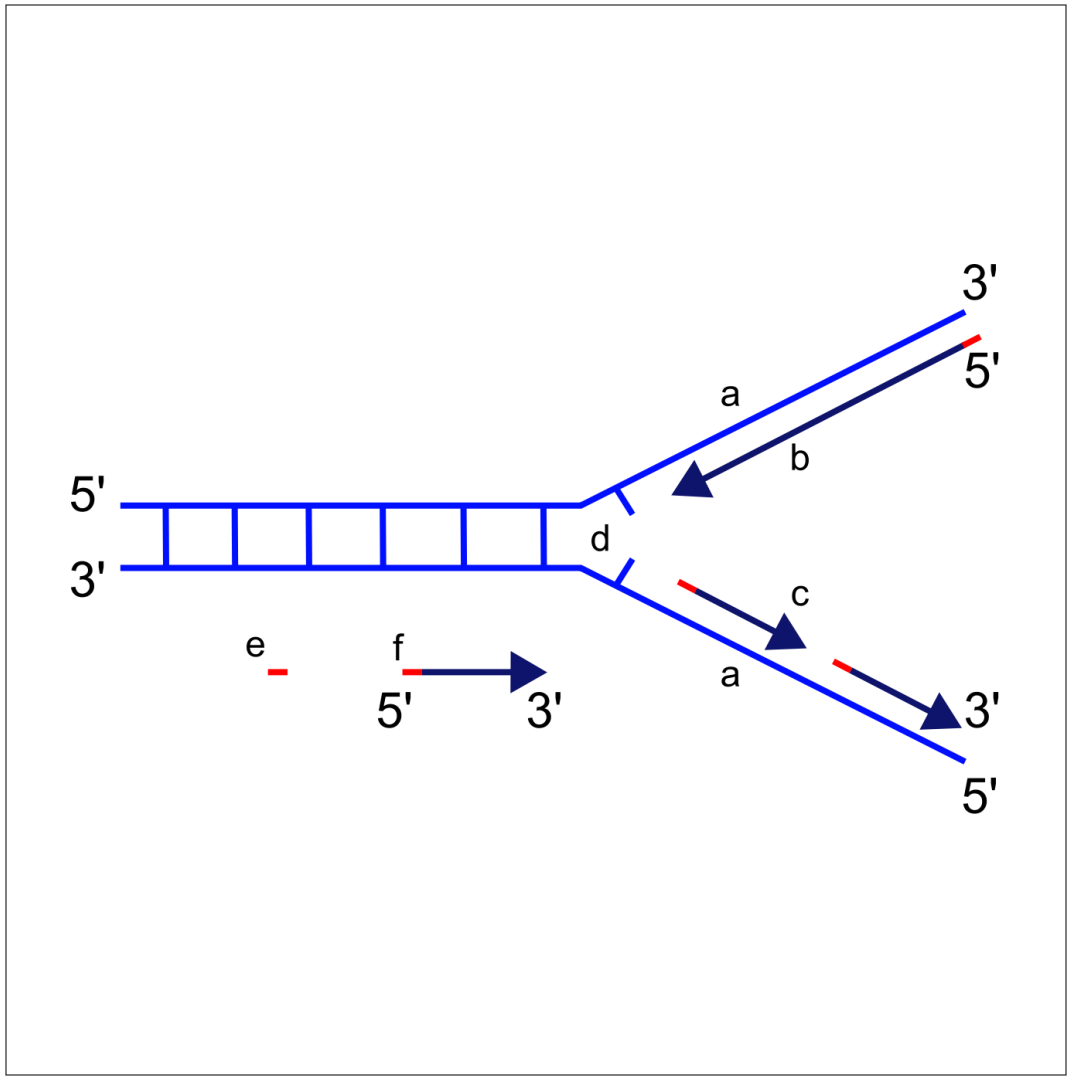
Methodology

- Western Blotting Analysis: detect proteins in a sample of extract
  - Samples of an identical amount of protein were separated using electrophoresis
  - RNA Isolation and Reverse Transcription: ensuring the accuracy of gene expression analysis



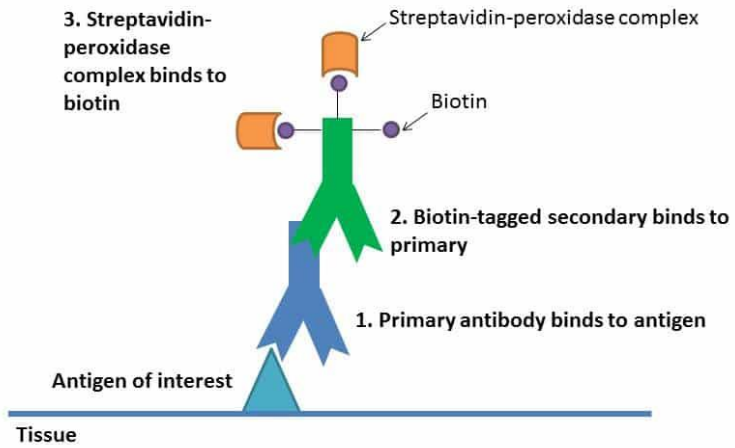
Anticipated results

- PARP inhibition expected to result in fork collapse
  - Combined effects of forming destabilizing forks by preventing their restart
- DSBs would not be repaired thus causing synthetic lethality



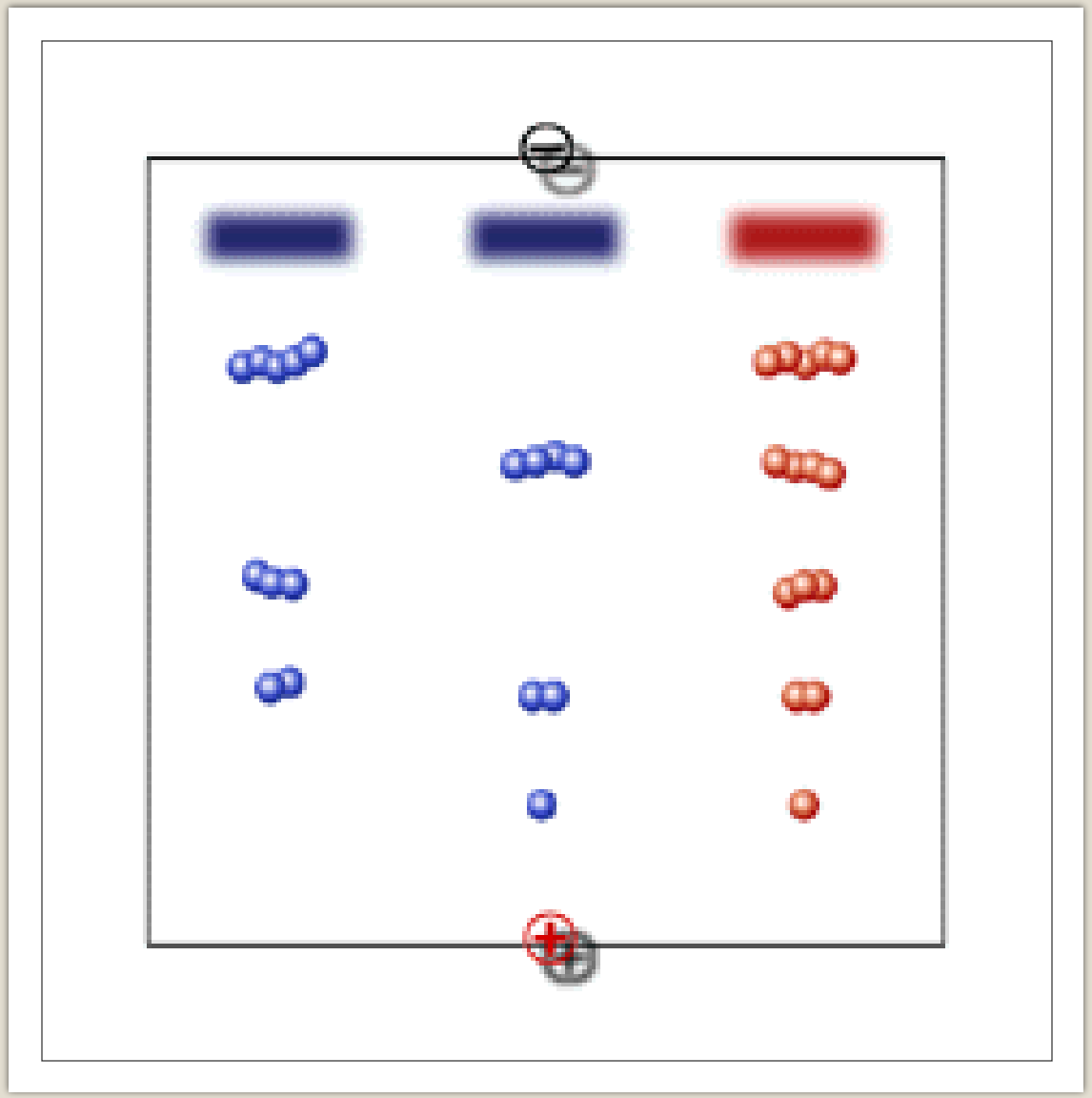
Anticipated Results

- Protection of replication forks by limiting PARP1 access to forks
  - Stabilize replication forks
  - Result in lower levels of genomic instability
  - synthetic viability



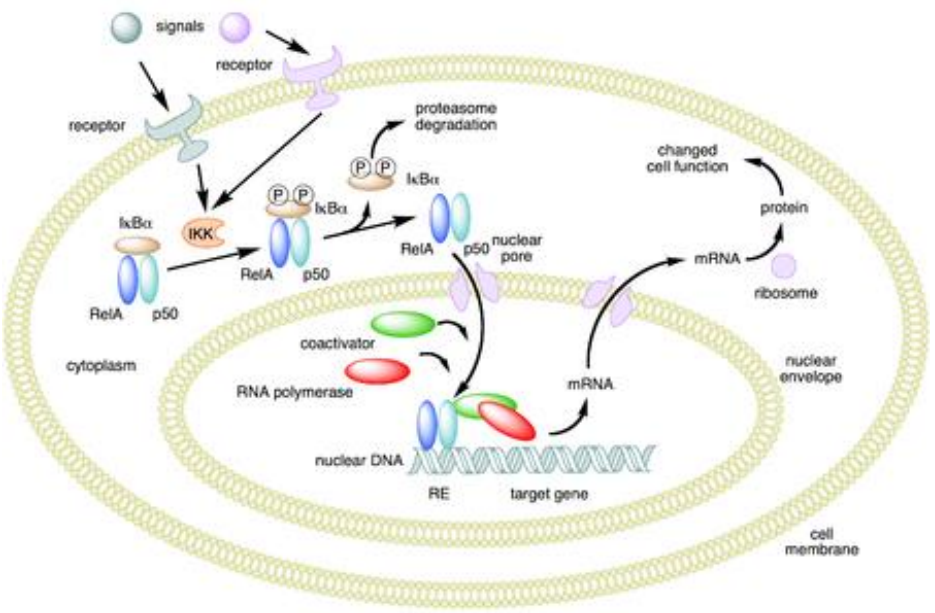
Previous results

- In vitro molecular biology demonstrated enhanced PARP1 expression in cutaneous melanoma
  - Confirmed by the immunohistochemical study



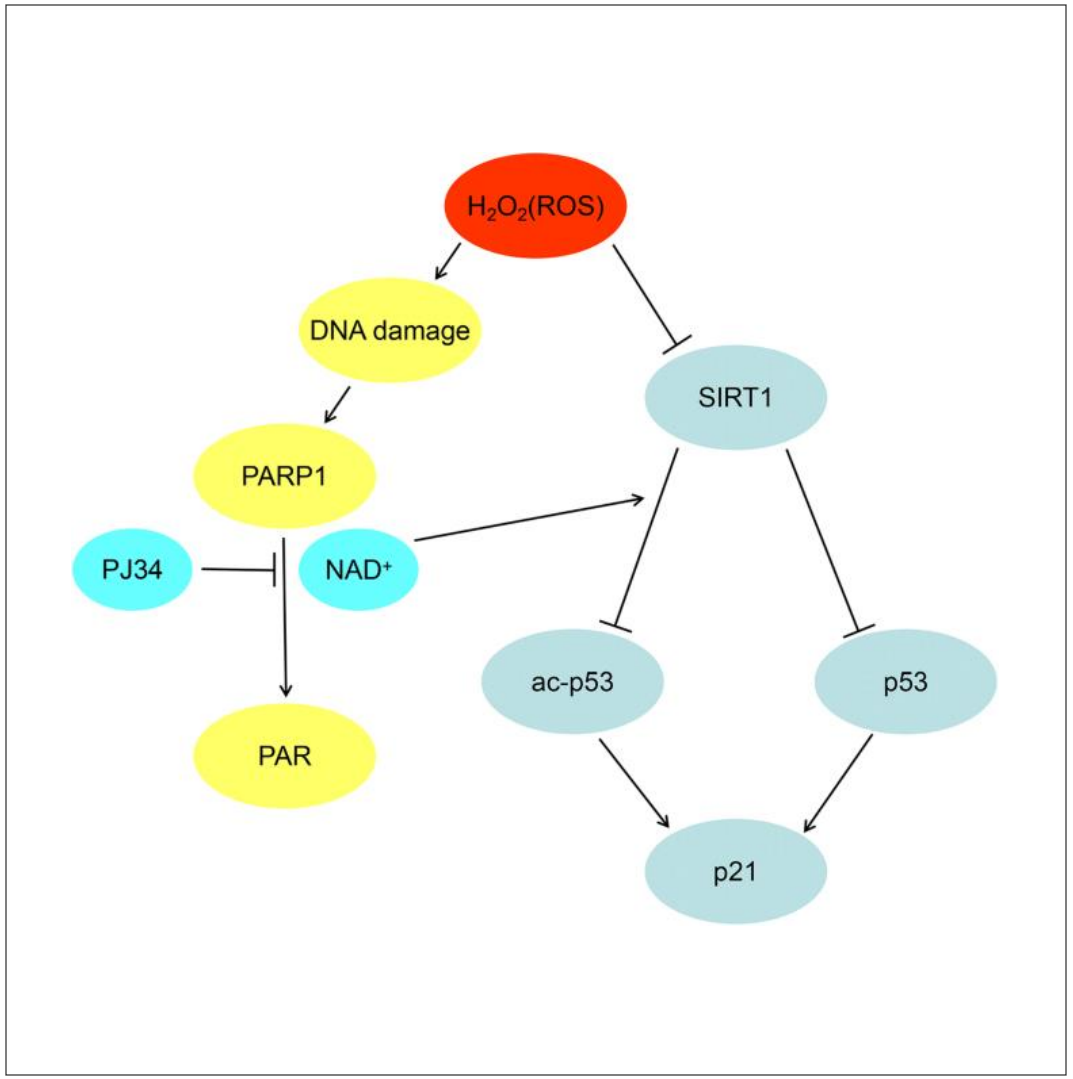
Previous Results

- Melanoma cells exhibited higher PARP1 expression at mRNA and protein levels than normal melanocytes
  - Performed Westen Blotting analysis to confirm results



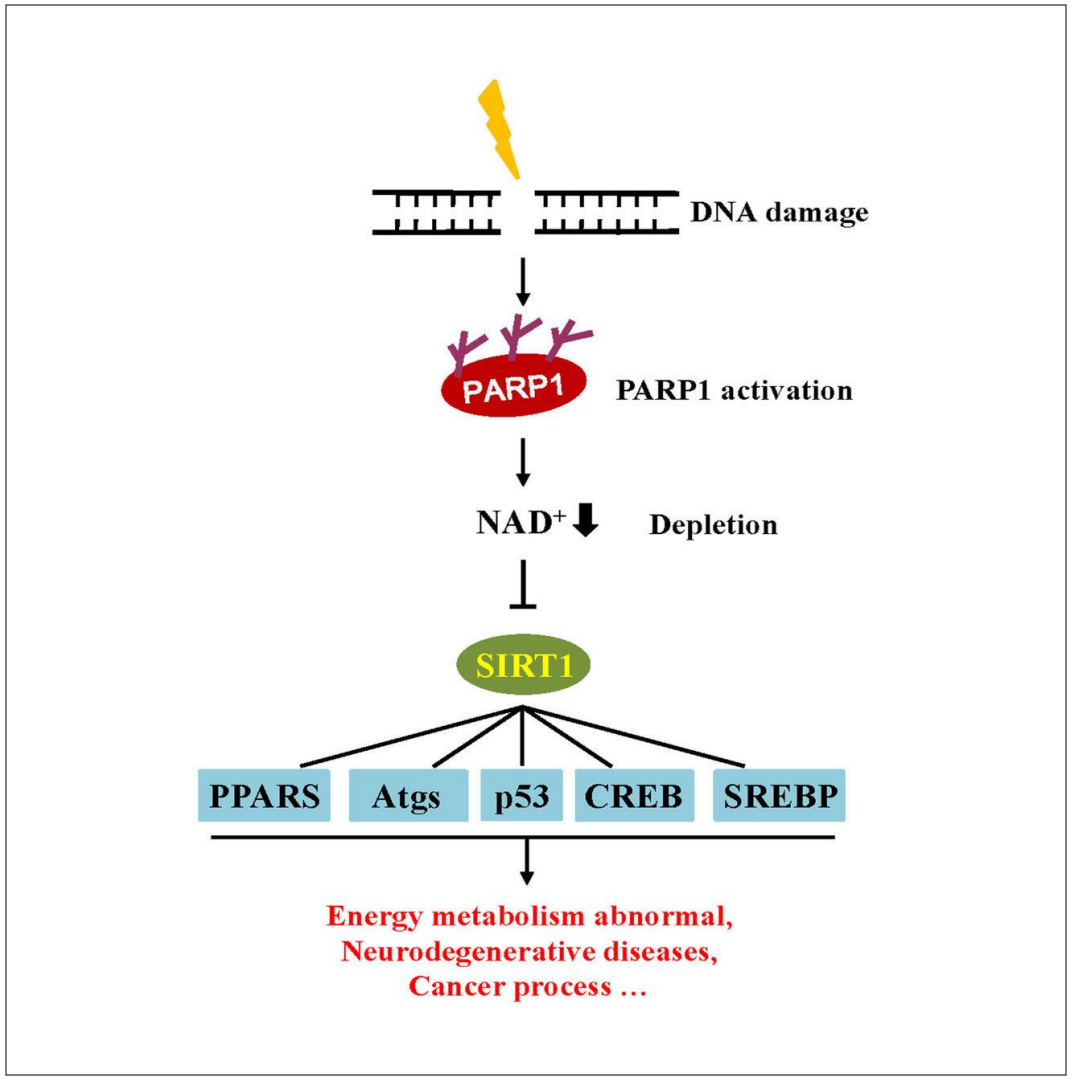
Implications

- PARP1 regulates downstream cytokine production
- PARP family regulate viral infection, replication, and virulence



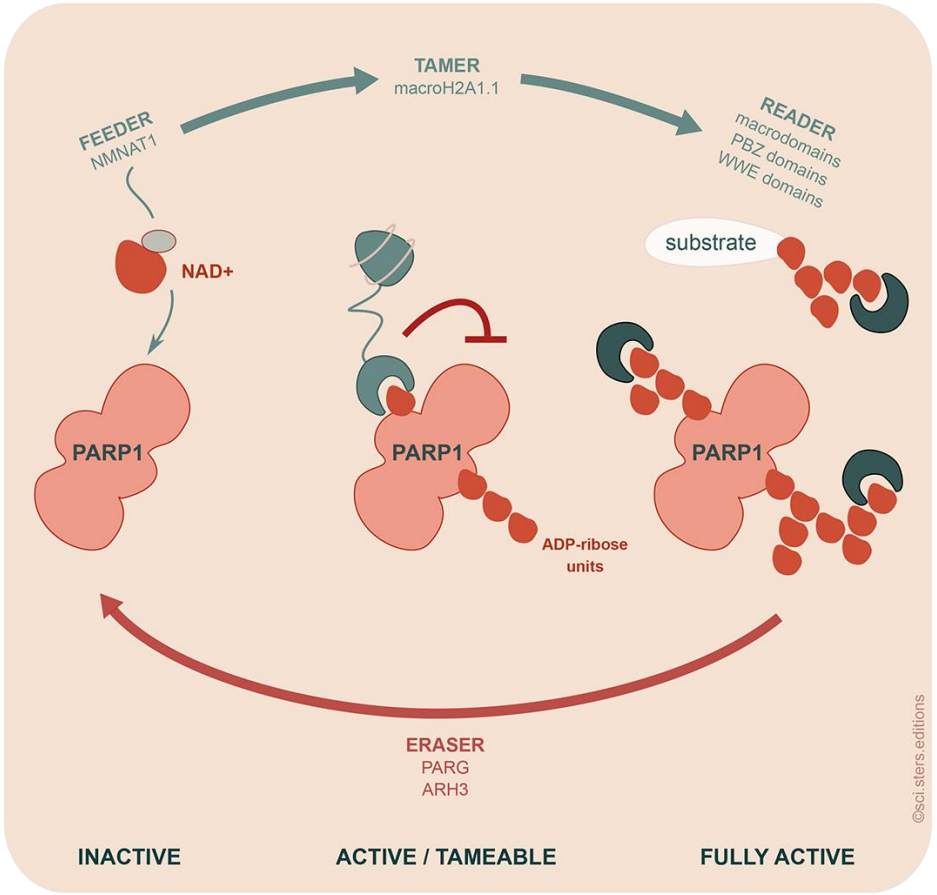
Limitations

- A five-year survival rate of 50% in patients with metastatic cutaneous melanoma
- Effectiveness is limited due to the appearance of many different mutations in the tumor cells
  - Varying from patient to patient
  - within the tumor in the same patient



Conclusion

- In vitro molecular biology approaches PARP1 expression in cutaneous melanoma
  - Confirmed by the immunohistochemical study
- Showed that a high level of PARP1 correlated with unfavorable clinical outcome



Future Research

- Disentangle the biology of PARP by dissecting the structural and functional relationship of PARP1

- Future treatment of human diseases, including cancer, diabetes, and stroke, as well as inflammation

References

- Murai, J., Shar-yin, N. H., Das, B. B., Renaud, A., Zhang, Y., Doroshow, J. H., ... & Pommier, Y. (2012). Trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer research*, *72*(21), 5588-5599.
- Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG. PARP inhibition: PARP1 and beyond. Nat Rev Cancer. 2010 Apr;10(4):293-301. doi: 10.1038/nrc2812. Epub 2010 Mar 4. PMID: 20200537; PMCID: PMC2910902.
- Ji Y, Tulin AV. The roles of PARP1 in gene control and cell differentiation. Curr Opin Genet Dev. 2010 Oct;20(5):512-8. doi: 10.1016/j.gde.2010.06.001. Epub 2010 Jun 28. PMID: 20591646; PMCID: PMC2942995.

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