Code Breaking: Inhibition of DNA Repair Pathways Enhances Radiotherapy in Colorectal Cancer

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Introduction

- Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer related mortality. [1]
- Radiotherapy (RT) is an important pillar of cancer treatment. [2]
- RT promotes cell death and immune responses by inducing breaks in DNA strands and promoting the release of damage-associated molecular patterns (DAMPs). [2]
- DNA released from dying cells are phagocytosed and activate the cyclic GMP-AMP synthase –stimulator of interferon genes (cGAS-STING) pathway which can promote anti-tumor immune responses. [3]
- Immune responses in CRC due to RT remain low due to the activation of DNA repair mechanisms via ATR. [3]
- Activation of the ATR pathway upregulates the expression of anti-phagocytic “don’t eat me” signals such as PD-L1 and CD47. [3]
- Inhibition of PD-L1 and CD47 can promote cell death in radioresistant colorectal cancer through activation of cGAS-STING. [3]
- **We hypothesize that inhibition of components of the ATR pathway can improve cell death and immune responses in CRC cells after RT.**

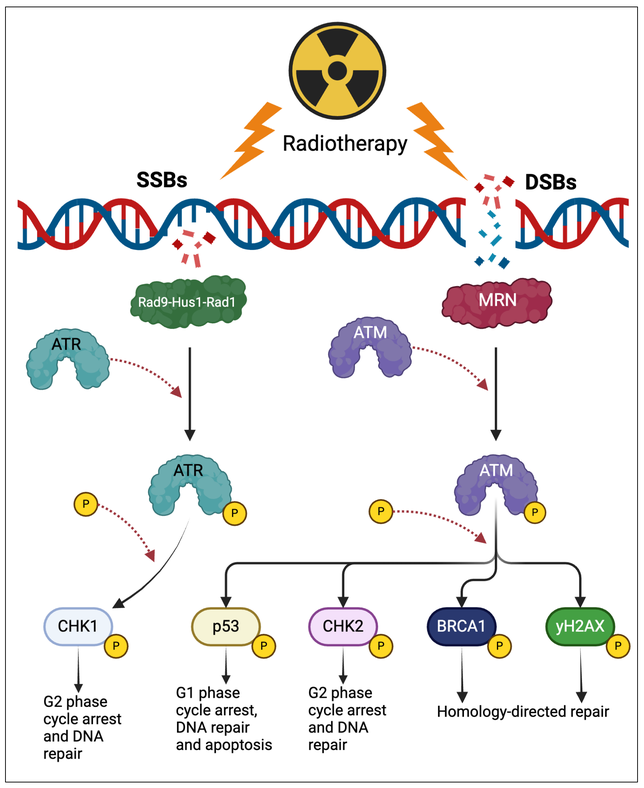

Fig. 1 DNA repair mechanisms activation after radiotherapy promotes radioresistance in CRC cells. ATR and ATM are activated in the presence of DNA breaks and promote cell cycle arrest and DNA repair. Activation can promote the upregulation of anti-immune responses that can lead to radioresistance.

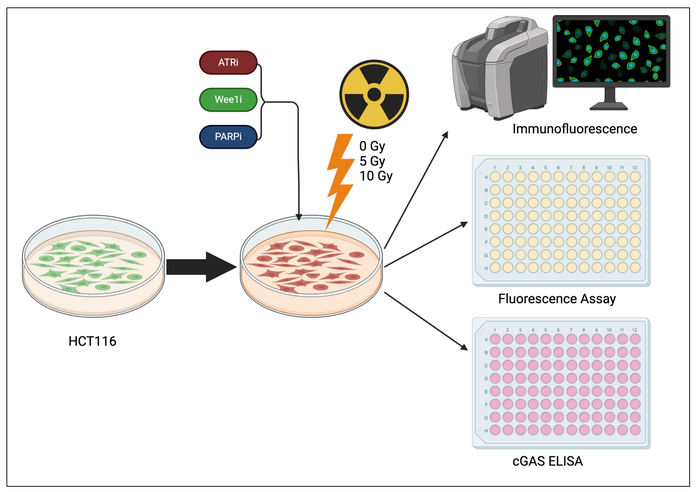

Fig. 2 Method to inhibit DNA repair pathways in colorectal cancer cells. Cultured HCT116 cells were treated with one of three inhibitors targeting ATR, Wee1 and PARP. The cells were then dosed with radiation at 0, 5 and 10 Grays. Cells were imaged using immunofluorescence, cytosolic DNA quantity via luciferase assay and cGAS activation determined by ELISA

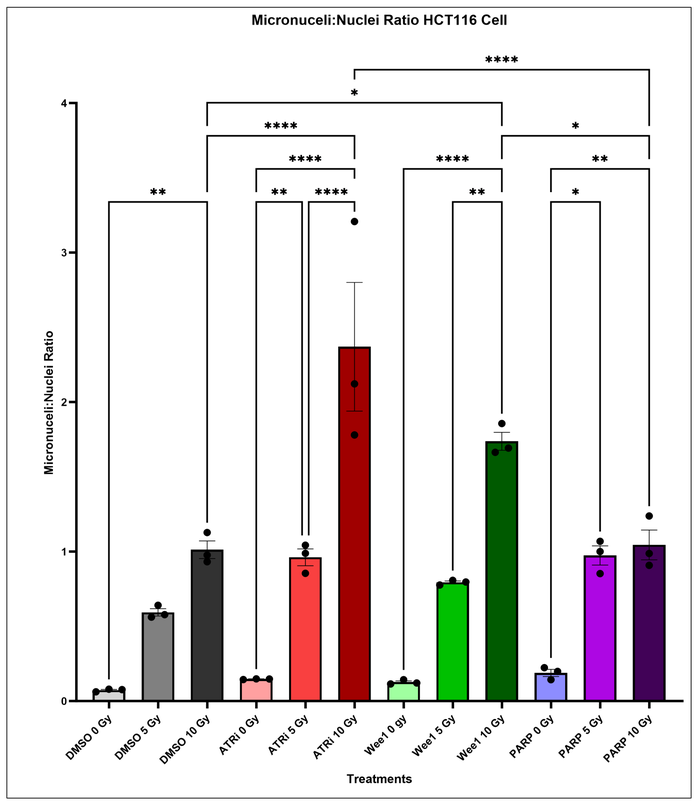

Fig. 3 Inhibition of DNA repair components enhances chromosomal instability and DNA damage in radiotherapy. Radiation-treated HCT116 cells demonstrated higher formation of micronuclei with inhibition of ATR, Wee1 and PARP. Statistical significance was determined using Tukey’s multiple comparisons test. * p < 0.05, ** p< 0.01, **** p< 0.0001.

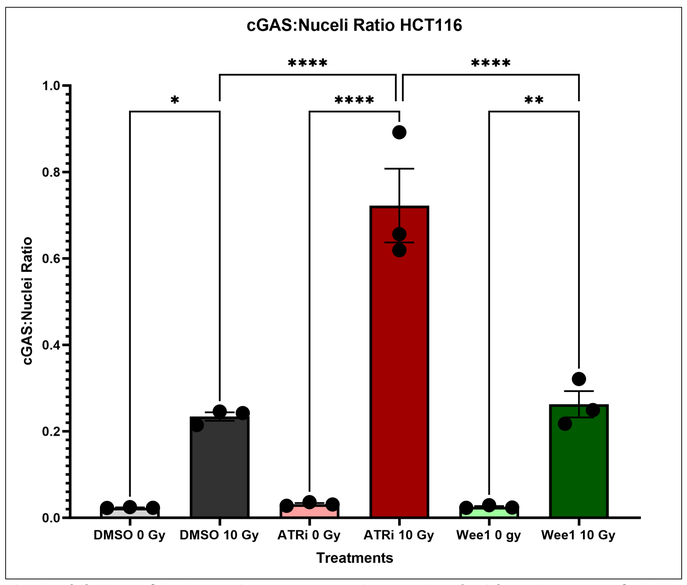

Fig. 3 Inhibition of DNA repair components is associated with an increase of cGAS activation. Radiation-treated HCT116 cells demonstrated higher association of cGAS with nuclei upon inhibition of ATR, and Wee1. Statistical significance was determined using Tukey’s multiple comparisons test. * p < 0.05, ** p< 0.01, **** p< 0.0001.

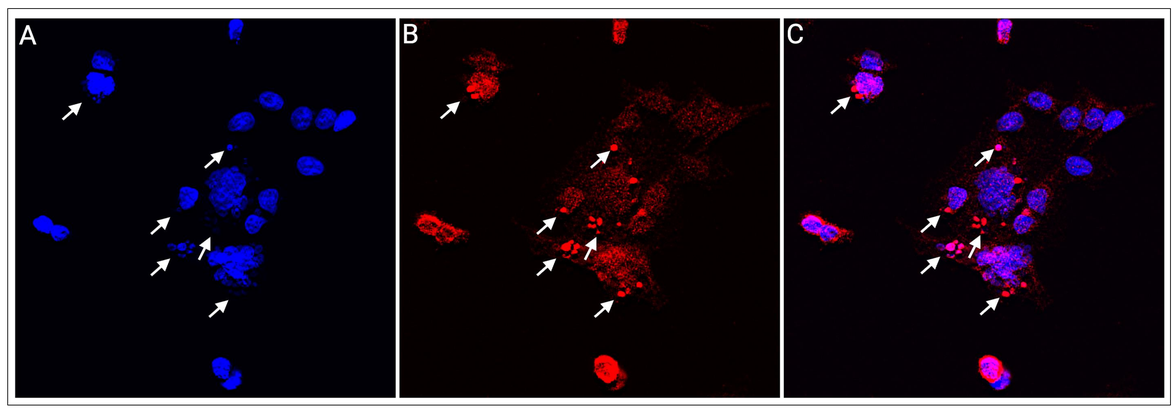

Fig. 4 Inhibition of DNA repair components is associated with increased colocalization of micronuclei with cGAS. HCT116 cells was treated with 10 Gys of radiation and 250 nM of ATR inhibitor for 24 hours and imaged using immunofluorescence microscopy. HCT116 cells were stained with DAPI (4A) and cGAS (4B). Imaging demonstrates colocalization of micronuclei with cGAS (4C).

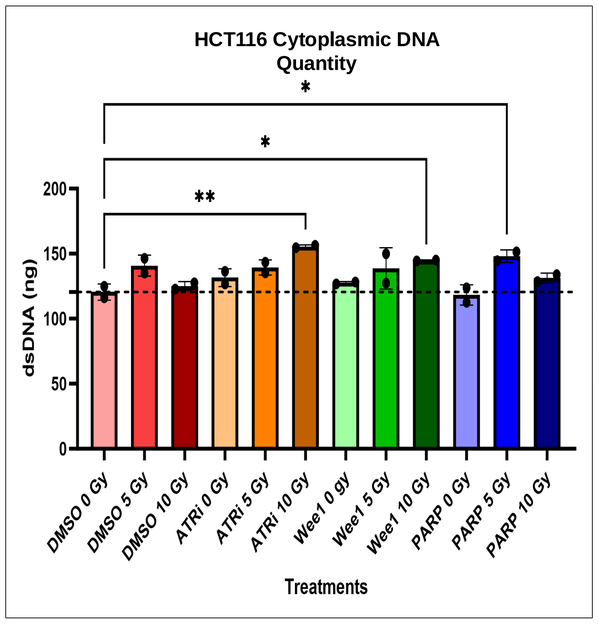

Fig. 5 Inhibition of DNA repair components increases cytosolic DNA quantity. HCT116 cells were treated with inhibitors targeting ATR, Wee1 and PARP, and with increasing doses of radiation. Cytosolic DNA was isolated using a cell fraction kit and quantified using a DNA quantification fluorescence kit. Statistical significance was determined using a Dunnett’s multiple comparisons test. * p < 0.05, ** p < 0.01.

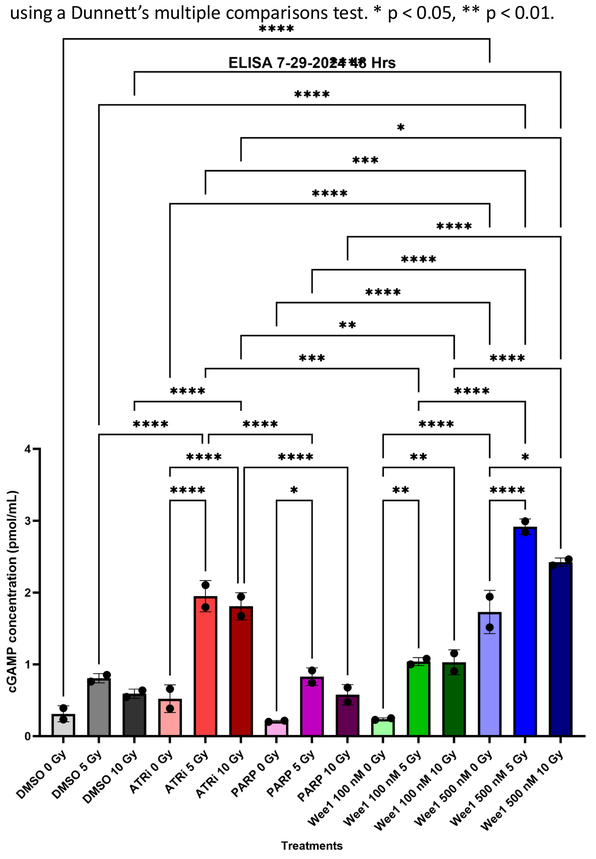

Fig. 6 Inhibition of DNA repair components is associated with increased activation of cGAS. Protein samples were harvested from treated HCT116 cells and activated cGAS was quantified using ELISA. Statistical significance was determined using Tukey’s multiple comparisons test. * p < 0.05, ** p< 0.01, *** p< 0.001, **** p< 0.0001.

Conclusions

- Inhibiting components of DNA repair mechanisms increases the formation of micronuclei in HCT116 cells *in-vitro.*
- DNA repair inhibitor enhances radiotherapy-induced damage in a dose-dependent manner.
- Combined DNA repair inhibition and RT demonstrates increased quantities of cytosolic DNA in treated cells.
- Combining DNA repair inhibition with RT demonstrates an increased association with activated cGAS.

Future Directions

- Inhibit DNA repair pathway components using RNAi or shRNA.
- Observe the activation of downstream targets of the cGAS-STING pathway such as type I IFNs, chemokines and cytokines.
- Determine the effects of DNA repair inhibition and RT using *in-vivo* models.

References

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3. Hsieh, R. C.-E. *et al.* ATR-mediated CD47 and PD-L1 upregulation restricts radiotherapy-induced immune priming and abscopal responses in colorectal cancer. *Sci. Immunol.* **7**, eabl9330 (2022).

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