Combination therapy of DNA repair inhibitors and ionizing radiation to enhance DNA damage in 4T1 murine breast cancer and H1299 non-small cell human lung carcinoma cell lines

Vijay Patel, Broderick X. Turner Scott J. Bright, David B. Flint, David Martinus, Mandira Ben Kacem, Simona F. Shaiilman and Gabriel Sawakuchi

Department of Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston, TX

Background
Cancer treatments are often non-selective and non-specific towards the cancer cell line. An intentional pairing of drugs with radiation could yield improved and synergistic treatment. Both radiation types, protons and photons, yield double strand breaks, but protons deliver more clustered damage, which is more difficult to repair than photon damage. DNA repair inhibitors prevent radiation damage from being undone. We investigated the combination of DNA-repair inhibitors with radiation therapy in non-small cell human lung carcinoma H1299 and murine breast cancer 4T1 cell lines. Ceralasertib is a drug that inhibits ataxia telangiectasia and Rad3-related (ATR) kinase, a protein prominent in homologous recombination. Since protons inflict greater DNA damage, they yield the most damage to cells when paired with a DNA repair inhibitor.

Methods
Cell seeding
200,000 cells were grown and seeded at a density of 200,000 and left to incubate for 24 hours.

Drug Treatment and Radiation
1 µM of either DMSO, Ceralasertib, or Olaparib was added to the cell condition. After one hour they were irradiated with either 5 Gy of Protons or Photons and incubated for 24 hours.

Comet Slides
An Alkaline Comet assay was performed. Cells were placed on comet slide in agarose gel. Once dry, gel electrophoresis was performed at a pH of 14.

Imaging
Cells were stained with sybr gold and imaged using Cytation 5.

Analysis
Images were analyzed using Comet Score. Overlapping cells and debris were removed to maintain data integrity.

Results
- H1299 had increased DNA% damage
  - Proton+Ceralasertib was 1.27x more effective than photons+Ceralasertib
  - 4T1 had a similar trend
  - Proton+Ceralasertib was 1.14x more effective than photons+Ceralasertib
  - More trials are needed to determine significance

Conclusions
- Combined DNA repair inhibitors with radiation was the most effective
  - Protons with inhibitors was the most effective
  - Similar trends were seen between both cell lines
  - Could imply applications to other cell lines
  - DNA repair inhibitors could be tailored to cell lines

Future Directions
- Test PARP inhibitors in 4T1
- Additional proton trials for 4T1
- Determine efficacy in additional cell lines
- Test treatment viability in animal models

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