Is proton radiation more effective than photon radiation at inducing senescence?

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Background
Breast Cancer is ...
- the most diagnosed type of cancer for women in the United States.
- the 2nd most deadly type of cancer for women in the United States.
BRCA = Breast Cancer genes
BRCA1 and BRCA2 encode proteins that repair damaged DNA, preventing cancer from developing — harmful mutations prevent this process and cause cancer.
- Everyone inherits 1 copy of BRCA1 and BRCA2 from each parent - harmful BRCA mutations can also be inherited.
- We use 2 human breast cancer cell lines: HCC1937 (BRCA-mutated), HCC1937-BRCA (BRCA-restored), and 1 mouse breast cancer cell line: 4T1.

Senescence - a state of permanent cell-cycle arrest
- Replicative senescence — caused by telomere shortening over many replication cycles.
- Stress-induced senescence — triggered by some type of external stress on the cell (such as radiation).

Radiotherapy
- Ionizing radiation — protons or photons — damages DNA and can result in apoptosis or senescence.

Methods (continued)
2) Flow Cytometry
- Plate cells in T12.5 flasks.
- Irradiate with 5 Gy protons (9.9 keV/μm) and photons (6 MV X-rays) or treat with H2O2 (200-400 μM) for 1-2h at 37°C when cells are sub-confluent.
- Allow 7 days for senescence to develop, then prepare cells for flow cytometry:
  - 1 hour incubation with β-galactosidase A1 to rid of background senescence (replicative senescence)
  - 2 hour incubation with C2-FDG (5-Dodecanoylaminofluorescein Di-β-D-Galactopyranoside), a substrate of SA β-gal that fluoresces when cleaved
- Run the samples in the flow cytometer

Results: Flow Cytometry

Hypothesis
We hypothesized that protons would cause greater senescence than X-rays for the breast cancer cell lines 4T1, HCC1937, and HCC1937-BRCA.

Methods
Senescence-associated β-galactosidase (SA β-gal) is a biomarker of cellular senescence, and we used two methods of SA β-gal detection to identify and quantify senescent cells.

1) Histological Assay
- Plate cells in 6-well plates using glass coverslips.
- Irradiate with 10 Gy protons (9.9 keV/μm) and photons (6 MV X-rays) when cells are sub-confluent.
- Allow 7 days for senescence to develop, then stain cells with the blue violet β-gal Staining Kit, which uses:
  - X-Gal = substrate of SA β-gal that releases an insoluble blue compound when cleaved. Senescent cells detected by presence of blue spots.

Results: X-gal Staining

Discussion
We are still working on optimizing the X-gal staining protocol. The current images are inconsistent and difficult to highlight differences by microscopy. We are trying to obtain clearer images by using glass coverslips and decreasing the seeding number.

Further replicates of these experiments are currently being conducted. Based on the current cytometry results, it appears that photons are more effective at inducing senescence for 4T1 but X-rays are more effective at inducing senescence for HCC1937-BRCA. We have significantly more senescent cells for HCC1937 after radiation compared to 6 Gy but no differences after proton and X-rays groups.

We expected protons would induce greater senescence because they are more massive than photons and have a higher linear energy transfer (LET), resulting in more double strand breaks and thus greater replicative stress.

After completing our data sets in vitro, we plan to design experiments to evaluate our results in vivo. We hope that our in vitro results are also reflected in vivo and that greater senescence results in better tumor control.

References

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