The 4T1 Cell Line Shows Increased Radiosensitivity When Treated With ATR Inhibitors

Ilsa Qureshi, Akanksha Acharya, Rishab Kolachina, David Flint, Scott Bright, Simona Shaitelman, Gabriel Sawakuchi

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

Background

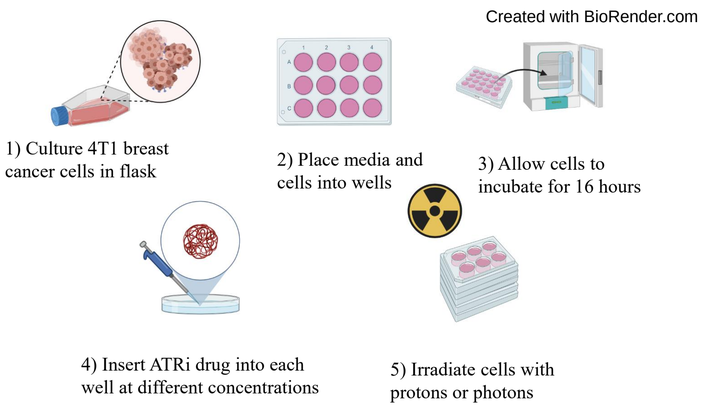
The ataxia telangiectasia and Rad3-related (ATR) protein has several roles in DNA damage response, including DNA double-strand break (DSB) repair through homologous recombination (HR) and cell cycle checkpoint control at intra-S and G2 checkpoints (Mei et al.). Combining ATR inhibitors (ATRi) with x-ray or proton radiation may lead to synthetic lethality in cancer cells. In particular, protons may provide synergistic effects with ATR inhibition due to their capacity to create more clustered DSB lesions, which require a higher contribution of HR, compared to less clustered DSB lesions induced by x-rays. In this work, we quantified the effectiveness of ATRi in sensitizing the 4T1 cell line, a murine triple-negative breast cancer cell line, to x-rays and protons.

Hypothesis

Increasing concentrations of ATRi added to the 4T1 cell line will radiosensitize cells to proton and photon radiation.

Methods

We performed clonogenic assays with 4T1 cells, treated with and without an ATRi (AZD6738). This was done at concentrations of 0.1 μM and 0.5 μM, with DMSO used as vehicle. The cells were then irradiated with 6 MV x-rays or 9.9 keV/μm protons. At least 3 independent repeats were done for each condition. To quantify drug sensitization at a given radiation quality, we used the sensitization enhancement ratio, SER: $SER=D_{10\%,DMSO}/D_{10\%,AZD6738}$, where $D_{10\%}$ is dose at 10% survival fraction.



Analysis

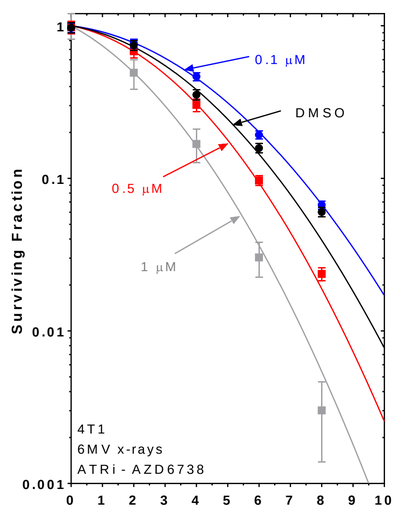


Figure 1: 10% survival is achieved using lower doses of proton therapy with the higher concentration of ATR (0.5μM).

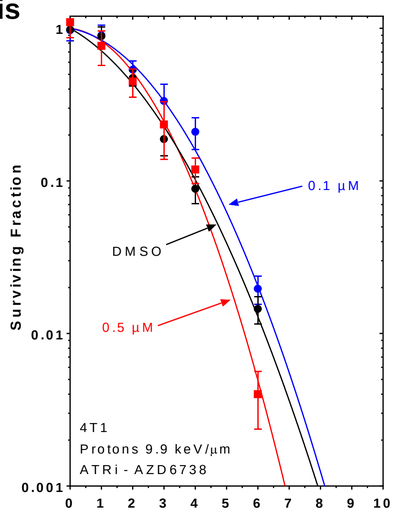


Figure 2: The higher concentrations of ATR (1 μM and 0.5 μM) achieve 10% survival with lower doses of radiation in comparison to the vehicle and lower concentration (0.1 μM)

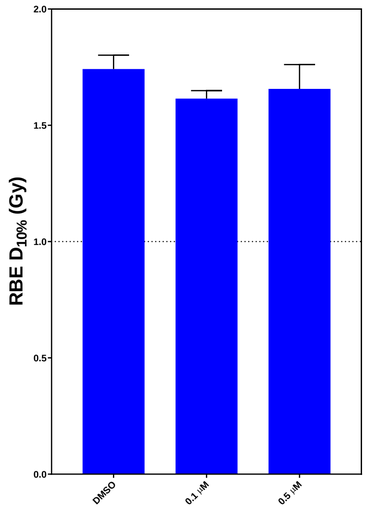


Figure 3: The RBE values across all concentrations and the vehicle (DMSO) is above 1.5 indicating proton therapy is effective.

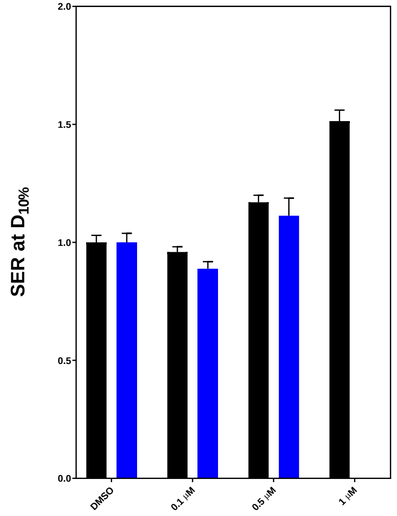


Figure 4: The low concentration of ATR (0.1μM) is below 1, indicating that smaller concentrations of ATR do not sensitize cells when treated with proton or photon therapy. At a concentration of 0.5 μM, the ATRi sensitizes cells, and more so in proton therapy.

Results

The SER value for the 4T1 at $D_{10\%}$, when treated with x-rays and ATRi (0.1 μM), was found to be 0.96 ± 0.16. With the higher concentration of ATRi (0.5 μM), the SER value was found to be 1.17 ± 0.12. This indicates that 4T1 cells are more radiosensitive at higher concentrations of ATRi. The SER value for the 4T1 cells treated with the low concentration of the ATRi (0.1 μM) and 9.9 keV/μm protons was 0.88 ± 0.13 while the SER value for the higher concentration (0.5 μM of AZD6738) was 1.26 ± 0.14.

Conclusion

With the higher concentration of ATRi (0.5 μM), 4T1 cells are more radiosensitive than the lower concentration of ATRi (0.1 μM) and the vehicle for both x-rays and protons. We found that the ATR inhibitor is a more effective radiosensitizer to protons than to photons likely because protons are more densely ionizing resulting in more complex DNA breaks. Additionally, 1 μM of the ATR inhibitor greatly senstizes 4T1 cells to photons.

Discussion

Utilizing photon radiation therapy contributes towards approximately 40% of curative cancer treatments (Baskar et al.). Furthermore, proton radiation therapy allows for greater specificity towards targeting carcinogenic tissue, decreasing irradiation of healthy tissue (Suit and Urie). When considering tumors located in delicate areas of the body, such as the brain, it is vital to apply the greatest direct dosage, while keeping irradiation to non-target areas to a minimum. Hence, The addition of inhibiting drugs, such as ATRi, sensitizes cells, allowing for cell death, and therefore tumor shrinkage, to be achieved using smaller, less toxic doses.

References

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Suit, Herman, and Marcia Urie. “Proton Beams in Radiation Therapy.” Oxford Academic, Journal of the National Cancer Institute, 5 Feb. 1992, academic.oup.com/jnci/article-abstract/84/3/155/895613.