Radiosensitivity of Cervical Cancer Cells as a factor of L. iners presence
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Abstract

Studies have identified some HPV+ Cervical Cancers as resistant to x-ray radiation treatment, which is responsible for most of the Cervical Cancer (CC) treatment and the standard of care for most treatment facilities with chemotherapy. Patient-derived CC samples have shown to have upregulated L-Lactate consumption, especially when in the presence of Lactobacillus iners (L. iners), a gram-positive bacteria found in cervical tissue, which does not consume L-Lactate. The objective of experimentation was to analyze inherent sensitivity to DNA damage and repair in cervical cancer cells.

Using patient-derived L. iners supernatant samples, we were able to test whether L. iners contributes to the recovery of CC cells after varying doses of radiation via clonogenic cell survival assays. Additionally, we were able to utilize antibodies observe γH2AX DNA damage repair foci. Overall, the results from clonogenic assay experimentation were inconclusive with respect to cancer cell proliferation post-irradiation and with or without L. iners supernatant treatments. The lack of conclusive results may be the result of identified mycoplasma contamination within RPMI media used to grow the CaSki cells during treatment. Ergo, additional studies should evaluate the proliferation of CaSki cells after supernatant treatments and x-ray doses. Despite these results from clonogenic assays, γH2AX foci formation was markedly greater with x-ray radiation compared to without x-ray radiation treatment. Additional studies should focus on whether L. iners significantly influences the intensity of foci formation.

Introduction

Cervical cancer is the 4th most common cancer in women with approximately 600,000 new cases annually, with ~95% of those cases shown to be HPV+ infections. Across HPV+ CCs, most have been shown to be resistant to radiation treatments which correspond to most clinical standard of care procedures. Higher Gray radiation treatments are consistent with worse side effects after treatment. Additionally, x-ray irradiation is responsible for most of CC treatment, supplemented with chemotherapies as necessary.

L. iners, a bacteria identified as essential to a cervix’s normal microbiome, has a mechanism for added digestion of L-Lactate (Fig. 1) in normal cervix tissue and CC samples.

Materials and Methods

Clonogenic Cell Survival Assay Protocol

- Treat cells in suspension
- Incubate overnight
- Count colonies and obtain plating fraction (%)
- Allow cells to reattach for 2-4 h
- Rinse with PBS and dry
- Add mitomycin C (if needed)
- Incubate at 37°C
- Fix cells in 3:1 methanol:glacial acetic acid

Results

The γH2AX foci immunofluorescence studies yielded viable data which demonstrated that irradiation prompted more γH2AX foci formation than without radiation treatment (control). The γH2AX foci stained as circular green dots were markedly greater with radiation treatment with respect to without radiation treatment as seen by both visual analysis as well as γH2AX foci intensity analysis (displayed in Arbitrary Units (AU)) as shown in Figs. 6 and 7.

Conclusion

Experimentation via the clonogenic assay protocol shown in Fig. 3 did not yield conclusive results indicating whether L. iners has a significant effect on CC cell recovery post-irradiation. However, the assay did prove to be informative for further γH2AX foci assays using CaSki cells such that it improved the seeding densities used for irradiation treatment. After the seeding density of this assay was optimized, the colonies formed, and distribution of these colonies appeared to be meaningful for this assay. However, the data obtained from the revised assays was not viable due to inconsistent colony distribution and less than 50 cells per colony. Additionally, reassessing this assay allowed us to exploit a potential source of experimentation error in the form of mycoplasma contamination via using new, untriminated RPMI media for cell growth.

The γH2AX foci immunofluorescence studies by supernatant treatment allowed for the visualization of the damage response of CaSki cells in vitro with respect to control. As anticipated, the untreated treated cells which were irradiated demonstrated a markedly greater DNA double stranded break response compared to control. Visual analysis via antibody staining enabled the clear visualization of the damage response in the control group, rather a lack thereof, and the γH2AX foci formed in response to the x-ray radiation stressor. Intensity analysis of the average foci formed per supernatant treatment at each radiation dose demonstrated greater foci formation in cells which were treated with L. iners supernatants with respect to non-L. iners groups. ATCC L. iners treated CaSki cells formed significantly more foci post-exposure to x-ray radiation compared to both controls (NYC and RPMI), however, the ATCC group observed the smallest difference between ATCC L. iners x-ray treatment and ATCC L. iners exposed to 4 Gy radiation dose, evidenced by Figs. 6b and 7.

Discussion

L. iners has previously been demonstrated to significantly improve HPV+ CC cell recovery post-irradiation. As shown in Figs. 6 and 7, groups exposed to x-ray radiation experienced markedly greater foci formation with respect to groups without radiation treatment (control). Interestingly, the intensity of γH2AX foci formed by supernatant treatment with the greatest difference between x-ray control and x-ray exposed being patient 366 supernatant sample. Also, the L. iners treated cells exhibited a larger cell size despite the same magnification and exposure settings used on all samples. This does correlate with the greater intensity of L. iners treated cells (Fig. 7); however, there is no established link between the two observations. While there is a suggested mechanism for the formation of γH2AX foci in CC cells, there is no such mechanism illustrating the effect of L. iners on the DNA double-stranded break recovery of CC cells. Ergo, it would be useful to accurately determine whether L. iners are responsible for the higher γH2AX foci intensity, demonstrated in Figs. 6, via additional immunofluorescence studies using the protocol outlined in Fig. 4.

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Citations