Investigating a Potential Relationship Between Distinct Cancer-Associated *Lactobacillus iners* and Chemoradiotherapy Resistance in Cervical Cancer Patients

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Background & Introduction

- Chemoradiotherapy (CRT) is the combined efficacious administration of both chemotherapy (Cisplatin) and chemoradiation as an antineoplastic and cytotoxic approach.[1]
- However, instances of poor clinical response to treatment, chemoradiotherapy resistance, and incurable relapse in cervical cancer patients are alarming, prompting the investigation of potential markers and underlying sources of CRT resistance.
- Previous research suggests that tumor microbiomes predominated by cancer-associated *Lactobacillus iners* are significantly associated with poor patient outcomes, unfeasible recoveries, and decreased recurrence free-survival rates.
- Cancer-associated *Lactobacillus iners* do not exhibit protective capabilities in the vaginal microbiome as this species cannot maintain intravaginal acidity, contributes to the onset and progression of infectious conditions, and lacks the genes capable of producing hydrogen peroxide and D-lactic acid.[4]
- *Lactobacillus iners*' association with poor response to radiation is potentially attributed to its distinctively adaptive, altered, heterogeneous, and small genome size, which is notably unique in contrast to other strains of the *Lactobacillus* species.[3]

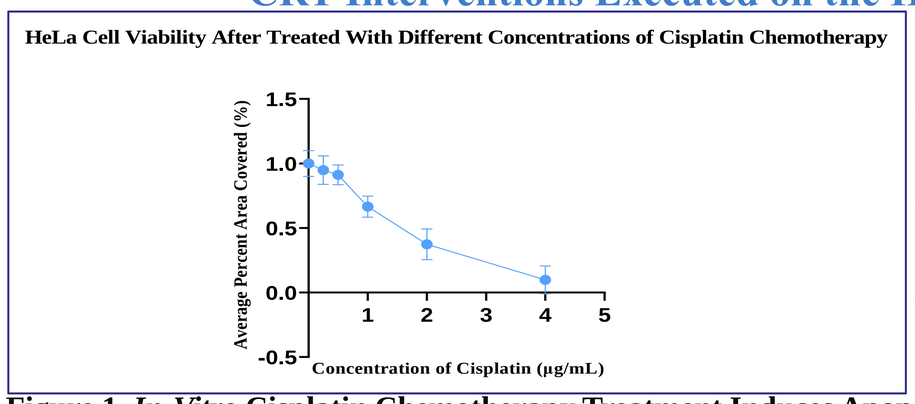
Purpose & Hypothesis

- Cervical cancer is the fourth most common cancer amongst women worldwide and is a major cause of patient morbidity and mortality.[2]
- There is an insufficient amount of identified and substantiated molecular markers to pinpoint patients who will respond poorly to treatment.
- The tumor microbiome serves as a manifestation of the potential radio-sensitivity of cervical cancer cells, which further indicates how cervical cancer cells will react to CRT interventions.
- Clarifying the damaging *in-vitro* effects of cancer-associated *L. iners* on cervical cancer patients promotes the detection and removal of *L. iners* from tumor microenvironments, leading to increased recurrence free survival rates and improved patient outcomes.
- Knowledge of this complex and ambiguous relationship between cancer-associated *L. iners* and cervical cancer cells in the context of CRT empowers the generation of enhanced prevention measures and low-risk, focused interventions, such as probiotics, bacteriocins, and lytic phages.
- After performing Clonogenic Assays and Cell Titer Glo Luminescent Cell Viability Assays for HeLa cells co-cultured with 50% supernatants produced by cancer-derived *L. iners* isolated from patients 366 and 370, the HeLa cells will increase in survival after irradiation alone and after both chemotherapy (Cisplatin) and chemoradiation treatment in comparison to the control group of NYC III broth and the ATCC *L. iners* strain treatment group. Thus, cancer-derived *L. iners* instigate resistance to both radiation and chemotherapy interventions.

Methodology

To establish validated treatment groups for effective Cisplatin chemotherapy as well as chemoradiation dosages and to institute negative control groups for comparison and analysis, clonogenic assays were performed through crystal violet staining in addition to Cell Titer Glo Luminescent Cell Viability Assays. HeLa cells were co-cultured with diverse *L. iners* supernatant groups isolated from cervical cancer patients (PT 366 and PT 370) or a BV patient (ATCC Strain), subjected to potent doses of Cisplatin, irradiated with 1.5 Gy or 3.0 Gy in the X-RAD, and subsequently incubated for 2 weeks.

CRT Interventions Executed on the HeLa Cervical Cancer Cell Line



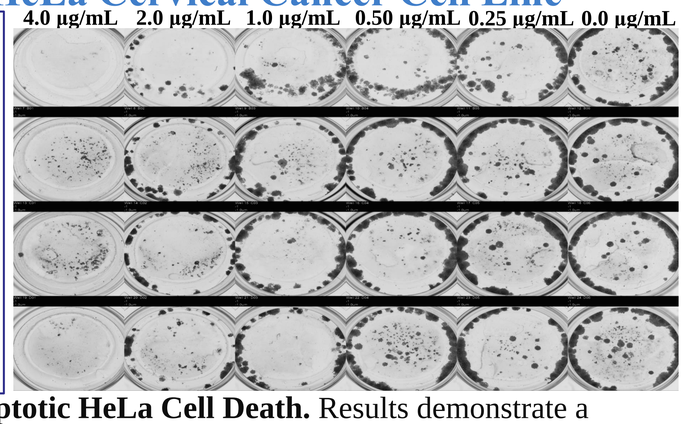


Figure 1. *In-Vitro* Cisplatin Chemotherapy Treatment Induces Apoptotic HeLa Cell Death. Results demonstrate a negative trend in HeLa cell viability after 1.0 µg/mL of Cisplatin chemotherapy was administered. The HeLa cells illustrated a substantial degree of sensitivity to the Cisplatin, which provides a verified standard for the *L. iners* supernatant experiment to determine if they impose a potential resistant effect, enhancing HeLa cell survival.

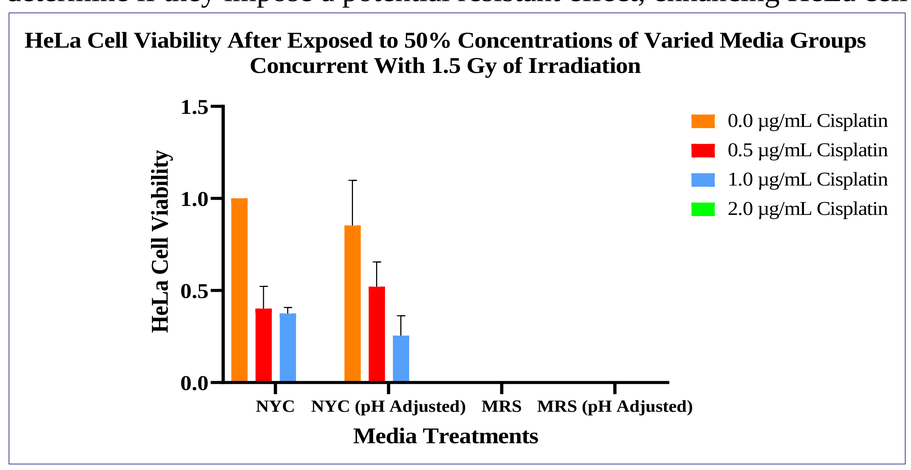
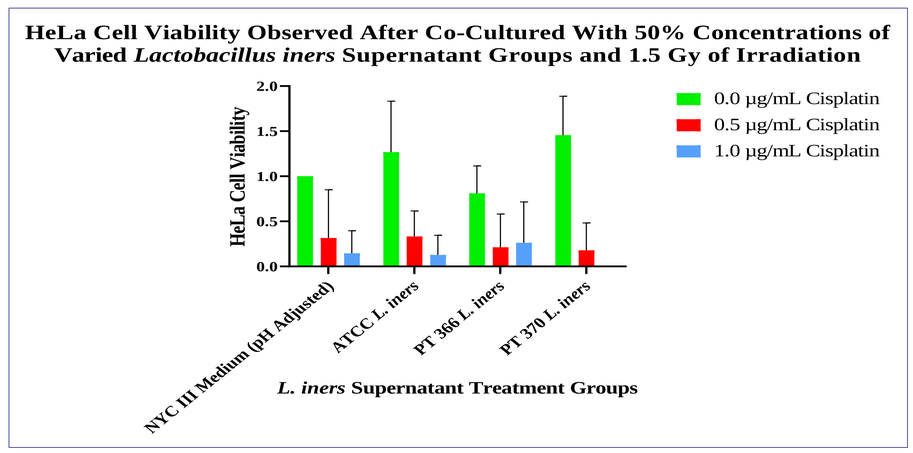


Figure 2. Cell Titer-Glo Luminescent Cell Viability Assay of HeLa Cells Apportioned 50% Concentrations of Disparate NYC III or MRS Media Groups and 1.5 Gy of Irradiation. There was a sharp decline in HeLa cell survival after 1.0 µg/mL of Cisplatin chemotherapy combined with 1.5 Gy of radiation was distributed. This reinforces the idea of sensitivity of cervical cancer cells to CRT interventions at low dosages. Results clarify that the 50% pH-adjusted NYC III Media is a validated negative control concentration that effectively reduces HeLa cell viability while preventing the complete elimination of all cells present in the wells, establishing a baseline for evaluation.



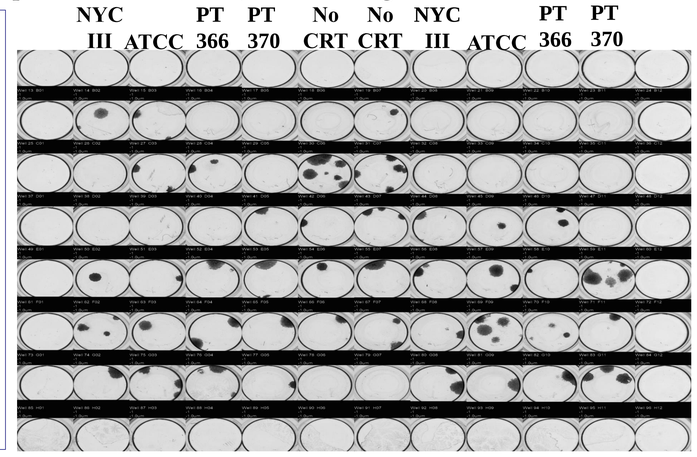


Figure 3. Clonogenic Assay of HeLa Cell Survival Observed After Exposure to 1.5 Gy of Chemoradiation and Co-Cultured with Varied Supernatant Groups of 50% Concentrations Derived From *Lactobacillus iners*. The grouped bar-chart and representative image suggest a notable form of CRT resistance ascribed to the cancer-associated *L. iners* supernatant groups isolated from Patient 366 and Patient 370. HeLa cells co-cultured with PT 366 *L. iners* demonstrated increased propagation through its numerous sustained colonies unparalleled by other treatment groups after treated with 1.0 µg/mL of Cisplatin (the highest dose), and the *L. iners* supernatant derived from PT 370 minimized the radio-sensitivity of the cervical cancer cells to the low level of chemoradiation (1.5 Gy), suggesting that cancer-associated *L. iners* confer CRT resistance.

Literature Cited

1) Rallis, K., *et al.*, Anticancer Research 2021; 1-7
2) Buskwofie, A., *et al.*, J. Natl. Med. Assoc. 2020; 229-232
3) Zheng, N., *et al.*, Frontiers 2021; 1-12
4) Nilsen, T., *et al.*, Appl. Environ. Microbiol. 2020; 1-20

Discussion & Conclusions

- This novel pathotype of cancer-associated *Lactobacillus iners* promotes the *in-vitro* resistance of cervical cancer cells against CRT while modifying the local tumor immunologic microenvironment, sustaining dysfunctional immune responses to introduced therapeutic agents and curtailing the radio-sensitivity of cervical cancer cells.
- Thus, this proposed detrimental *in-vitro* influence of cancer-associated *Lactobacillus iners'* supernatants on cervical cancer cells must be further scrutinized to corroborate evidence of it as a validated marker in the tumor microbiome that signals treatment resistance and predicts poor clinical responses to chemoradiotherapy modalities
- Discerning this association equips researchers with the insight to ameliorate recurrence-free survival rates, overall survival rates, and the quality of life of all cervical cancer patients.

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