DNA Repair Dynamics In Colon Adenocarcinoma Cell Lines Following UVC Treatment

Tristan O'Donoghue, Andrej Podlutsky

1. Department of Biology and Wildlife, University of Alaska Fairbanks

Background

-More than half of patients in the United States who are diagnosed with cancer are treated with radiation therapy.
-Colorectal cancer is one of the deadliest cancers worldwide, responsible for approximately 750,000 death per year.
-The goal of this therapy is to damage cancerous cells by destroying genetic components that facilitate cell growth and division.
-Damaging cells with UV-C shows how well they are able to perform Nucleotide Excision Repair, a fundamental DNA repair pathway.
-Collecting this data will gain a further understanding of how cancerous cell lines respond to DNA damage.
-Exact cause of the disease is not completely understood.

Objectives

-Two Colon adenocarcinoma cell lines, HT-29 and HTC-116, were examined. These cells lines are established in-vitro models for colon cancer.

-Little research has been done on how these cell lines are able to perform nucleotide excision repair (NER, UV-light), a pathway affected by UV radiation.

-Understanding these repair pathways is important for basic knowledge on the biology of cancer, being important not only to human health and wellness, but to that of cancers affecting animal health also.

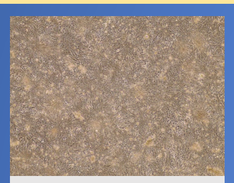

Image 1. HTC-116 cells attached to culture dish at 100% confluence.

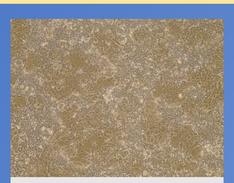

Image 2. HT-29 cells attached to culture dish at 100% confluence.

Methods

The design of this experiment consists of two elements; cell culturing and comet assay methodology.
-Cell culture involves ensuring uniform, sterile growing environments in an incubator set to 37°C.
-Cells are subjected to the comet assay experiment with human fibroblast cells being used as the control.
-Doses of UV-C treated in the experiment are 10 and 20J/m^2 and data will be collected on post-exposure times of 1, 2, 4, and 6 hour increments.
-Slides are then placed in lysis solution to unwind cellular DNA. Following electrophoresis, the slides will be fixed in ethanol.

Image 3. Three well slides used in experiment. Cells are mixed with agar and then pipetted into each well before being irradiated.

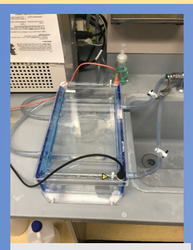

Image 4. Electrophoresis chamber used to aid in visualizing DNA damage from radiation.

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Single Cell gel Electrophoresis

-The comet assay uses the single cell gel electrophoresis technique to measure DNA damage in the form of irradiation, chemical, and drugs.
-In this case, UV radiation will damage the cell and DNA will move through the agarose as the smallest fragments are pushed further away. This process creates a comet-like visual where the damaged portion of the cell creates a "tail" following the head of the cell which is the undamaged DNA.
-Because of the straightforward way to measure damage from treatments, this makes the comet assay a useful technique to use in cancer research in order to test the efficacy of various external factors.

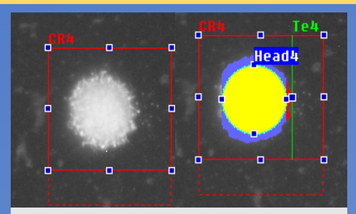

Image 5. Vis-Comet analysis software measuring a cell with no damage.

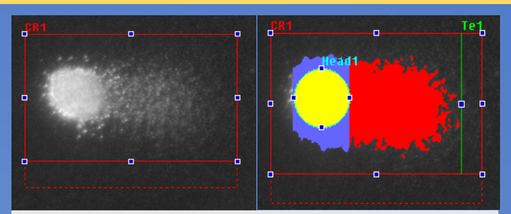

Image 6. Vis-Comet analysis software measure a cell with approximately 40% DNA damage exhibited in the tail section.

Results

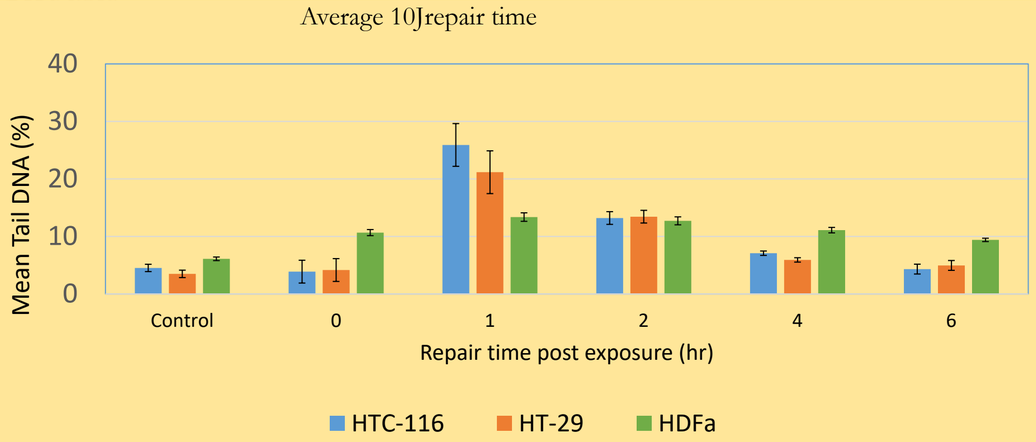

Figure 1. Average mean values of three experiments from DNA damage of HT-29, HTC-116, and HDFa following 10J/m^2 of UV irradiation. All error bars of same repair times overlap between the two cancer cell lines while some of HDFa repair times appear to show no overlap.

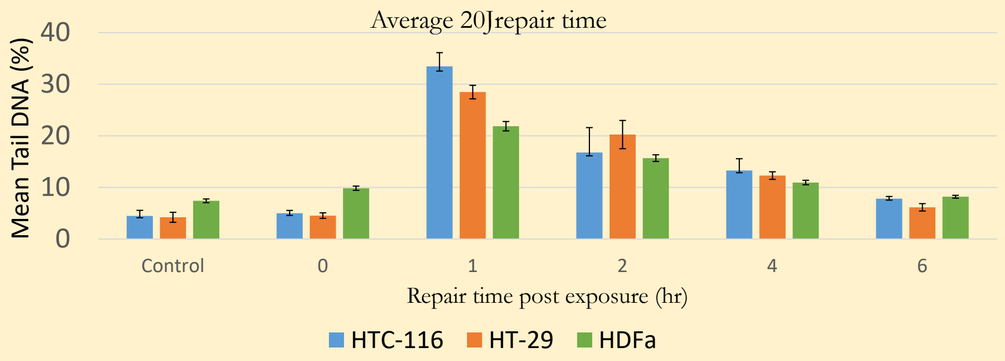

Figure 2. Average mean values of three experiments from DNA damage of HT-29 and HTC-116 following 20J/m^2 of UV irradiation. All but the 1 hour exposure time reveal overlapping error bars between the two cancer cell lines while some of HDFa repair times appear to show no overlap.

Conclusion

Our final results are indicating:
-Because of overlap in error bars within almost all expressed repair times, there cannot be a statistically significant difference between the NER repair pathways of HTC-116 and HT-29. This implies that UV damage will be corrected in similar manners between both colon cancer cell lines.
-Both Colon Cancer cell lines responded to the UV radiation with more variance in the amount of damage to the cell compared to HDFa cells. This indicates colon cancer cells may have dissimilar NER mechanisms compared to normal cells.

Future Directions

-This data will be formatted as two entries into a developing online database that will contain approximately one hundred cancerous cells lines used in cancer research in the United States and around the world. This database can be found at https://sites.google.com/alaska.edu/podlutskylab/cancer-cell-line-database
-More cell lines, such as ovarian carcinoma cells, will be tested under the same standard conditions compared to HT-29 and HTC-116. This comparison will inform whether different types of malignant cancer are similarly damaged when treated with the same dosage of UV radiation.

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References

Declan J. McKenna, Stephanie R. McKeown, Valerie J. McKelvey-Martin, Potential use of the comet assay in the clinical management of cancer, *Mutagenesis*, Volume 23, Issue 3, May 2008, Pages 183–190

Pietro T. Inhibition of Base Excision Repair Potentiates Iododeoxyuridine-induced Cytotoxicity and Radiosensitization. Cancer Res. 2003;63(4):838–846.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu J-C, Sasaki YF. Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. Environmental and Molecular Mutagenesis. 2000;35(3):206–221.

Bray, F. , Ferlay, J. , Soerjomataram, I. , Siegel, R. L., Torre, L. A. and Jemal, A. (2018), Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68: 394-424. doi:10.3322/caac.21492