DNA Repair in Virally Infected Cells

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BLaST BIOMEDICAL LEARNING AND STUDENT TRAINING

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Background

- UV-C and X-ray radiation induces damage on cells [3]
  - UV-type of DNA damages (helix-distorting lesions) are repaired by nucleotide excision repair (NER)
  - Small, non-helix distorting lesions are repaired by base excision repair (BER)
- If NER and/or BER are not successful in repair, double strand breaks occur. Resulting mechanisms for repair are non-homologous DNA end joining and homologous recombination mediated repair, both of which can lead to cancerous mutations [1]
- BCBL-1: Body Cavity Based Lymphoma cell line infected with Human Herpes Virus 8 (HHV8)[4]
- Kaposi's sarcoma is directly
- caused by HHV8 [2]
- Kaposi's sarcoma is 3,640 times more likely in HIV/AIDS patients than in healthy populations [5]

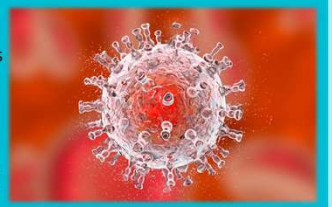

Figure 1. Computer generated image of Kaposi's Sarcoma Herpes Virus

Objectives

- Identify differences between repair times of latent and active BCBL-1 cells following UV-C and X-ray exposure
- Establish an understanding of the effects of active viral production on DNA repair and formation of cancer

Methods

- BCBL-1 cells were grown in suspension within specialized growth media
- Cells were treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) to activate the production of viral proteins 48hrs prior to UV treatment [6]
- Comet assay protocol was followed for UV-C treatment and repair
- Fluorescent microscopy was used for imaging
- Analysis was performed using Comet IV software

Figure 2. Sabrina checking BCBL-1 stock viability

Figure 3. Samantha splitting BCBL-1 stock

ACTIVE VIRUS → MORE DNA DAMAGE

Results

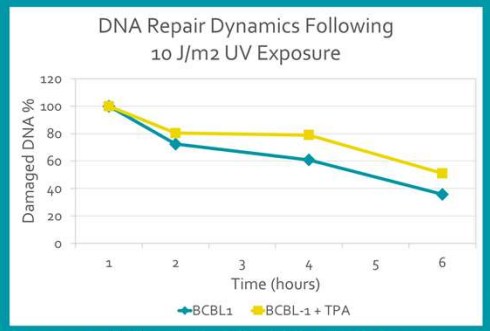

Figure 4. Following 10J/m² UV-C exposure, TPA-treated BCBL-1 appear to retain more DNA damage than untreated BCBL-1 *

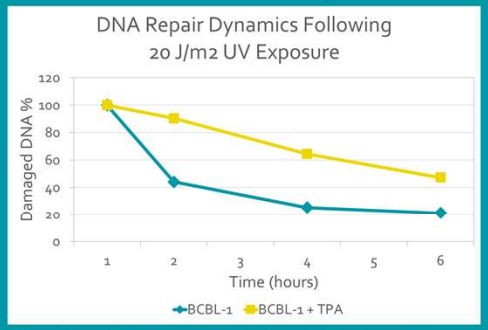

Figure 5. Following 20J/m² UV-C exposure, TPA-treated BCBL-1 appear to retain more DNA damage than untreated BCBL-1 *

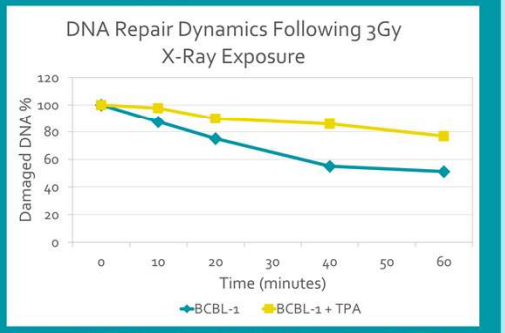

Figure 6. Following 3Gy X-ray exposure, TPA-treated BCBL-1 clearly retain more damaged DNA than untreated BCBL-1 *Based on preliminary data

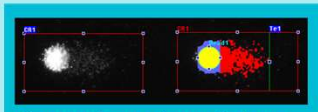

Figure 7. Comet shown of a latent BCBL-1 cell after 20J exposure and 4 hours of repair during Comet IV analysis. Exhibits 12.51% damaged DNA

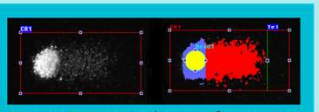

Figure 8. Comet shown of an active BCBL-1 cell after 20J exposure and 4 hours of repair during Comet IV analysis. Exhibits 26.37% damaged DNA

Conclusions

- Active BCBL-1 cells repair DNA damage significantly slower than latent cells following UV-C and X-ray exposures
- Active viral production in TPA-treated BCBL-1 cells appears to lower DNA repair efficiency by both NER and BER pathways

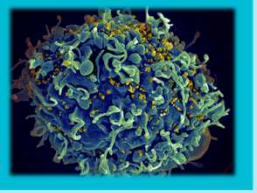

Figure 9. Scanning electron micrograph of HIV-infected CD4 cell

Future Studies

- Continue to perform experimentation and analysis comparing latent and active BCBL-1 following UV-C exposure to obtain final results on current project
- Utilize VolTRAX V2 library prep device and MinION Nanopore DNA sequencer to detect genomic variation in latent and active BCBL-1 cells

References and Acknowledgments

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