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OBJECTIVES

- Obtain gene expression profile of BCBL-1 cells using Oxford's Nanopore MinION sequencer
- Determine differences in gene expression between latent and lytic BCBL-1 cells following X-ray exposure
- Identify DNA repair-associated gene expression in each treatment

BACKGROUND

- Faulty DNA repair leads to cancer formation¹
- We previously found viral activity affects DNA repair
- Exact viral influence on DNA repair is unknown²
- BCBL-1 cells are infected with cancer-causing virus³
- Nanopore technology generates full length reads of viral transcripts⁴⁻⁶



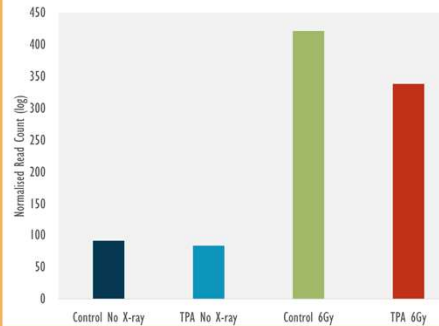
Figure 1. Computer generated image of Kaposi's Sarcoma Herpes Virus

METHODS

- Cultured BCBL-1 cells
- Activated viral production with 12-O-tetradecanoylphorbol-13-acetate (TPA)
- Treated half the flasks with 6Gy X-ray to induce DNA damage
- Extracted RNA 4 hours following X-Ray treatment
- Sequenced transcriptome using the Nanopore MinION Sequencer (PCR-cDNA Barcoding kit)
- Used DESeq2 for differential expression analysis

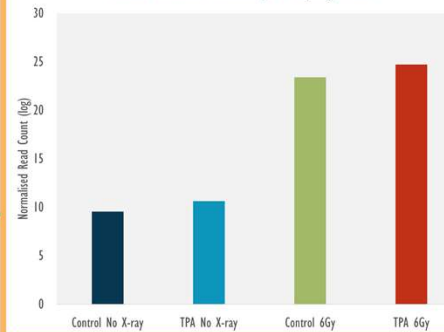
RESULTS

Figure 2. Following X-ray exposure, Stress-Inducible Scaffold Protein transcript is upregulated



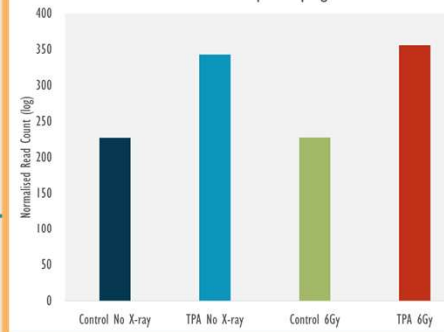
- SQSTM1—stress-inducible scaffold protein
- Regulates endosomal trafficking, apoptosis, and inflammation
- Known protein diagnostic and prognostic marker for many cancers⁷

Figure 3. Following X-ray exposure, Cyclin-Dependent Kinase Inhibitor transcript is upregulated



- CDKN1A—cyclin-dependent kinase inhibitor
- Arrests cell at G₁ stage to repair damage or induce apoptosis⁸

Figure 4. Following viral induction by TPA, Galectin-I transcript is upregulated



- LGALS1—Galectin-I
- Modulates cell-cell and cell-matrix interactions
- Regulates cell proliferation⁹

INTERPRETATIONS

- Proof of principal concept demonstrated transcriptomic sequencing ability
- SQSTM1 expression may be due to its role in the apoptotic pathway in response to damage⁷
- CDKN1A expression likely related to cell cycle arrest at G₁ in order to repair damaged DNA or lead to apoptosis⁸
- Viral induction upregulates LGALS1 perhaps due to protein's ability to inhibit infectivity and viral production⁹

FUTURE DIRECTIONS

- Analyze and interpret remainder of 16 genes identified
- Improve validity through separate replication of each time sample for treatments
- Examine isoforms of genes through analysis of long reads of mRNA from Nanopore
- Induce DNA damage with UV-C radiation to seek out repair pathway differences between UV and X-ray

REFERENCES AND ACKNOWLEDGEMENTS

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