

Using Viruses to Kill Cancer: Investigating the role of in vitro cell differentiation in HSV-1 double-mutant KM110red infection of hFOB and U2OS cells

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Background

In the successful development of a cancer treatment, there are two fundamental requirements that must be met: the treatment must be able to selectively induce the death of cancerous cells and it must not harm normal cells. Drugs used in chemotherapy not only affect cancerous cells but also cause harmful and unwanted toxicity to normal cells. The therapeutic index of chemotherapy is reported as 6:1 which means that for every 6 tumour cells killed by chemotherapy, one normal cell is killed¹. Thus, it is essential to design a method that accurately targets only cancerous cells so that cancer can be treated effectively with minimal side effects. One such approach is to harness the natural properties of viruses to aid in the fight against cancer. The exciting field of oncolytic virus therapy is now being tested in limited trials around the world. One of the benefits of oncolytic virus therapy is that the therapeutic index has been speculated to be as high as 100,000:1¹ which makes it appear to be a superior cancer treatment.

Herpes Simplex Virus type I (HSV-1) is an excellent candidate for cancer therapy as it has broad tissue specificity, replicates to high titres, kills infected cells, is easy to manipulate and can tolerate large insertions into its genome. From a safety standpoint, anti-herpes drugs are available as a safeguard against unwanted replication and the virus does not insert into host cell nucleic acid, eliminating the fear of insertional mutagenesis². During tumor evolution, diminished interferon (IFN) responsiveness is a common genetic dysfunction³. IFN is a multi-functional cytokine with potent anti-proliferative and antiviral activities⁴. Thus viruses, or virus mutants, whose replication is inhibited by IFN should preferentially replicate in cancerous cells and not normal cells.

KM110red (KM110r) is a double mutant HSV-1 bearing lesions in the ICP0 gene, which has been shown to function in part to overcome IFN-mediated anti-viral activities, and VP16, a virion-

associated viral transactivator. Therefore KM110r should fail to replicate in normal fibroblasts, and efficiently propagate on cancerous cells, making it a potential oncolytic virus for cancer therapy.

Purpose

To determine whether a double mutant Herpes Simplex Virus type I, KM110red (KM110r), is an effective and safe oncolytic virus therapy by exploring its effects on cancerous bone (osteosarcoma or U2OS) cells and non-cancerous precursor bone (human fetal osteoblast or hFOB) cells, and to determine whether induced genetic differentiation alters the permissiveness of hFOB cells to KM110r.

Hypotheses

Genetically engineered KM110r will target and selectively destroy all U2OS cells, and will not be toxic to hFOB cells. KM110r will be an effective and safe potential cancer treatment when administered appropriately. U2OS living cell counts will decrease post KM110r infection, while undifferentiated hFOB cells will continue to grow at constant growth rates with no identified infection.

The point during *in vitro* cell differentiation at which the hFOB cells respond to infection by KM110r will be identified and the potential underlying genetic changes investigated. This knowledge will enhance understanding of which genetic changes permit oncolytic virus infection with the intention of identifying the point along the differentiation pathway to maximize treatment efficacy.

Procedure

U2OS cells are moderately differentiated bone cancer cells that are genetically recombined with bioluminescent green fluorescent protein (GFP) to make proteins in the cells glow fluorescent green in order to identify them under ultraviolet light microscopy. hFOB cells are normal undifferentiated pre-cursor bone cells containing a temperature sensitive mutation (tsA58) that

drives differentiation in response to temperature change. KM110r is also genetically recombined with a bioluminescent fluorescent protein called red fluorescent protein (RFP) which causes any infected cells to appear red under fluorescent microscopy.

The cells were cultured in 6-well dishes which contained two wells each of hFOB, U2OS, and MIX (50% U2OS, 50% hFOB) seeded at concentrations of 1.3×10^5 cells/mL. One of each group was infected using KM110r while their counterparts were mock-infected as the negative-control. Three experimental trials were conducted at incubation temperatures of 34°C, 37°C, and 39°C to induce genetic differentiation in the hFOB cells. Each temperature trial was completely repeated to ensure reliability of the data.

Each experimental trial employed fluorescent microscopy to conduct a proliferation assay over the course of four (4) time periods spanning a five (5) day infection (since full differentiation is expected in this time period). During each time period, three (3) areas in each of the wells were imaged in bright field light, GFP and RFP channels. Approximately forty (40) images were taken each day, totaling almost one-thousand (1000) images over the course of experimentation. The cells in each image were counted and morphologically analyzed.

Any necessary display equipment certifications were obtained and will be part of the display. Approval for tissue research, as well as approval from recognized institutions and/or scientific mentors were obtained and will be available at the display.

Results/Observations

General steady increases in cell counts were recorded for U2OS uninfected (U2OS⁻) at all 3 incubation temperatures. However the U2OS infected (U2OS⁺) cells resulted in steady decreases for both the

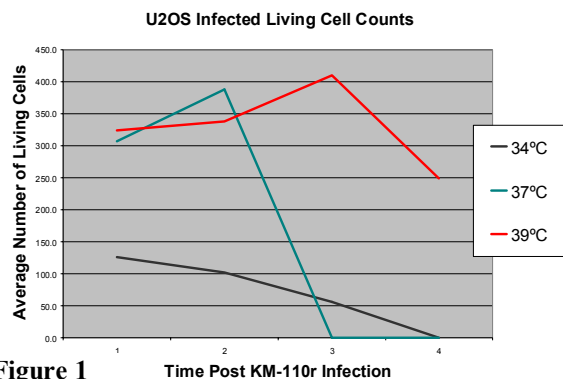


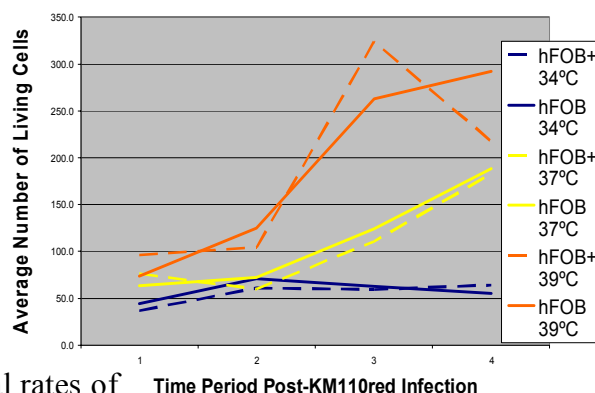
Figure 1

34°C and 37°C degree infections (**Figure 1**).

Under 34°C incubation all cells were showing infection and were dead by time period 4; however the same end results were observed at 37°C incubation by time period 3. Unexpectedly, under 39°C incubation, by time period 4 many U2OS+ cells did not show infection and were still alive. The results of an ANOVA showed statistically significant differences among U2OS+ growth trends at each temperature (significance <5%, and F-Ratio >1).

hFOB cells grew at almost identical rates regardless of incubation temperature or status of infection (**Figure 2**), as verified by a student's t-test. Throughout the entire experimentation hFOB+ cells were not permissive to KM110r.

Figure 2 hFOB Living Cell Counts



Uninfected co-cultures (MIX-) cells grew at general rates of increase regardless of incubation temperature and were consistently lower than U2OS- and higher than hFOB uninfected (hFOB-). Infected co-cultures (MIX+) cells, at 37°C and 39°C incubation remain almost constant, but MIX+ cells at 34°C incubation steadily decrease until time period 3 when it plateaus. Visual analysis of hFOB cells under bright field microscopy showed evidence of morphological changes among the 3 different incubation temperatures, indicating that genetic differentiation was occurring at 39°C incubation, as reported³.

Conclusions

The first major finding of this experiment confirms that KM110r is able to target and selectively destroy U2OS cells and is not toxic to hFOB cells over the range of temperatures 34°C, 37°C and 39°C where hFOB cells are differentiating. At 34°C and 37°C, it was clear that KM110r could successfully eradicate all of the cancerous U2OS cells within the experimentation period. However, KM110r did not effectively destroy all cancerous cells at 39°C. This strongly suggests that at 39°C KM110r infection of U2OS has been altered. Furthermore, at 37°C the cancerous cells

were completely eradicated at time period 3, one entire time period earlier than at 34°C. The significant practical application of this discovery is that as a cancer therapy, use of KM110r is optimal when administered at 37°C—or physiological body temperature—since 34°C conditions delay cell destruction and 39°C conditions do not permit successful termination of the cancerous cells. Therefore, the effectiveness of KM110r as an oncolytic virus therapy could be severely impaired if the body temperature of the cancer patient becomes hyperthermic or hypothermic, i.e. the patient spikes a fever or experiences dangerously low body temperatures, respectively.

The second major finding is that the results did not support identification of a specific point during *in vitro* cell differentiation that the hFOB cells were infected by KM110r. hFOB cells were not permissive to KM110r at any temperature condition. This reveals an advantageous quality of the virus from a gene therapeutic perspective. The differentiation that occurred in the hFOB cells at 39°C are changes that would generally occur in a normal precursor bone cells, meaning that the virus is able to continue to ignore these non-cancerous cells despite differentiation. The fact that KM110r was able to ignore hFOB cells over the course of full differentiation strongly affirms the safety of this genetically engineered virus. This finding is extremely valuable since non-cancerous cell death must be inhibited in order to avoid the negative side effects of conventional cancer therapies such as chemotherapy. Therefore this suggests that KM110r satisfies the highly sought-after criteria in the discovery of effective cancer therapies: the ability to completely terminate cancerous cells and the ability to ignore and avoid harm to non-cancerous cells.

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Appendix: Bibliography

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