A virus to treat cancer: A 3D cell model

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What is the problem?

Most cancer therapies are non-specific, for example chemotherapy or radiation. These approaches kill all growing cells in the body, not just the cancer cells. For this reason, the toxic effects cause severe adverse reactions in the body.

A more rational and directed approach to killing only cancer cells would benefit cancer patients and potentially save the NHS money as patients' health would be better during treatment.

Selected viruses, called oncolytic viruses, have been manipulated so they grow poorly in normal cells but grow well in the rapidly dividing cells that are characteristic of cancers. As a result, they selectively kill cancer cells due to replication of the virus in the cells. The mechanism of action of this new potential therapy is shown in Figure 1.

One such virus is herpes simplex virus (HSV) 1716, which is a modified version of HSV. HSV normally infects skin cells and causes cold sores, a benign condition. HSV1716 shows anti-cancer properties in a number of human cancers and has been shown to be well tolerated by patients who have been treated with HSV1716 in clinical trials. It can be used alone or in combination with chemotherapy agents as a rational approach to increase cancer cell killing. However, to date it has been difficult to study the possible beneficial effect of HSV1716 with chemotherapeutic agents in studies using cancer cells grown in the laboratory. This may be because cancer cells which are usually grown in a single layer in dishes in the laboratory possess very different properties to 3 dimensional (3D) cancers found in living organisms.

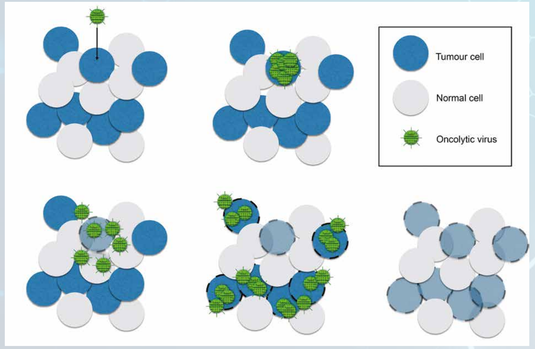
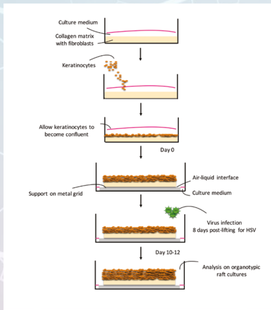


Figure 1 Mechanism of selective replication of oncolytic viruses and their killing ability. Virus (green) is able to enter and produce more viruses only in cancer cells (blue), leaving normal cells (grey) unharmed. When there are a lot of viruses inside the cell, the cell "explodes" enabling viruses to spread and infect more cancer cells.

What am I interested in?

I want to develop a 3D cancer model to analyse the effectiveness of HSV1716 alone or in combination with anti-cancer therapies for different cancer types. Such a model will improve the current methods of growing cells in the laboratory and avoid the use of the animal models.

What did I do?



I assessed whether HSV1716 and the unmodified HSV1 virus, which was used as a control, could infect, grow in and kill a range of cancer cells grown in the usual way in the laboratory. I then optimised the conditions to get the cancer cells to form tissue in 3D by a method called 3D organotypic raft (see Figure 2). I then went on to carry out preliminary tests of the effect of a range of chemotherapeutic drugs on the cancer cells grown in 3D.

Figure 2 Representation of organotypic raft culture method. A support structure (matrix), formed by collagen and cells (fibroblasts) in growth (culture) medium, is prepared on to which skin cells (keratinocytes) are added and grown until they form a complete layer of cells (they become confluent) on the matrix. The matrix is then moved on to a metal grid so that the cells can grow at the air-liquid interface and 3D tissue that is similar to the skin is formed. We can treat this tissue with the virus.

What did I find?

As previously seen with different cancer cell types, HSV1716 is able to replicate as efficiently as the normal HSV1. Viral replication was analysed using different methods, including analysis of the number of cells, the presence of viral DNA in the cells and the release of virus from infected cells. The last method is shown in Figure 3, which highlights that the viruses replicate in all the cells studied in a similar way but only the SiHa cancer cells showed a reduced ability to release HSV1716 from the infected cells compared to the HSV1 infected cells, but they still sustained the infection.

Moreover, I have shown that 3D cultured cancer cells can be infected with HSV1 and HSV1716 as shown in Figures 4 and 5 and that the effect of adding chemotherapeutic agents can also be tested.

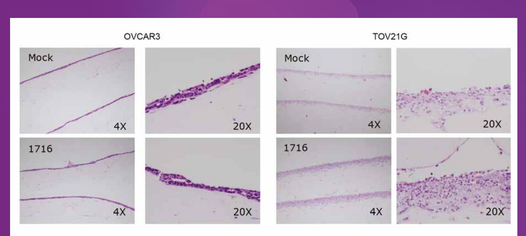


Figure 5 Examples of 3D tissue of ovarian cancer cells. Two different magnifications are shown to highlight the structure of the tissue. First row shows non-infected tissue while the second row shows tissue infected with HSV1716.

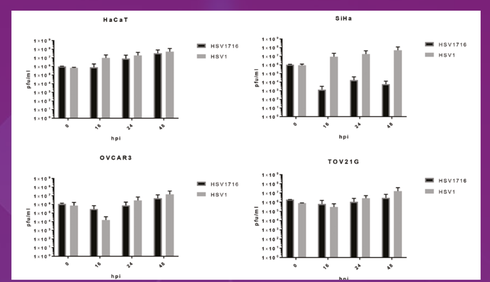


Figure 3 Virus release experiment performed in standard cell culture. The number of viral particles released (pfu/ml) at a given number of hours after infection (hpi) with either HSV1716 or HSV1 is shown for normal cells (HaCaT) and cancer cells (SiHa, OVCAR3, TOV21G).

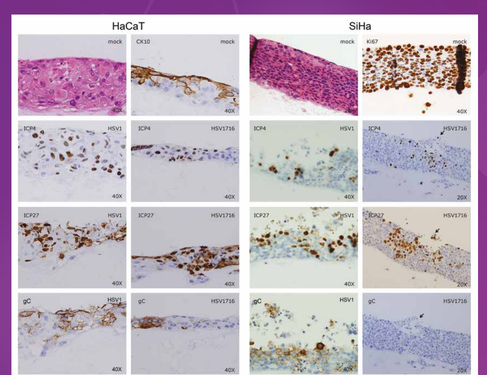


Figure 4 3D tissue grown from HaCaT (normal cells) and SiHa (cancer cells), infected with HSV1716. In the first row, uninfected tissue is shown with different staining aiming to identify the structure of the raft or cell differentiation (CK10) or cell growth (Ki67). In all these and in the following staining, the brown colour indicates the presence of the particular protein being studied. All the following rows indicate either HSV1, as a control, or HSV1716 infected tissue. The images show the presence of viral proteins at the different stages of protein production and demonstrate complete replication of the virus.

What does this mean?

I have established a powerful tool to assess the effectiveness of HSV1716. I have demonstrated that it is possible to create a 3D tissue, similar to human skin, in the laboratory under controlled conditions and that these cancer tissues can be infected with HSV1716 and can also be treated with chemotherapeutic agents.

I am currently investigating the potential combined effects of the virus with selected relevant chemotherapeutic agents in different cancer cells to find potential new treatments.

Who am I?

I am a third-year PhD student at the Centre for Virus Research, University of Glasgow where I am carrying out a project, funded by Medical Research Scotland, which I hope will result in my research findings being put into medical practice with beneficial health outcomes. My project allows me to explore the different fields of virology, cancer biology and immunity and fitted perfectly with my previous Bachelor degree in Biotechnology and Master Degree in Medical Biotechnology from the University of Milano-Bicocca.

Ilaria started her PhD project entitled *"A three dimensional tissue model for examining the mechanism of oncolytic herpes virus activity and synergy with chemotherapeutic agents in epithelial tumour cells"* in 2015 and while principally based at the University of Glasgow, supervised by Professor Sheila Graham, she is also working closely with Virttu Biologics Ltd.