Molecular and immunological correlates of latent versus productive reoviral infection in cancers

James R. Mansfield,1 Andrew Stiff,2 Matt Coffee,1 Gerard Nuovo,4 Flavia Picchioni,3 Craig C. Hofmeistera
1. PerkinElmer, Hopkinton, MA, 2. Ohio State University, Columbus, OH; 3. Oncolytics Biotech, Inc. Calgary, AB; 4. Phylgeny Labs Inc, Powell, OH

Abstract

An oncolytic virus, due to its ability to replicate and lyse cancer cells while leaving normal tissue intact, is an example of a targeted cancer therapy. Reovirus is immunologically stealthy for the need for any innate immunomodulators. While viruses can be detected inside hematopoietic cancer cells after exposure, the key question is whether a reoviral infection is productive or latent. We show that productive infection is associated with tumor cell killing by both direct apoptosis and increased T-cell and NK cell response to the tumor.

We analyzed several human multiple myeloma cell lines either sensitive or resistant to reovirus infection and a pancreatic melanoma model in mice after reoviral infection. Latent reoviral infection was defined by limited RNA production and cap production. C7HL66 mice were injected subcutaneously with RH51 or B16-NVMEC1 cells. Intracellular cell depletion avoided interventional injections of anti-CD3, anti-CD4 and anti-methylene blue control. For NK depletion, anti-asialo GM1 or rabbit IgG Fc epsilon control was injected intraperitoneally. Fluorescein-activated cell sorting of spleens and/or lymph nodes confirmed subsite-derivative splits. Pleotfried was injected intraperitoneally at 10 mg/kg per injection.

Myeloma cell lines resistant to reoviral infection showed that the majority of the cancer cells had latent reoviral infection with very weak productive infection (productive/infectious latent infection 6%). Myeloma cells with the majority of productive latent infection showed many cells with a productive viral infection (productive/infectious latent infection 78%). FISH expression was identified in the resistant and sensitive myeloma cell lines; two molecular markers that defined the sensitive myeloma cell lines were high CUG expression and low and ampicillin production in the metastatic melanoma model. Latent reoviral infection was dominant in mice treated with reovirus alone; productive infections were dominant in mice treated with reovirus and chemotherapy. In both the cell lines and metastatic melanoma model, productive infection was seen in the fibroblast-like or melanoma-like morphology and non-melanotic melanoma model. Infection was associated with increased T-cell and NK cell infiltration of the tumor. Myeloma cells in the tumor were investigated using a combination of multispectral and immunohistochemical. Multispectral imaging and unmixing enables the separation of interest, which can then be enhanced individually in the tissue. This can greatly improve throughput, saving time and money for standard pathology scoring.

Methodology

Key Component #1: Multistaging reoviral infection

Multispectral imaging and unmixing of reoviral infection is a critical step. Multispectral imaging separates the interest, which can then be enhanced individually in the tissue. This can greatly improve throughput, saving time and money for standard pathology scoring.

- Immunochemistry (patient #1) of OP cells treated with reovirus infected and stained for CD45, CD8, FOXP3, and CD20. The cell populations are quantitated by automated cell quantitation with deconvoluting the immunofluorescence data to separate the immune cells from the tumor cells.

Reoviral and caspase 3 expression in two cell lines

Reovirus expression increases apoptosis

Reovirus expression is known to induce caspase 3-4 and -7 related to the increased apoptotic death of cancer cells in clinical samples. The cancer cell apoptotic death was due to a marked viral-induced expression of needle-like C3-microtubule activity. In summary, reovirus is a potent tumor cell lysis, and is highly cytotoxic to both the virus and host.

Conclusions

1. Resistant cell lines show reoviral RNA and not protein
2. More sensitive cell lines showed increased reoviral RNA and protein with a concomitant up regulation of caspase 3
3. Chemotherapy enhances productive reoviral infection
4. A combination of multispectral imaging and software can quantify this information.