Investigating the importance of immunogenic cell death in the context of cancer immunotherapies.

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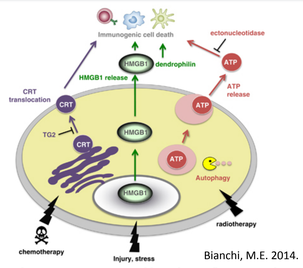
Introduction

- Oncolytic viruses (OVs) possess immune stimulating properties that can prolong survival when used as cancer therapies, but there are many gaps in our understanding of the underlying mechanisms.[1]



Bianchi, M.E. 2014.

- Irradiated tumor cells infected with an OV, called Infected Cell Vaccines (ICVs), have shown promising efficacy in animal models yet the immunogenicity of these treatments is poorly understood and they still fail to provide a durable cure.[1]
- The immunogenicity of dying tumour cells is reliant upon the externalization/release of three Immunogenic Cell Death (ICD) markers: Calreticulin, ATP, and High Mobility Group Box 1 (HMGB1).[2]
- Currently, the level of immunogenic cell death within the context of ICVs and its influence on the treatment's efficacy remains unknown.
- This project aims to characterize the release of one ICD marker (ATP) and elucidate its role in determining the immunogenicity and efficacy of ICVs.

Hypothesis

- Infected cell vaccines elicit immunogenic cell death signals that contribute to their efficacy and understanding this effect can reveal potential targets to improve their efficacy.

Methodology

- Murine melanoma cells (B16F10) and colon carcinoma cells (MC38 and CT26) were treated with ICD inducers or controls for 4 hours.
- Treated cells were stained with quinacrine (fluorescent compound that binds nucleic acids) to assess intracellular ATP release by microscopy and flow cytometry.
- Supernatants of treated B16F10 cells were collected to measure extracellular ATP using a commercially available kit (Promega) based on a luciferase reaction.

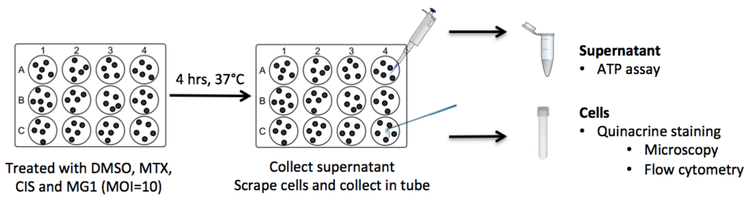


Figure 1. Method of visualizing and measuring ATP release.

Results

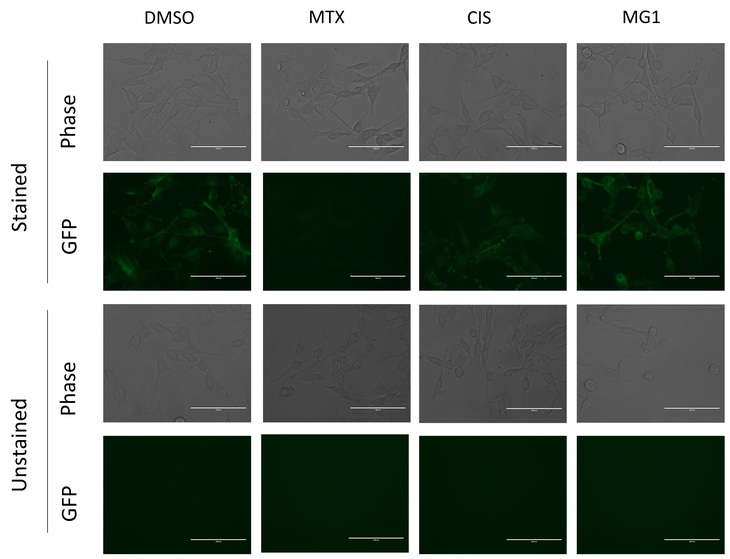


Figure 2. Fluorescent microscopy of B16F10 cells stained with quinacrine (GFP) and treated with 10uM MTX (ICD inducer), 50uM Cisplatin (non-ICD inducer), or infected with MG1 (MOI=10) for 4 hours. N=3.

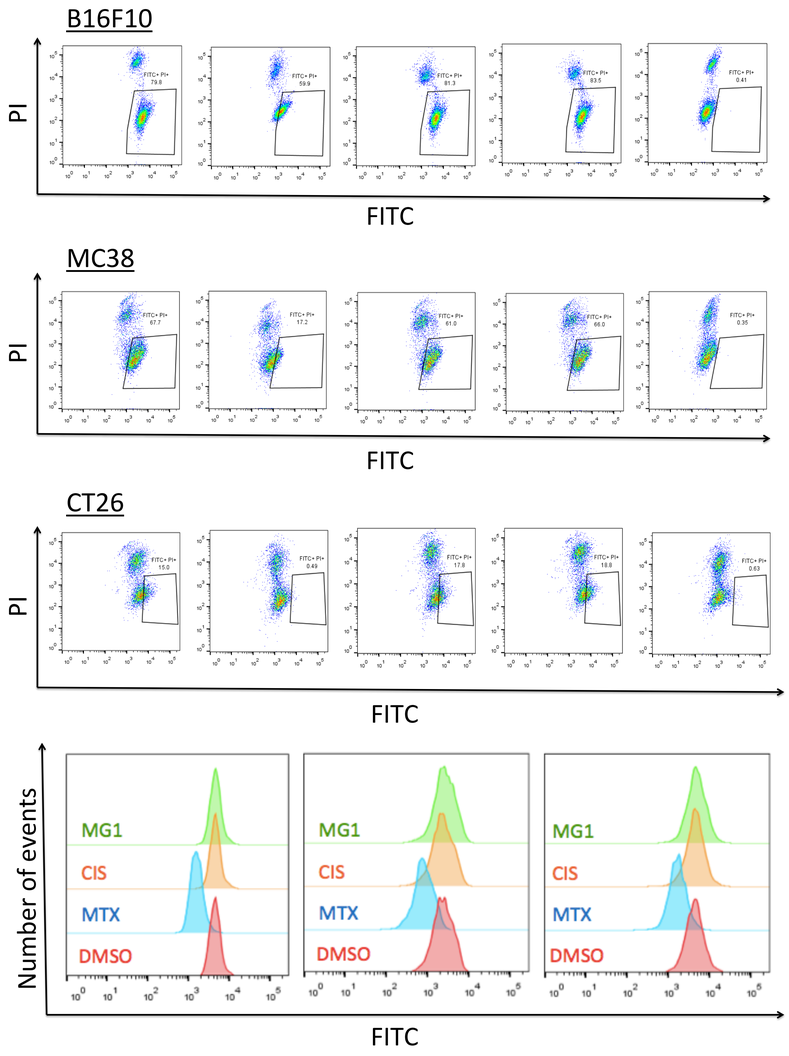


Figure 3. Flow cytometry analysis of B16F10, MC38 and CT26 cells stained with quinacrine (FITC) to label intracellular ATP and propidium iodide (PI) to assess cell viability after treatment with 10uM MTX (ICD inducer), 50uM Cisplatin (non-ICD inducer), or MG1 (MOI=10) for 4 hours. N=3.

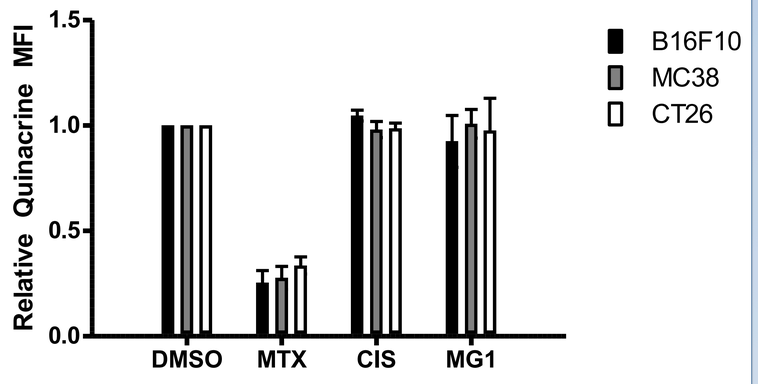


Figure 4. Relative Mean Fluorescent Intensity (MFI) for quinacrine-stained B16F10, MC38 and CT26 cells treated with 10uM MTX (ICD inducer), 50uM Cisplatin (non-ICD inducer), or infected with MG1 (MOI=10) for 4 hours

Conclusions

- MG1 infection does not result in a significant decrease in quinacrine signal when compared to control, suggesting minimal or no ATP release through ICD mechanisms.
- These results suggest that there are low levels of immunogenic cell death within the context of the infected cell vaccine, thus identifying a potential target for improvement to increase the treatment efficacy.

Future Directions

- Extracellular ATP release will be measured in B16F10 cells treated with ICD inducers and controls at later time points.
- Methods of the collection and measurement of another ICD marker (HMGB1) will be developed and optimized.

Acknowledgements & References

1. Alkayyal, A *et al*. NK-cell Recruitment Necessary for Eradication of Peritoneal Carcinomatosis with an IL-12-Expressing Maraba Virus Cellular Vaccine. Cancer Immunol Res. 2017;3:211-221. 2. . Kroemer, G *et* al. Immunogenic Cell Death in Cancer Therapy. Ann Rev Immunol. 2013;31:51-72.

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