Enucleated Cells: A Novel Platform for Delivering Oncolytic Viruses to Treat Metastatic Cancer

Bei Liu[1,2,*], Huawei Wang[1,2,*], Christina N. Alarcón[1,2,3], Felicia Watson[1,2], and Richard L. Klemke[1,2,+]

[1]Department of Pathology, [2]Moores Cancer Center, [3]Biomedical Sciences Program, University of California, San Diego, La Jolla, California
*Co-first authors; HW, presenter; +Corresponding Author: Richard L. Klemke Ph.D., Department of Pathology, School of Medicine, UCSD 9500 Gilman Drive #0612, La Jolla, CA 92093. Phone: 858-822-5610; Fax: 858-822-4566; E-mail: rklemke@ucsd.edu

Abstract

Metastatic disease remains the most significant life-threatening event for cancer patients. Oncolytic virus-mediated therapies in clinical trials hold great promise to treat metastases by stimulating endogenous immune reactions against metastatic tumor cells. However, these therapies have limited success due to the difficulty of delivering the oncolytic viruses specifically to the tumor sites. We have developed a new delivery platform in which hTERT-immortalized mesenchymal stem cells (MSCs) are genetically engineered and then enucleated to form nucleus-free cytoplasts. These cytoplasts have the ability to deliver different cargos such as small molecule drugs, proteins, and RNAs to diseased tissues in pre-clinical animal models. To test if cytoplasts can also deliver oncolytic viruses, we screened a group of oncolytic viruses currently used in clinical trials, including measles virus (oMV), herpes simplex virus (oHSV) and vesicular stomatitis virus (oVSV). We found oVSV can efficiently infect, propagate, package and be released from cytoplasts. Cytoplast-released oVSV can also infect tumor cells in vitro and cause virus-induced tumor cell lysis. We have established a pre-clinical metastatic tumor model using the triple-negative (ER-, PR-, HER2/neu-) murine breast cancer cell line E0771. In vitro experiments suggest engineered cytoplasts can migrate towards the conditioned medium of E0771 cells through CCL2-CCR2 and SDF1a-CXCR4-mediated chemotaxis. Furthermore, the oVSV-infected cytoplasts maintain their migratory capacity. Presently, we are testing if engineered cytoplasts in a syngeneic animal model can deliver oVSV to metastatic E0771 cells and trigger the immune responses against the tumor cells. Our novel delivery strategy has the potential to greatly enhance the efficiency of current oncolytic virus therapies for treating deadly metastatic disease.

Background

➢Triple Negative Breast Cancers (TNBC: ER$^-$, PR$^-$, HER2/neu$^-$) are often highly metastatic with poor prognosis under current therapies[1].

➢Oncolytic viruses (OV) such as oMV (Measles Virus), oHSV (Herpes simplex virus), and oVSV (Vesicular stomatitis virus) hold great promise as anti-cancer treatments, including metastatic TNBC[2,3].

➢Systematic administration of OVs into patients is highly inefficient because the immune system rapidly clears unprotected viruses [4].

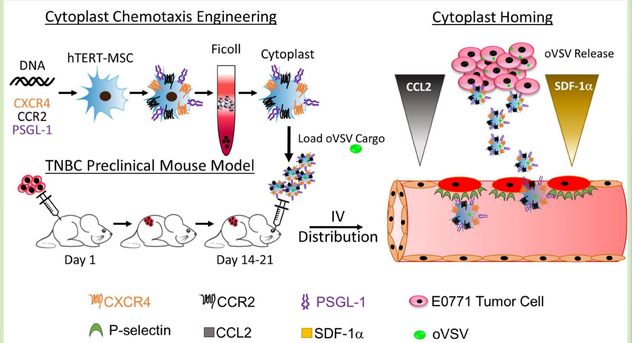
➢Therefore, there is a major need to develop biocompatible carriers that improve systemic delivery of oncolytic viruses and target them to primary and metastatic tumors in a safe manner [5,6].

➢ To study the *in vivo* tumor-delivery of OVs, a syngeneic TNBC model in immuno-competent mice is ideal.

➢Our platform technology enucleates hTERT-immortalized MSCs to generate nucleus-free cytoplasts, which can be extensively engineered with improved homing abilities to safely deliver oncolytic viruses to tumors.

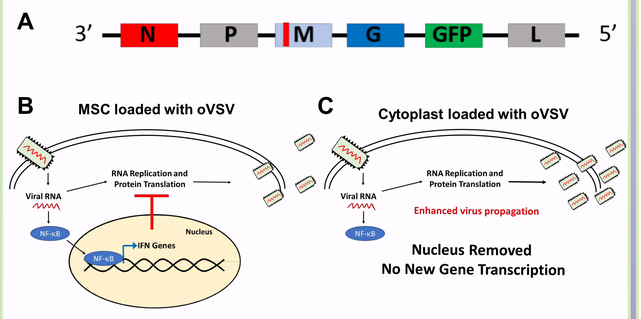
Strategy

Cytoplasts can be engineered for optimal homing and delivery of oncolytic viruses to treat TNBC.



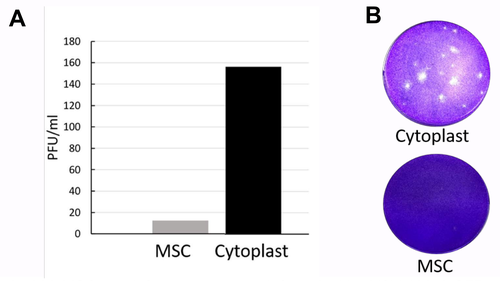
Cytoplasts can be engineered with chemokine receptors such as CXCR4, CCR2, or adhesion molecules such as PSGL-1. The engineered cytoplasts can efficiently home and deliver oVSV to treat syngeneic TNBC.

oVSV-ΔM51-GFP propagation in MSC or MSC-derived cytoplasts.



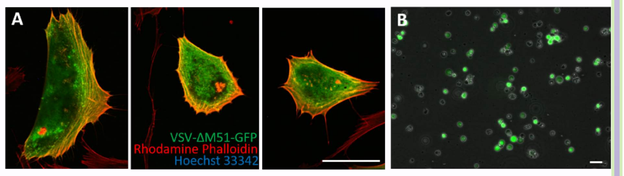
A) Schematic design of oncolytic VSV-ΔM51-GFP virus. Methionine 51 was deleted from the M protein and GFP was inserted upstream of the L polymerase gene. Wild-type M protein suppresses host gene expression in infected cells and inhibits antiviral responses. This activity is abrogated by the deletion of M51 site. **B)** In normal cells such as MSCs, oVSV infection will activate the antiviral response mediated by NF-κB and transcription of interferon α/β genes. **C)** In cytoplasts, as the nucleus has already been removed, there will be no new gene transcription, which leads to enhanced virus propagation.

Figure 1. Cytoplasts can be infected with oVSV- ΔM51-GFP at high efficiency and have functional virus propagation.



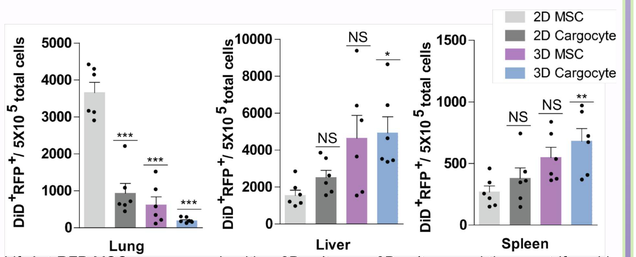
A) 25,000 MSCs and Cargocytes were infected with oVSV-ΔM51-GFP at MOI 0.1. 72 hours after infection, the plaque forming units (PFUs) from the supernatants of each group were determined by plaque assay. **B)** Representative image of Crystal violet-stained plaque assay wells in which 600,000 Vero cells were seeded into each 6-well. 24 hours later, cells were infected with 10-fold dilution of supernatants from oVSV-ΔM51-GFP-infected cargocytes or MSCs. n=3

Figure 2. oVSV-ΔM51-GFP infects MSC-derived cytoplasts and E0771 cancer cells.



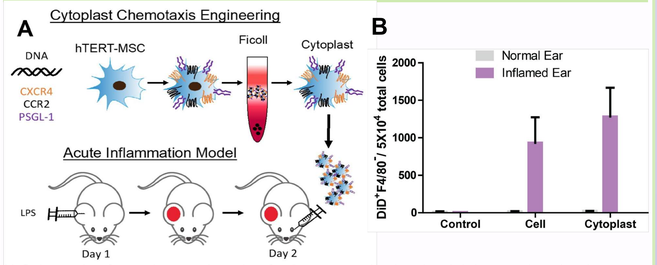
A) 25,000 cargocyte were infected with oVSV-ΔM51-GFP at MOI 0.1. After 48 hours, cargocytes were fixed, stained with anti-GFP, rhodamine phalloidin and Hoechst 33342 for confocal microscopy. Merged images of 3 representative cargocytes shown. Note the absence of Hoechst (no nuclei). Scale bar = 50 μm. **B)** 100,000 E0771 cells were infected with oVSV-ΔM51-GFP at MOI 0.1. Merged phase contrast and FITC channel images were taken 24 hours after infection. Scale bar = 50 μm.

Figure 3. 3D-cultured MSC-derived cytoplasts have improved *in vivo* biodistribution



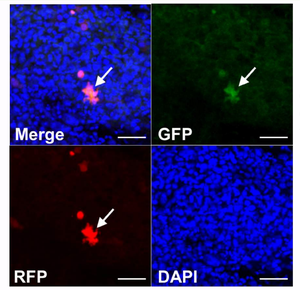
LifeAct RFP MSC were grown in either 2D culture or 3D culture, and then centrifuged in Ficoll to generate 2D or 3D-derived cytoplasts. Cells and cytoplasts were then washed and stained with DiD intravital dye. One million cells or cytoplasts in 2D or 3D were injected retro-orbitally into BALB/c mice. 24 hours after injection, organs were harvested for FACS analysis. Number of DiD stained, RFP+ cells per 5x10^5 events . NS= not significant; * = p<0.05, **=p< 0.01, *** = p<0.001.

Figure 4. 3D cytoplasts can be engineered to home to inflamed tissue *in vivo*.



A) Schematic of cytoplast engineering for 3 receptors/molecules (triple cytoplasts) to improve homing to LPS-induced acute inflammation in the mouse ear. Mice were retro-orbitally injected with 1M 3D-cultured Triple-engineered cytoplasts stained with DiD vital dye. B) Cells from mouse ears were analyzed by flow cytometry. Cells from each ear were stained with F4/80 to rule out macrophage phagocytosis. Bar graph quantifies DiD+, F4/80- cytoplasts in ears.

Figure 5. 3D-Triple-cytoplasts deliver oVSV to micro-metastases.



Syngeneic metastatic TNBC model was generated in immuno-competent C57Bl/6 mice by retro-orbital injection of 1X10^6 RFP-labeled E0771 cells. Cytoplasts infected with 0.5 MOI oncolytic VSV-ΔM51-GFP virus were retro-orbitally injected into the mice 14 days post tumor cell injection. Lung tissues were harvested and fixed 24 hours later. Confocal images were taken with the Olympus FV1000 microscope and analyzed in ImageJ. RFP=E0771 tumors; GFP=VSV-infected cells; Blue=Hoechst 33342-stained cell nuclei. Arrow indicates VSV-infected tumor cells. Scale bar =50 μm.

Conclusions

➢MSC-derived cytoplasts can be effectively loaded with oncolytic viruses

➢oVSVs extensively propagate in cytoplasts and can be transferred to cancer cells.

➢Cytoplasts can be extensively engineered to home to inflamed tissues, including tumors *in vivo*.

➢Cytoplasts are a novel delivery platform for oncolytic viruses, which may improve clinically-relevant delivery and efficacy of oncolytic virus-mediated cancer therapies.

References

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