Using Natural Killer Small Extracellular Vesicles (NK-sEVs) to Treat Glioblastoma

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Introduction

- Glioblastoma is a fast growing cancer that targets glial cells and the five-year survival rate for glioblastoma patients is only 6.8%.
- Currently there are only three drugs and one device that have been FDA approved for GPM therapy, and Blood Brain Barrier (BBB) serves as an obstacle for Glioblastoma Multiforme (GBM) therapy.
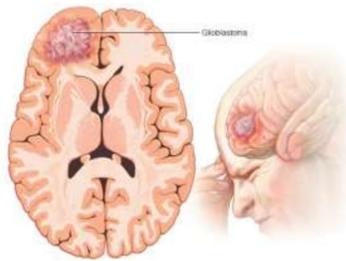

Figure 1. *Image showing GBM*

- NK cells can bind to tumor cells and release cytokines, yet NK cell activity is inhibited in the tumor microenvironment. As an alternative, NK-sEVs, made in endosomes and released from their parent cells, can cross the BBB and do not attack healthy cells.
- NK-sEVs share characteristics of their parent cells as it has NKG2D and apoptotic factors (GrzB, GrzA, FasL), which can be used to improve localization towards GBM.

Objective & Impact of Professor's Research

- Objective of the professor's research is to synthesize nanoparticles that can be used to improve localization and therapeutic delivery methods.
- Nanoparticles such as NK-sEVs, with their great anti-tumor potential and the addition of the targeting peptide, can increase treatment efficacy and reduce off-targeting effects.
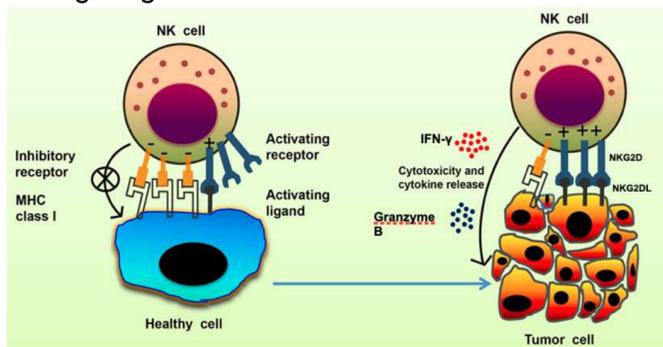

Figure 2. *Natural Killer cells in GBM. NK cell-derived IFN-γ promotes GBM cancer stem cell differentiation*

Methods

Isolation of Exosomes from NK Cells
- Centrifuge and filter to get sEV's with sizes of 80-150nm
- NTA, TEM, western blot for confirmation

Synthesis of Peptides
- Synthesize GBM targeting peptide (KKD-IL13αD) using solid phase peptide synthesis
- Use HPLC, ESI MS, PDA, MALDI for confirmation

Next steps: Add Peptides to sEVs

Skills and Techniques Learned

Peptides

High Performance Liquid Chromatography (HPLC)
- Used to isolate or purify substances

Electrospray Ionization Mass Spectrometry (ESI MS)
- Ions are produced using electrospray
- Marks which molecular weight was detected frequently and detects molecular weight of substance

Photodiode-Array Detection (PDA)
- Measure absorbance at 220nm to detect peptide bond presence

Matrix Assisted Laser Desorption/Ionization (MALDI)
- Used to measure molecular weight of substances and ensure sample is pure

sEVs

Nanoparticle Tracking Analysis (NTA)
- Provides specific characterization of nanoparticles (ex. Size)

Transmission Electron Microscopy (TEM)
- Electrons are fired through a specimen to form an image
- Confirm using size

Western Blot
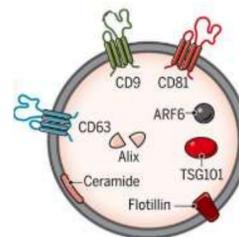- Used exo-specific protein and NK-cell to confirm presence of exosomes

Figure 3. *Protein biomarkers for EVs*

Results

Peptides
- ESI MS and MALDI results show expected peptide molecular weight (2400g/mol), indicating successful peptide synthesis
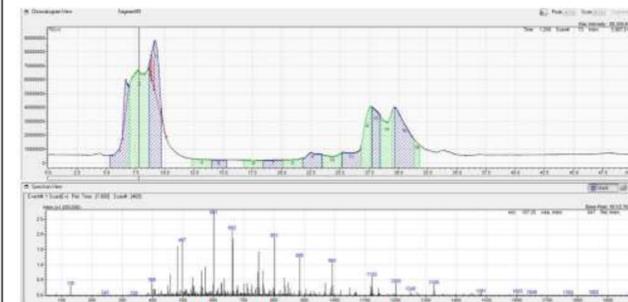

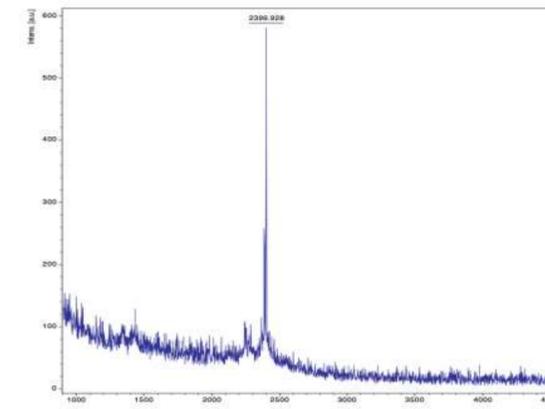**Figure 4.** *ESI MS results PC: Abby Lim*

Figure 5. *MALDI results PC: Abby Lim*

sEVs
- TEM image analysis shows expected size for EV and western blot expresses specific sEV marker, indicating successful isolation of sEVs
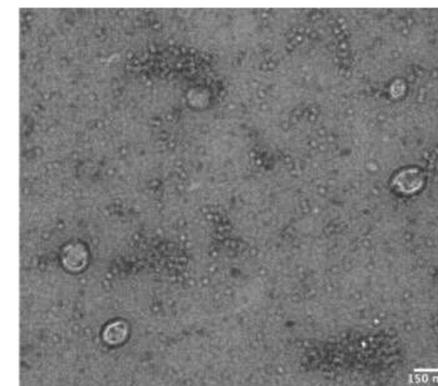

Figure 6. *TEM image PC: Abby Lim*

80kDa
60kDa
50kDa
TSG101

Figure 7. *Western blot for an EV specific protein*

How This Relates to My STEM Coursework

- **AP Chemistry**: chromatography, mass spectrometry, titrations
- **AP Biology:** peptides, binding sites, immune system, antibodies

Next Steps for You OR Advice for Future SHINE Students

This experience has furthered my interest in the medical field and I will continue to pursue research and medicine in college.

Advice for Future SHINE Students
- Make use of all opportunities at hand
- Don't be afraid to reach out for help to your PhD mentor
- If you don't understand your topic, read articles or helpful resources in your own time

Acknowledgements

References

[1] National Brain Tumor Society. July 2020.
[2] Sourav et al, *Front. Immunol.*, September 2017.
[3] Kalluri, R., et al. *Science.* February 2020.