Internalization of Folate Receptor Targeting Nanoparticles into Ovarian Cancer Cell Lines

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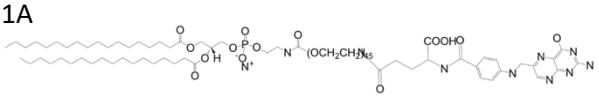
Statement of Purpose: According to the American Cancer Society, ovarian cancer is the leading cause of death from cancer of the female reproductive system in the United States.[1] Ovarian cancer cells are known to overexpress folate receptors on the cell membrane; this overexpression can be utilized to target and attack malignant cells by delivering a high dose of drug to the cancerous cells and minimizing effects to healthy cells. However, there is currently no data on the number of folate receptors expressed by individual ovarian cancer lines or how this variance affects nanoparticle (NP) internalization. Our goal is to determine how NP internalization is affected when the NPs have been conjugated with folic acid as a way to target folate receptors on the cell surface. In this study, we seek to quantify the expression of folate receptors by various ovarian cancer cell lines and examine how these differences in expression play a role in folate receptor targeting NP internalization.

Materials and Methods: Samples were prepared by lysing cells and extracting the proteins from cultured ovarian cells. The cell lines used in this study were A2780, OV-90, OV-SAHO, SKOV-3, OVCAR-3, OVCAR-4, and HOSE. In order to quantify the amount of folate receptors expressed by each cell line, the samples were tested using Quantikine Human FOLR1 Immunoassay from R&D Systems according to manufacturer's instructions. Self-assembling DSPE-PEG NPs labeled with fluorescent Cy5 dye, both conjugated to folic acid and unconjugated, were incubated with the cells (**Fig. 1A**). After washing, the association of NPs to cells was determined using flow cytometry. We then used confocal imaging and ImageJ analysis to determine the number of nanoparticles that were internalized by the cells.

Results: Our current results show that different ovarian cell lines have varying expression of the folate receptor, as indicated by the ELISA study (**Fig. 1B**). We are currently completing the calculations of cellular uptake of folate receptor targeting NPs for each line based on these detected amounts of folate receptor for the flow cytometry

1A



1B

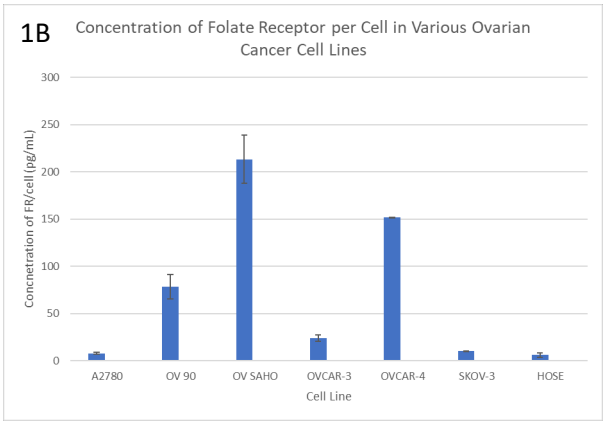


Figure 1. A) DSPE-PEG conjugated to folic acid. **B)** ELISA results indicating varying amounts of folate binding proteins between cell lines.

and confocal microscopy studies as a way to determine how the quantity of folate receptor proteins expressed on the cell membrane plays a role in nanoparticle internalization.

Furthermore, we are looking for characterization of variety of pathways to understand the internalization of the NPs with and without the folic acid.

Conclusions: Our current findings have indicated the variances of expression of folate receptors between different ovarian cancer cell lines. This data will allow us to continue to study how these varying quantities of folate receptors affect the internalization of folic acid conjugated NPs.

Acknowledgements: Katherine Haddad has been partially funded by the STEM Workforce Development Award funded by NASA's Established Program to Stimulate Competitive Research and the Undergraduate Mentored Research Fellowship funded by the University of Oklahoma.

References: [1] American Cancer Society. Cancer Facts & Figures 2018. Atlanta, GA: American Cancer Society; 2018.