Surveillance of response to PARP inhibitors using characterized Extracellular Vesicles

Laura Nguyen[1], Sara Corvigno PhD[2]

Department of Gynecologic-Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA[1]

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Introduction

- Cancer cells release biological material into extracellular vesicles (EVs).
- Extracellular vesicles are defined as membrane-coated vesicles that vary in size. According to their size, there are small extracellular vesicles (sEVs) between 50 and 150 nm in diameter, and medium-large EVs (mlEVs) between 160-300 nm in diameter (ref)
- Both sEVs and mlEVs have been successfully isolated from biological fluids, such as blood, urine, or ascites (ref)
- Cancer cells-derived EVs have been investigated for their possible role as cancer biomarkers, particularly as early diagnostic markers or as predictive markers of response to therapy
- EVs may be a predictive marker of response to PARP inhibitors, a specific targeted treatment for ovarian cancer

Aims

- To isolate and analyze EVs secreted from ovarian cancer cells with higher or lower sensitivity to PARP inhibitors
- To analyze EVs with the gold standard techniques (nanotracking) and transmission electron microscopy (TEM)

Methods

- EV Isolation:
  1. Collect conditioned medium from cancer cells
  2. Remove cells, dead cells, and cellular debris
  3. Collect miEVs via ultracentrifugation at 10,000 X g for 40 minutes
  4. Wash and resuspend miEVs in filtered PBS
  5. Collect sEVs via ultracentrifugation at 100,000 X g for 120 minutes
  6. Wash and resuspend sEVs in filtered PBS
- EV Analysis:
  1. Resuspend 10 uL of isolated EVs in 1 ml of filtered PBS
  2. Load the sample into a 1 ml syringe and analyze at NanoSight
  3. Resuspend EVs pellet in TEM buffer to undergo microscopy

Results

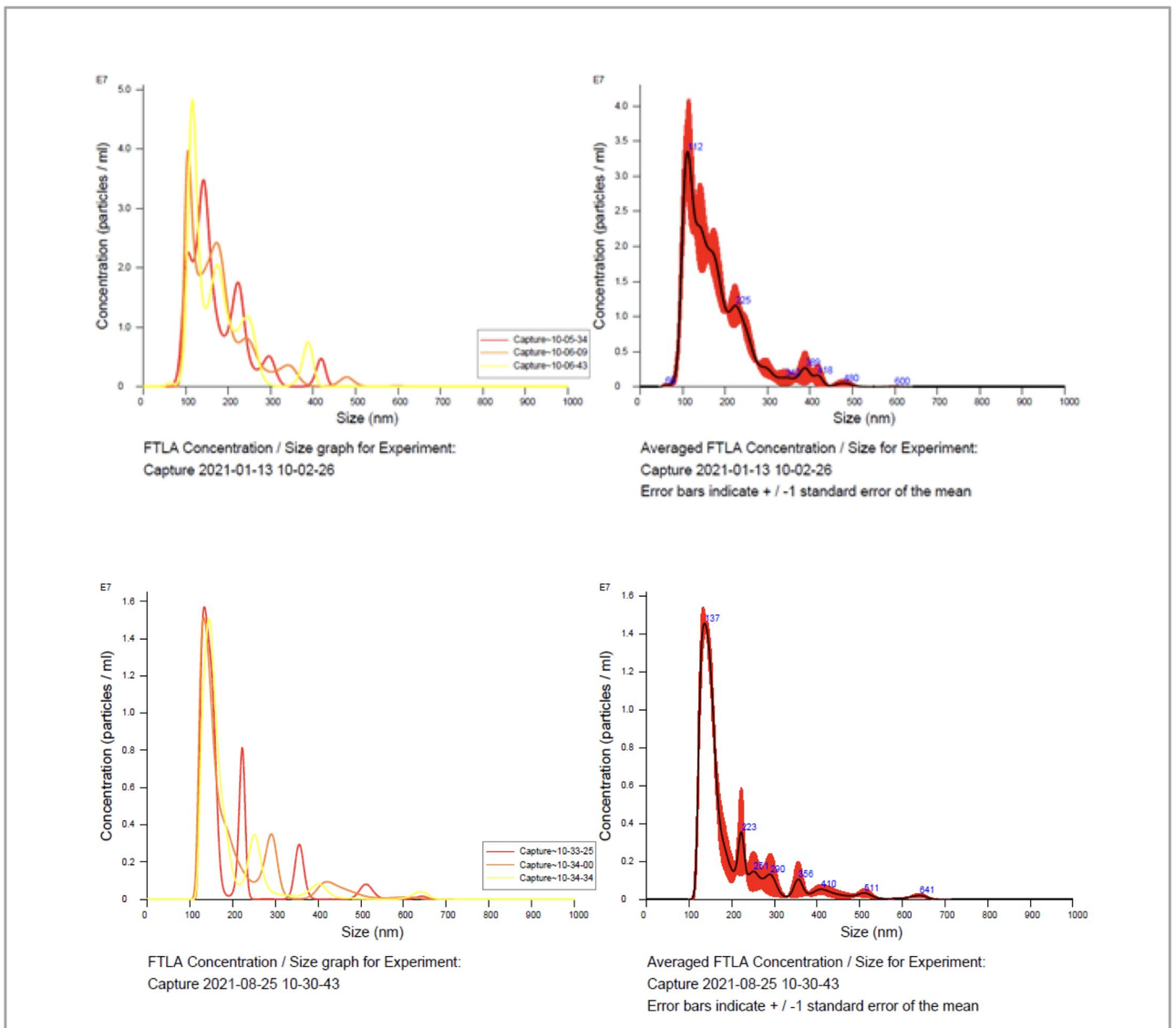

Fig. 1 Nanotracking analysis of small EVs from the ovarian cancer cell line OVCAR3 (A); Nanotracking analysis of medium-large EVs from the ovarian cancer cell line OVCAR3

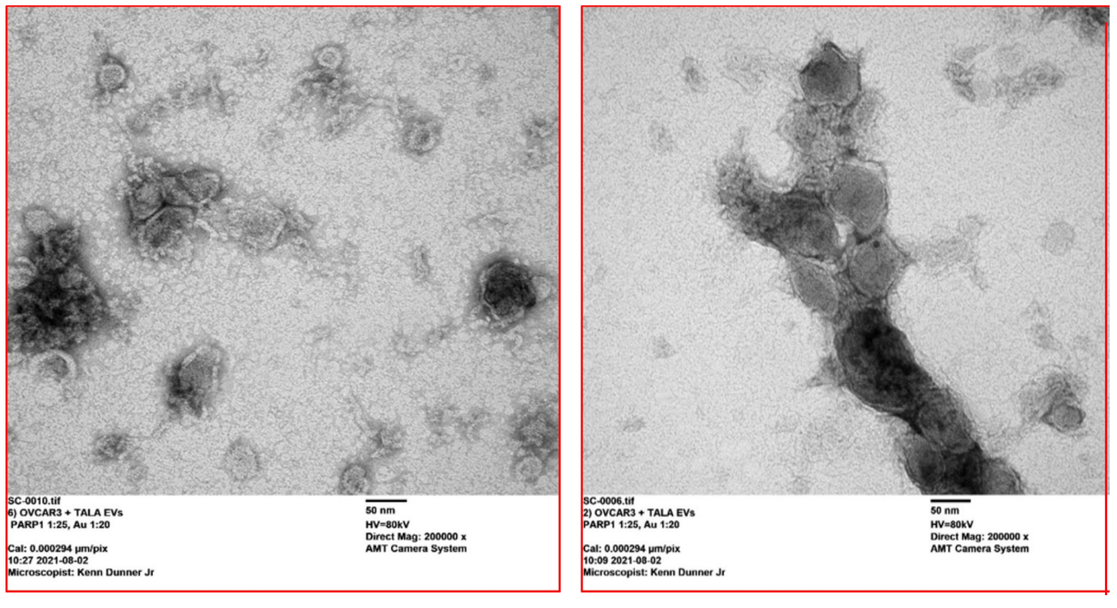

Fig. 2 transmission electron microscopy (TEM) analysis of the ovarian cancer cell line OVCAR3 after PARP inhibitor treatment

Results

- We isolated EVs from a sensitive and a resistant cell line to PARPi and identified them via *nanotracking*
- We studied the EVs content with flow cytometry to measure their content in PARP and DNA
- Sensitive cells to PARPi have an increase in PARP-DNA complexes after PARPi treatment, as compared to resistant cells

Conclusions

- We are currently assessing the differences in cargo of EVs from cells sensitive and resistant to PARP inhibitors through flow cytometry, western blot, and confocal imaging
- Further analysis will be performed on circulating EVs from in vivo models and patients affected by ovarian cancer who underwent treatment with PARP inhibitors

Acknowledgements

I would like to thank Dr. Anil Sood and his laboratory for their abundance of supplies, resources, and support.

References

1) Schlacher, Katharina. "PARPi focus the spotlight on replication fork protection in cancer." *Nature cell biology* vol. 19,11 (2017): 1309-1310. doi:10.1038/ncb3638

2) Sunetra Roy, Jessica W. Luzwick, Katharina Schlacher; SIRF: Quantitative in situ analysis of protein interactions at DNA replication forks. *J Cell Biol* 2 April 2018; 217 (4): 1521–1536. doi: https://doi.org/10.1083/jcb.201709121

3) Phelan K, May KM. Basic techniques in mammalian cell tissue culture. Curr Protoc Cell Biol. 2015 Mar 2;66:1.1.1-1.1.22. doi: 10.1002/0471143030.cb0101s66. PMID: 25727327.

4) Mekonnen, Negesse et al. "Homologous Recombination Deficiency in Ovarian, Breast, Colorectal, Pancreatic, Non-Small Cell Lung and Prostate Cancers, and the Mechanisms of Resistance to PARP Inhibitors." *Frontiers in oncology* vol. 12 880643. 17 Jun. 2022, doi:10.3389/fonc.2022.880643