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

➢ 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

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References