Utilization of Exosomes for the Early Detection of Ovarian Cancer

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Ovarian Cancer

- Difficult to diagnose because the disease is largely asymptomatic until it has reached a late stage of development
- 5-yr survival rate for women diagnosed at an advanced stage is ~15%
- 5-yr survival rate for women diagnosed at Stage 1 is ~90%
- 67% of women are diagnosed at an advanced stage

TURN TEAL

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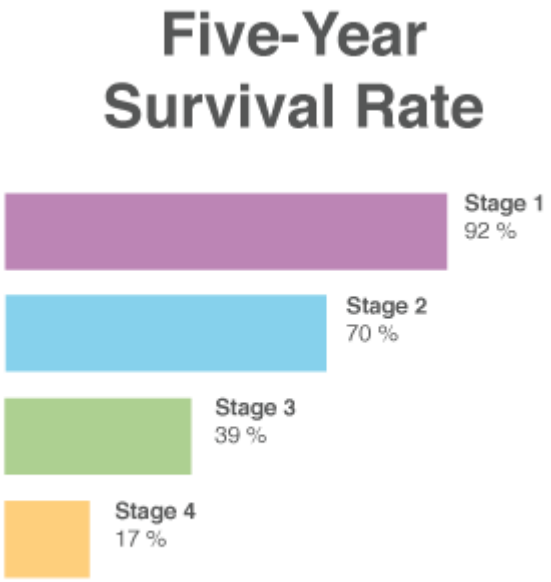
Early Diagnosis is Key

- Diagnosis at an early stage increases the 5-yr survival rate significantly

- There is currently no standard early detection method for ovarian cancer

- There is a need for a simple, non-invasive, inexpensive, reliable method of screening for early stage ovarian cancer that can be incorporated into a standard physical



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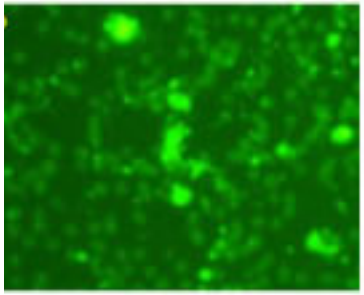
Utilization of Exosomes for the Early Detection of Ovarian Cancer

- Ovarian cancer-derived exosomes have been shown to have specific marker proteins (fingerprints)
- They have been found in urine, blood and cervical mucus
- They can be detected using antibodies to the specific marker proteins



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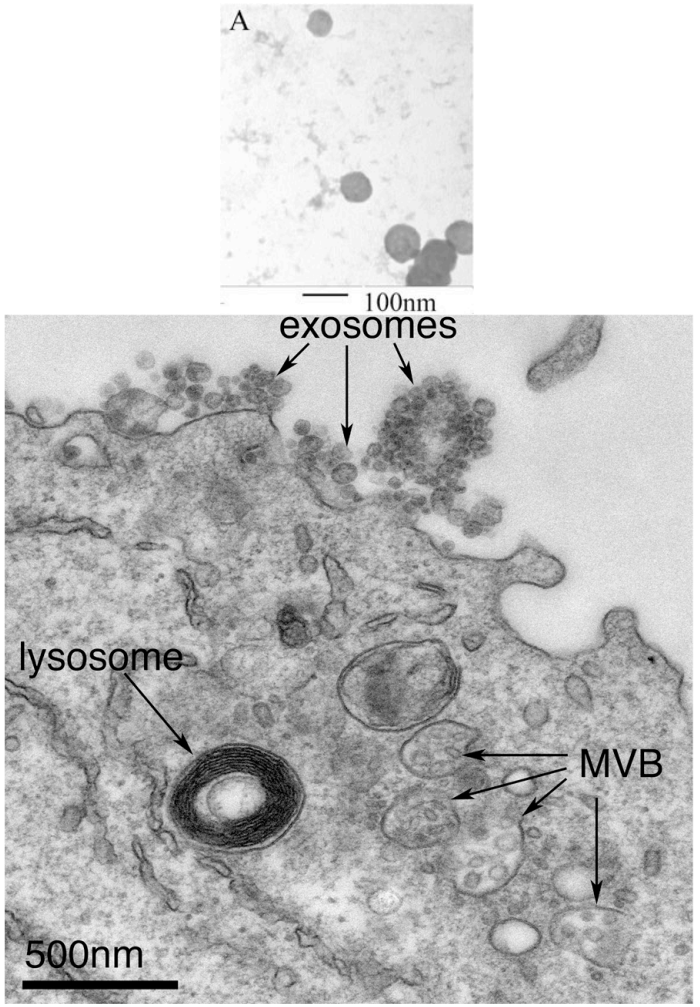
What are Exosomes?

- Very small, membrane-bound cargo vesicles (sacs) that are released from cells
- Once thought to be cellular debris, exosomes have now been shown to be “messages in bubbles”
- Form of communication between cells and other cells and/or cells and their microenvironment



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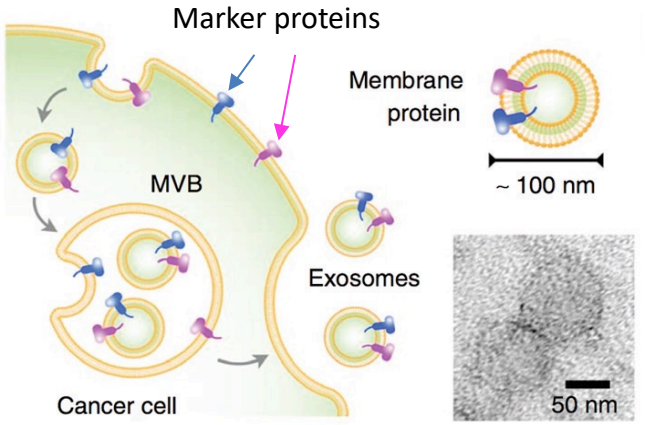
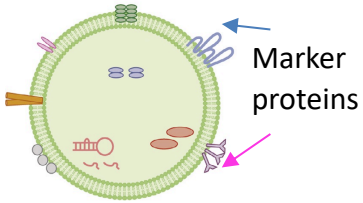
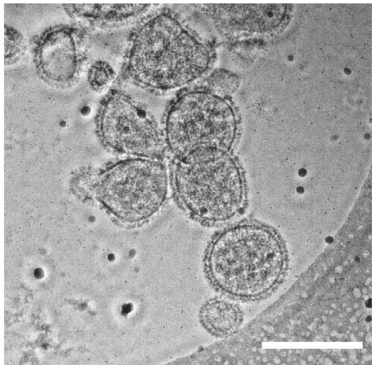
What are Exosomes?

- Exosomes are formed from the plasma membrane of the cell
- Because they are made by "pinching off" part of the plasma membrane, they contain the same membrane proteins as the host cell
- These marker proteins are like a "fingerprint" as each type of cell has some unique proteins on their plasma membrane
- These marker proteins allow us to determine what type of cell produced the exosome (like a home address label)







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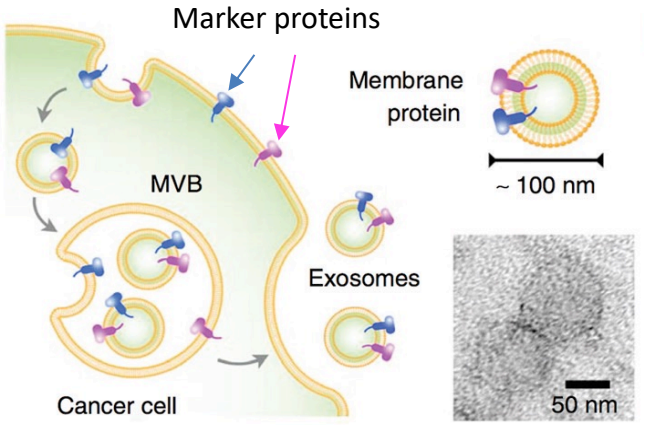
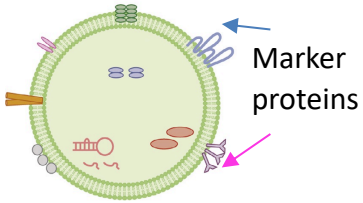
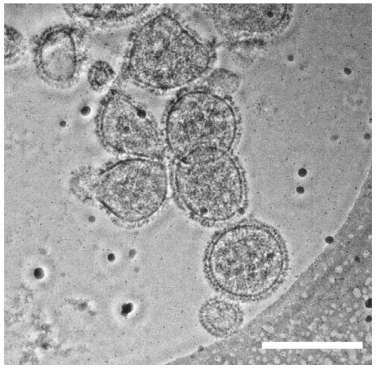
What are Exosomes?

- The exosomes are filled with cargo (messages) within the MVB (multi-vesicular body)
- They are then released from the "host" cell
- They can travel throughout the body and be taken up by other cells (recipient cells)







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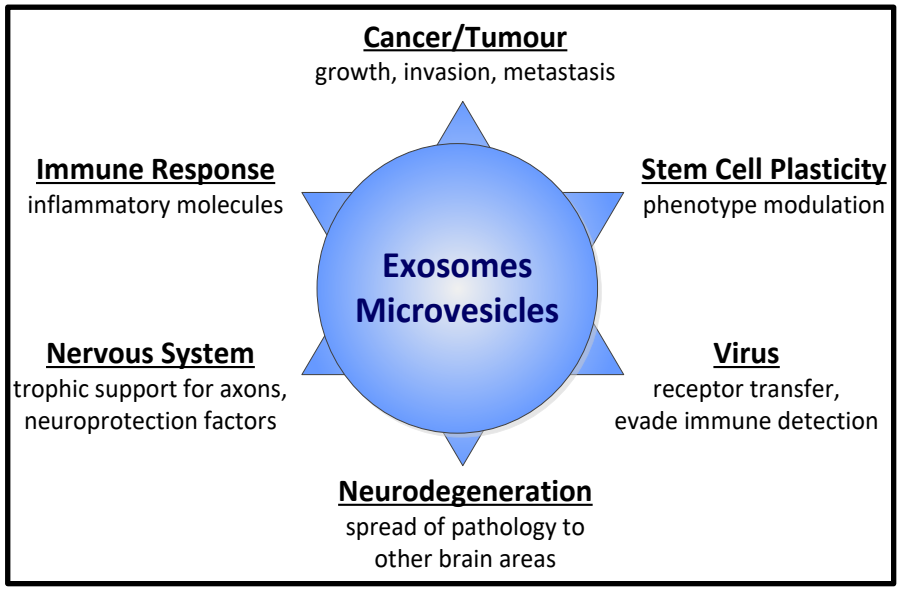
What Can Exosomes Do?

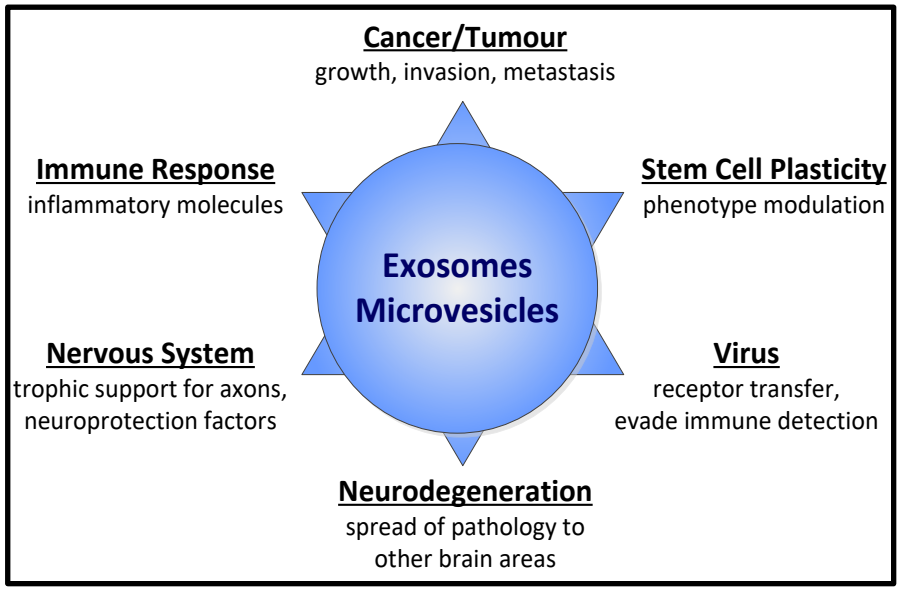
- Exosomes can contain all kinds of different "messages"

- Some messages are good…for example, during fetal development, they help stem cells know what type of cell to become



What Can Exosomes Do?

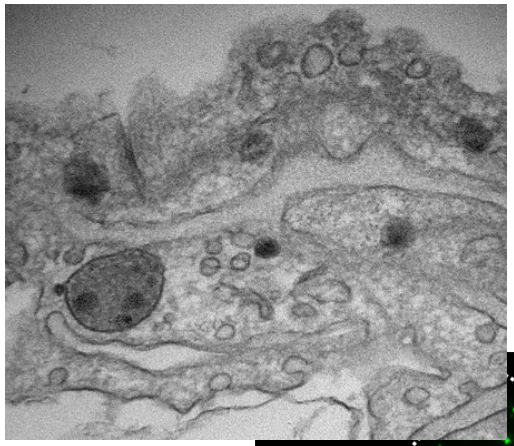
- Some messages are NOT good…
- Exosomes have been shown to play a role in arthritis (inflammation) and the spread of neurodegenerative disease symptoms
- Can play a role in cancer

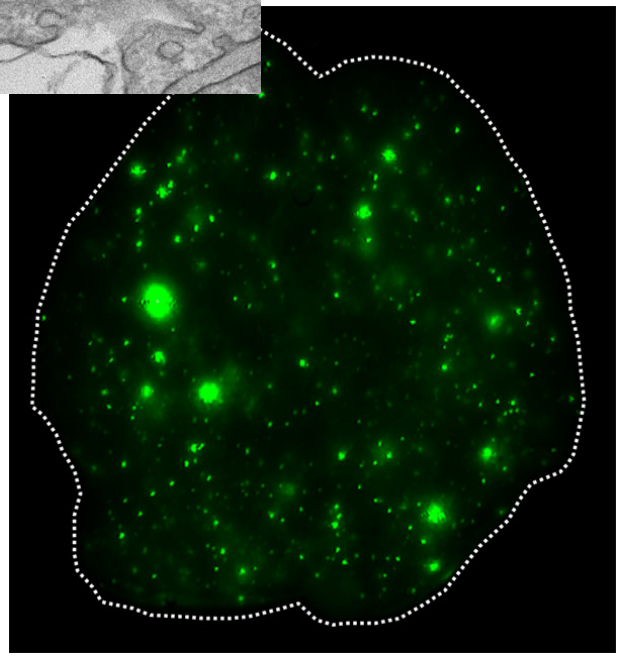


What Can Exosomes Do?

- Exosomes can deliver messages to normal cells that instruct them to change in ways that make them more susceptible to cancer cell invasion (metastasis)

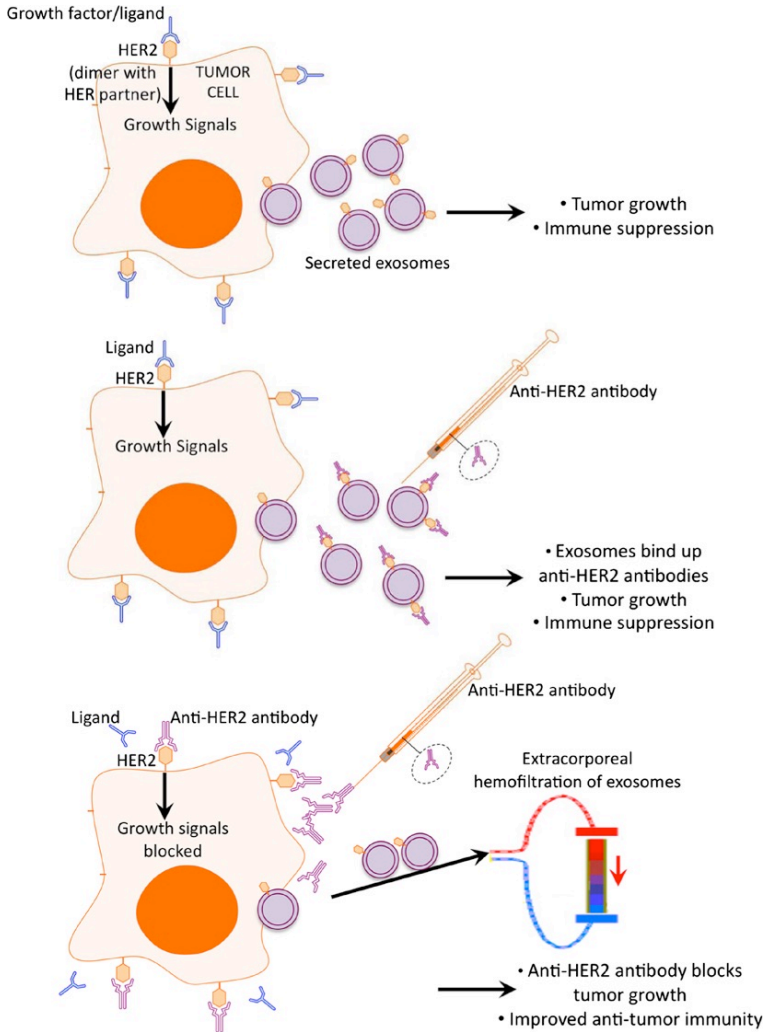
- They have been found in primary tumor sites and in metastatic sites





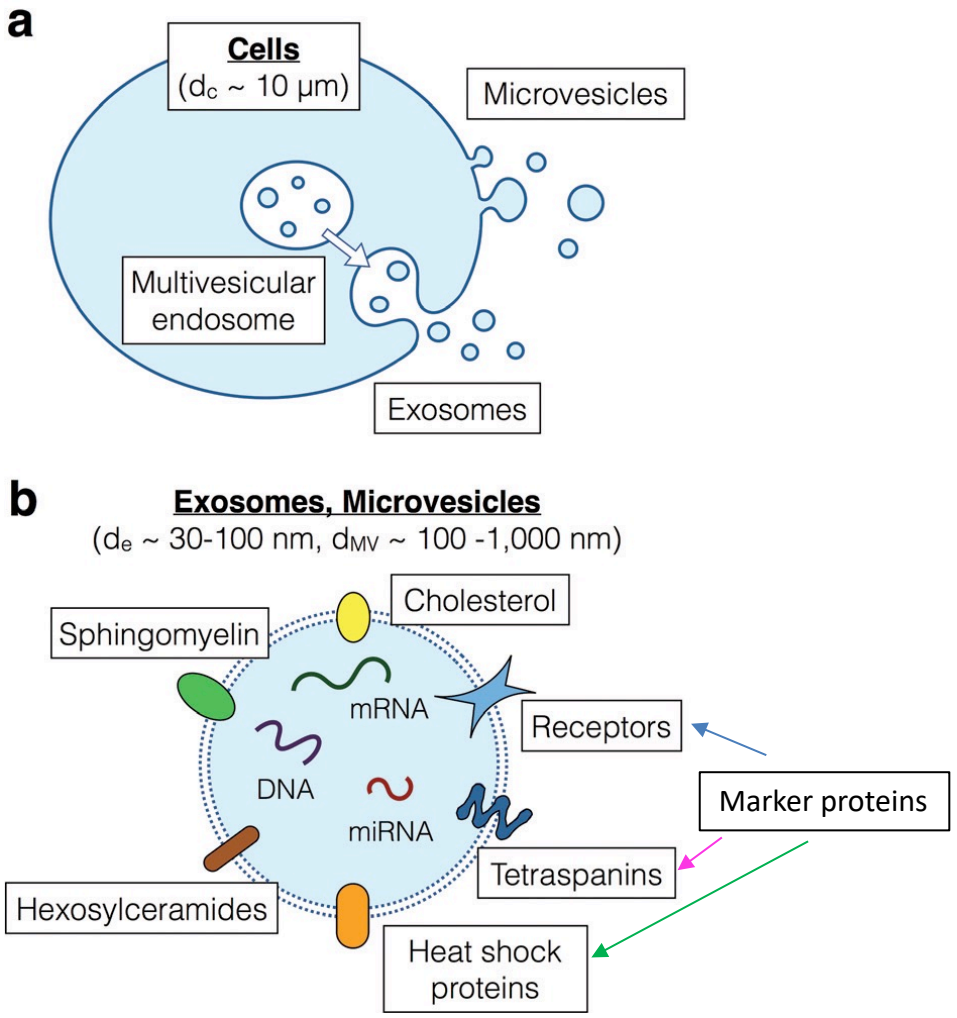
What Can Exosomes Do?

- Exosomes can interrupt treatments
- They can confer drug resistance to cancer cells (e.g., cisplatin)
- They can bind up antibodies used for immune therapies



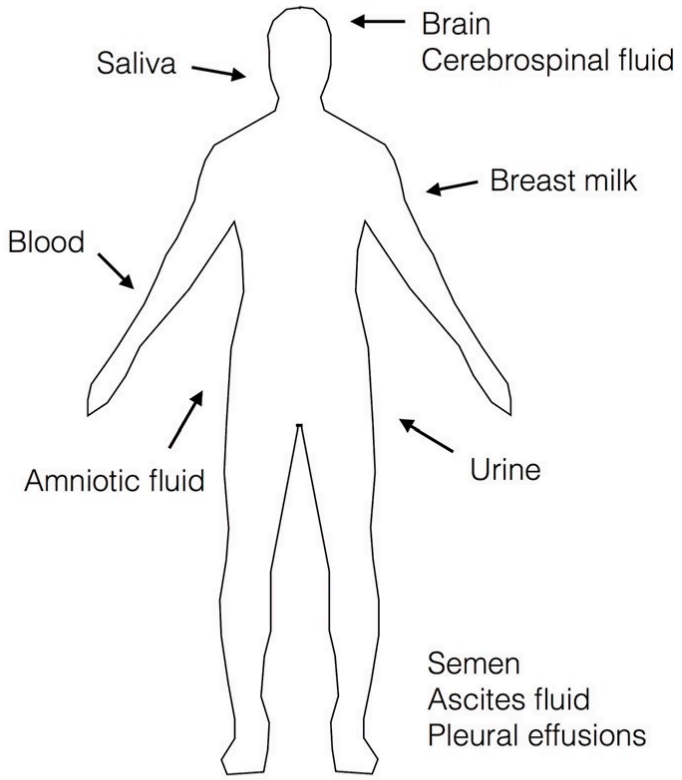
Which Cells Produce Exosomes?

- ALL cells produce exosomes
- Tumor-derived cells have been shown to produce more exosomes than normal cells
- They all have unique marker proteins based on their cellular origin (where they came from)



Where Are Exosomes Found?

- In all types of body fluids
- Saliva, blood, urine, CSF have all been shown to contain exosomes
- This makes it easier to access exosomes through normal lab sampling procedures



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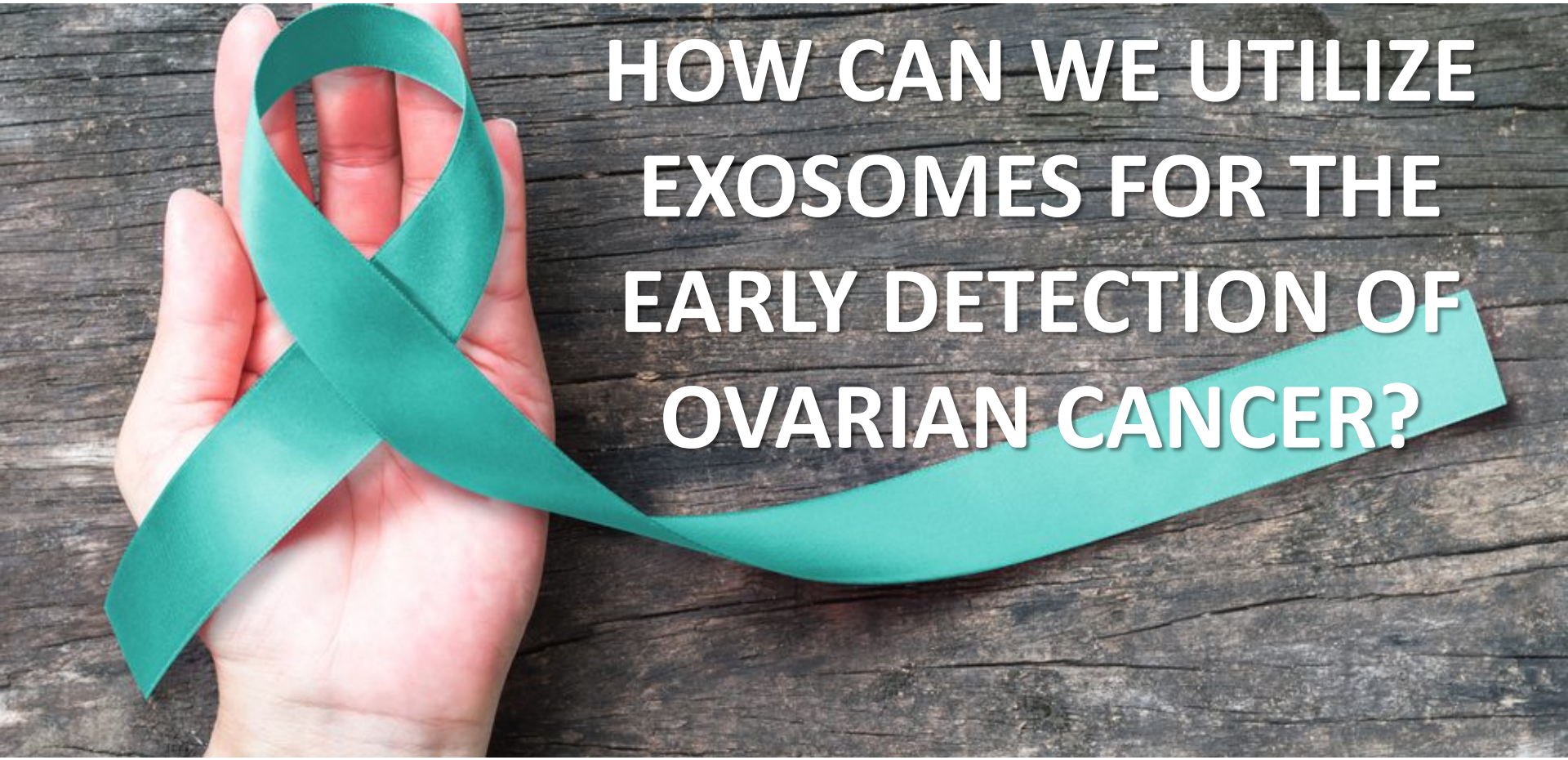
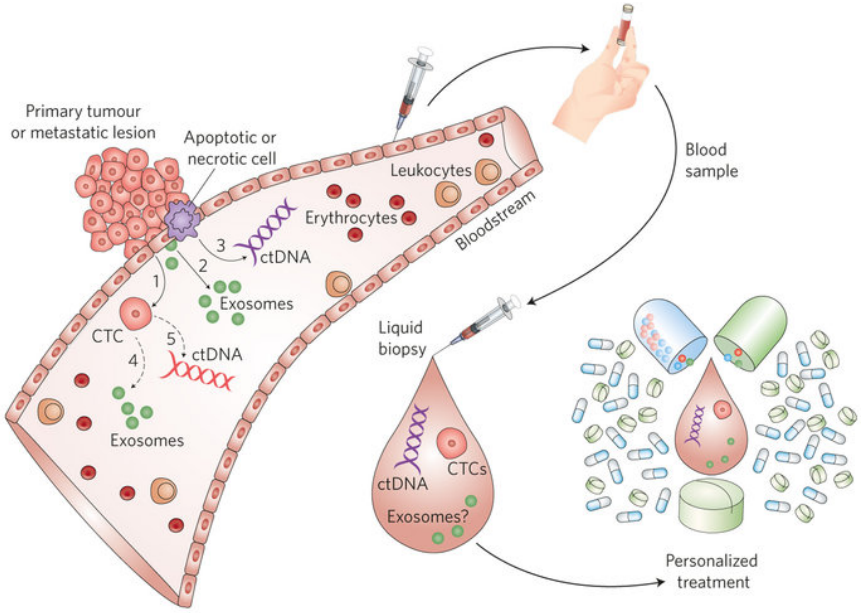

HOW CAN WE UTILIZE EXOSOMES FOR THE EARLY DETECTION OF OVARIAN CANCER?

Liquid Biopsy

- Because exosomes carry specific protein markers (fingerprints) on/in them, the type of cell that they came from can be identified
- As they are in body fluids, this opens the possibility of conducting “liquid biopsies”
- Liquid biopsies are non-invasive and can be conducted as part of a routine medical examination



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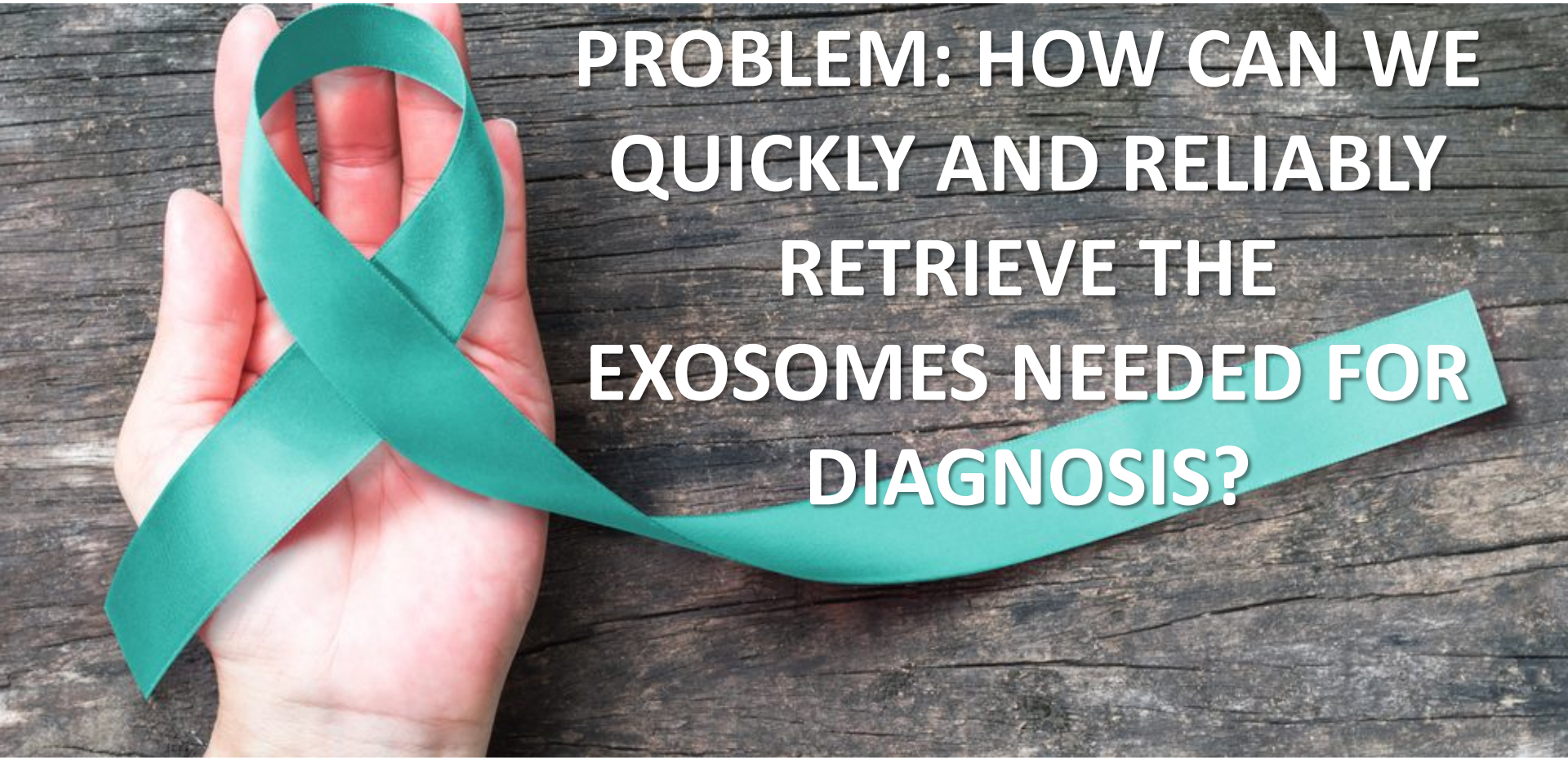
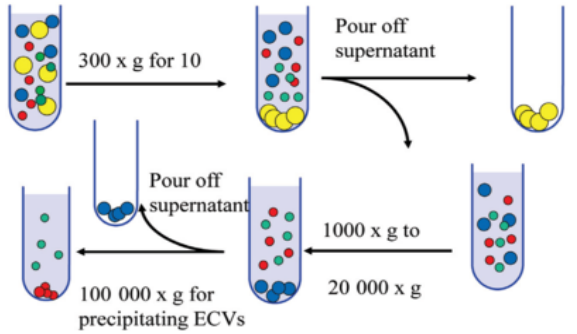

PROBLEM: HOW CAN WE QUICKLY AND RELIABLY RETRIEVE THE EXOSOMES NEEDED FOR DIAGNOSIS?

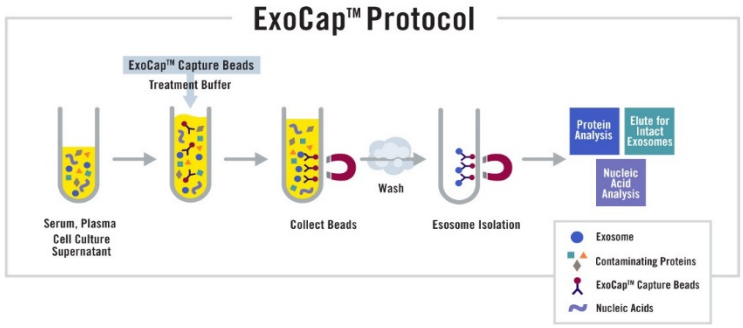
Standard Exosome Retrieval Methods

- Various retrieval methods exist, but all have issues with reliability and/or time to conduct the isolation
- Differential Centrifugation
- Magnetic Beads

Differential Centrifugation ~ 2 hours



Magnetic Beads ~ 1-2 hours



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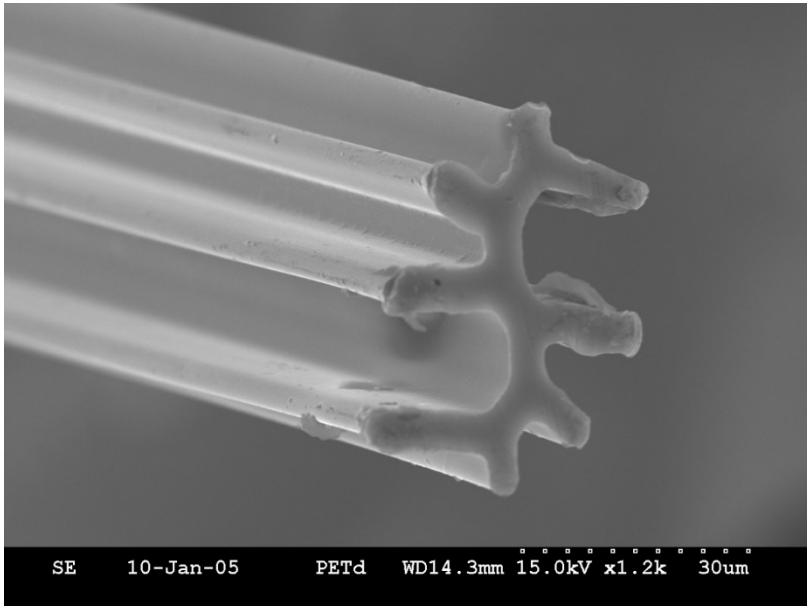
The Idea – Exosome Retrieval Using Functionalized Polymer Fibers

- A simple method to retrieve exosomes from diverse biological samples

- Utilizes novel capillary-channeled polymer (C-CP) fibers to selectively capture exosomes from ovarian cancer cells



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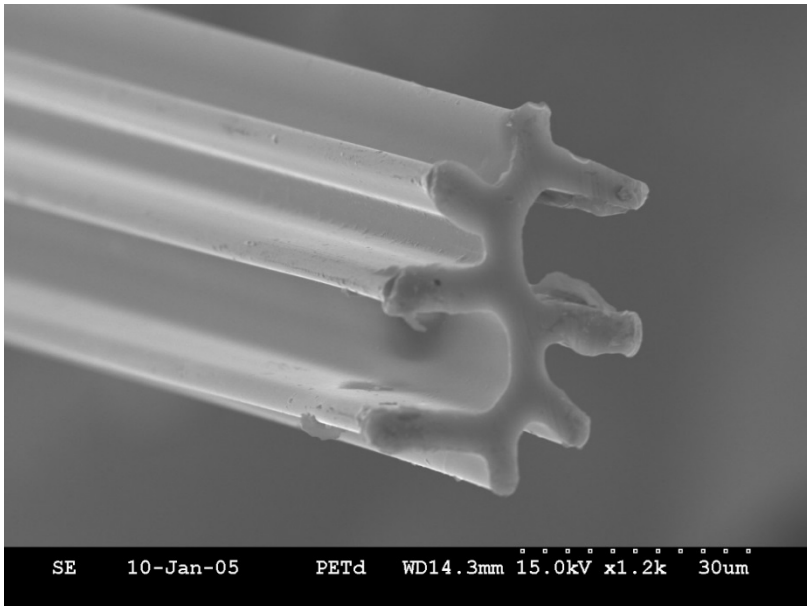
The Fibers

- Made from inexpensive plastics: polyester, polypropylene, and nylon
- The unique capillary channel design gives the fibers a large surface area due to their cross-sectional shape
- Surface can be modified with antibodies to interact with specific marker proteins for selective capture



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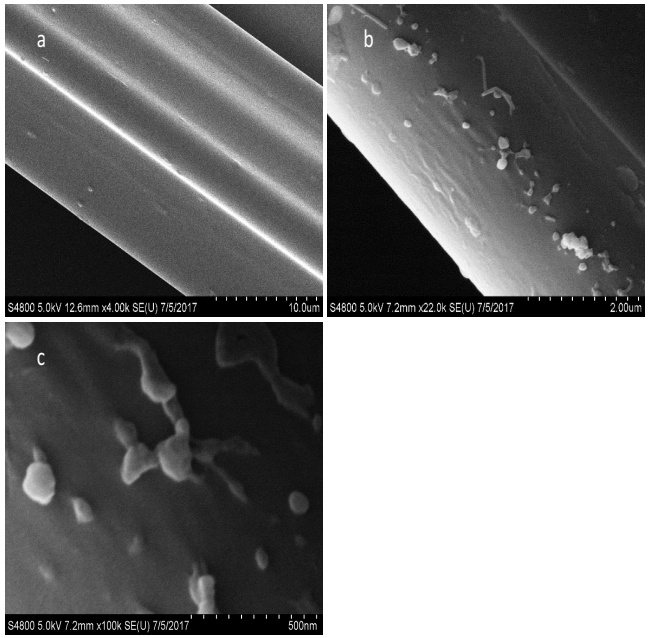
The Initial Results

- Exosomes have been isolated and purified from diverse matrices (fluids) using the fibers in a simple column format and spin-down tips
- Matrices have included urine, human serum/plasma, seminal fluid, blastocoel fluid, and cervical mucus.
- Processing times have been reduced from multiple hours to <10 minutes
- Exosomes have been isolated with no substantial loss of exosomes or damage to the exosomes.
- Exosomes can either be selectively captured and counted/recovered, or generically collected and selectively labeled for identification



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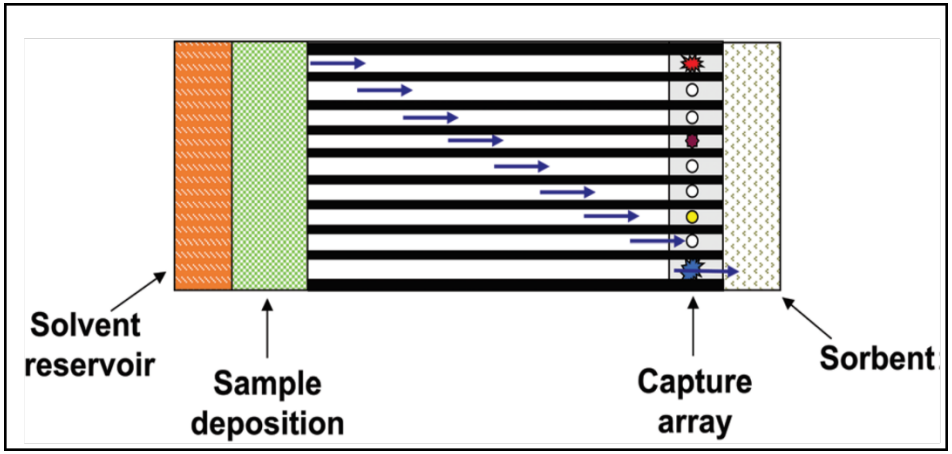
DEVELOPMENT OF
COLORIMETRIC OC
SCREENING PLATFORM

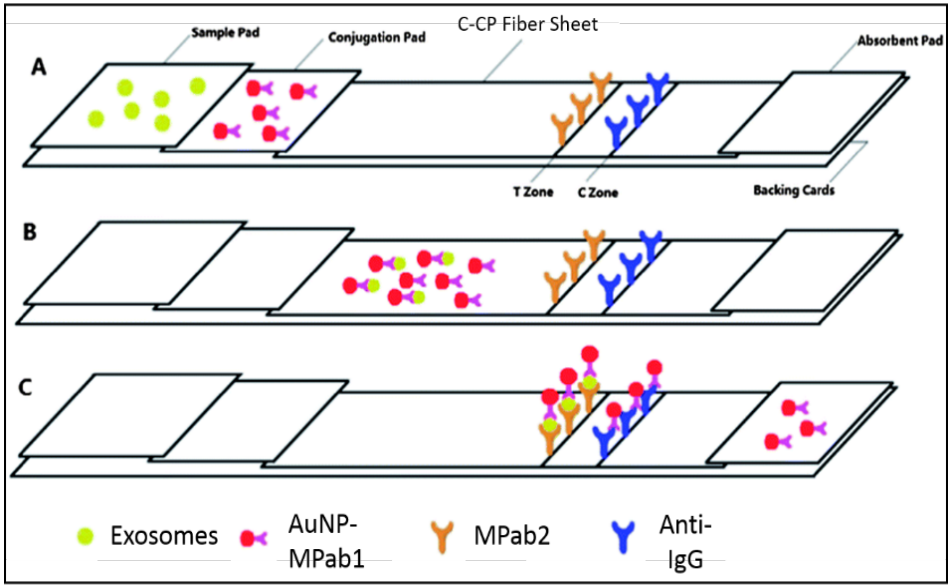
The Basic Platform

- Films with same capture abilities made with individual channels
- Film channels may be conjugated with antibodies to specific exosomal biomarkers for exosome capture
- Exosomal capture will cause a color change when they are bound to Abs on the film surface



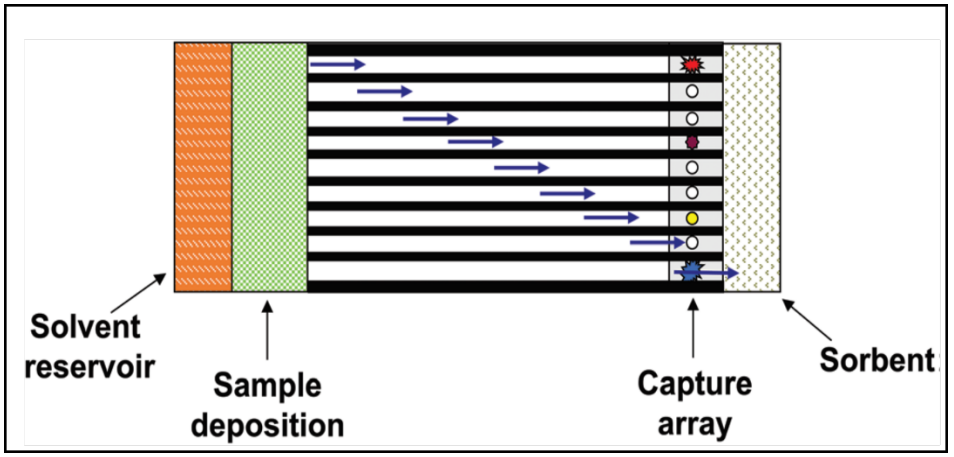
The Ultimate Goal

- Simple, quick, cost-effective test designed to detect exosomal biomarkers associated with early stage OC
- Platform similar to a pregnancy test; envision multiplexed tests for multiple biomarkers on the same film
- Utilizes C-CP film to isolate and capture exosomes with specific marker proteins on their surface



The Ultimate Goal

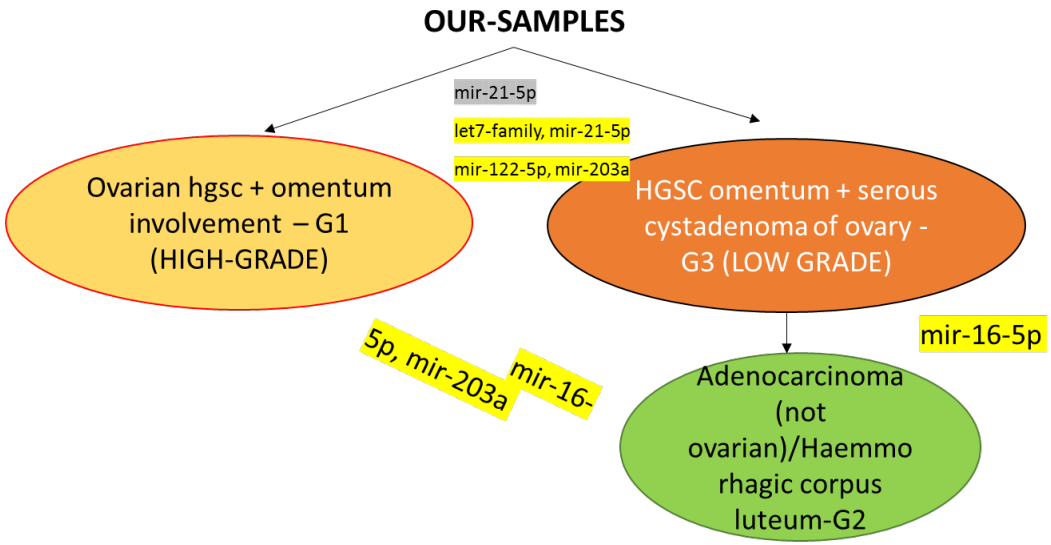
- If positive for any markers in the subset, alerts physician to refer patient for further, more extensive/invasive testing for OC
- Collection could also include isolation of exosomes for further profiling for patients with presence of specific biomarkers
- Exosomal biomarkers are currently the subject of a large prostate cancer study




PROGRESS –
WHERE ARE WE NOW?

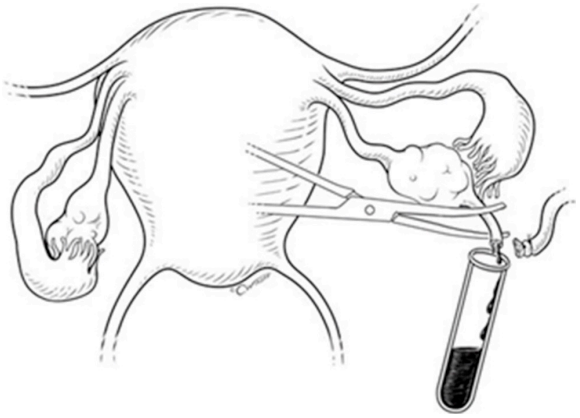
Where Are We Now?

- Initial study of exosomes from 46 patients
- Bioinformatics
  - New machine learning algorithms have been established
  - Clustering studies
  - Small RNA profiling
  - Identification of involved pathways
- Panel of biomarkers
  - Proteomics have been done
  - Junction plackoglobin



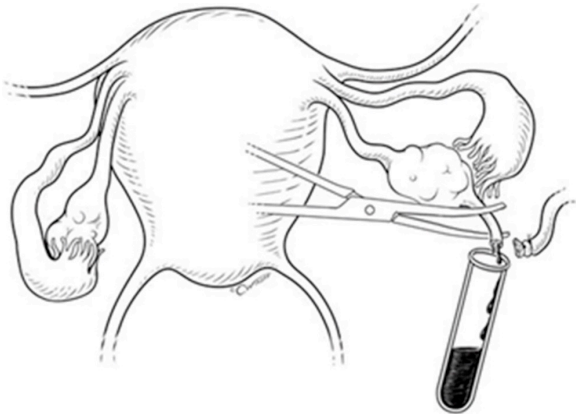
Of Note: Junction Plakoglobin

- Found to be in veinous blood samples downstream of early stage epithelial ovarian tumors
- Breast cancer – High levels enable tumor cells to cluster and travel together in the bloodstream, enhancing metastasis possibilities
- Could play similar role in OC



Of Note: Junction Plakoglobin

- Diagnosis – Surgical
  - Only ~10% are OC
  - JUP with CA-125 for FIGO Stage 1
  - 75% screening sensitivity (100% specificity)



- Liquid Biopsy
  - CA-125 is exosomal
  - Preliminary proteomics show JUP is exosomal!

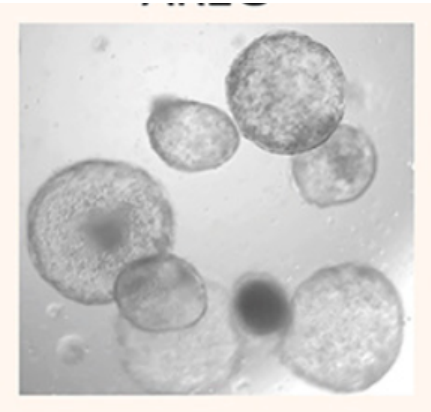

WHAT'S NEXT?

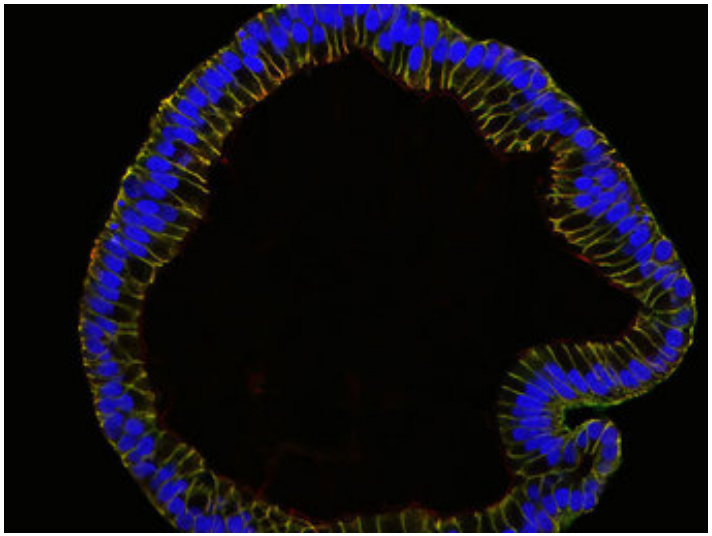
Phase II Patient Study

- Recruitment is ongoing
- New 50 patient cohort
- BRCA1/2 patients
- RNA Seq/Proteomics

Fallopian Tube Tissue Models

- Primary Cell Cultures/Organoids from fallopian tube tissue
- Exosome retrieval from spent media
- Exosome feeding
- RNA seq/proteomics





Our Group: Sisi Huang, Kaylan Kelsey, Lei Wang, Rhonda Powell, Tyler Slonecki, Tykia Blakney, Paritra Mandal, Brian Dean, Billy Bridges, Larry Puls, Lorie Allen, (not pictured - Tina Pettry, Ken Marcus

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