DISCLOSURE

THE FOLLOWING POTENTIAL CONFLICT OF INTEREST RELATIONSHIPS ARE GERMANE TO MY PRESENTATION.

EQUIPMENT: N/A
SPEAKERS BUREAU: N/A
STOCK SHAREHOLDER: N/A
GRANT/RESEARCH SUPPORT N/A
CO FOUNDER: CLINICAL PEPTIDE SOCIETY

STATUS OF FDA DEVICES USED FOR THE MATERIAL BEING PRESENTED: N/A
STATUS OF OFF LABEL USE OF DEVICES, DRUGS OR OTHER MATERIALS THAT CONSTITUTE THE SUBJECT OF THIS PRESENTATION: N/A
Most Common Cancers Worldwide

[Cases per year]

1. Lung: 1,825 K
2. Breast: 1,677 K
3. Bowel (inc. anus): 1,361 K
4. Prostate: 1,112 K
5. Stomach: 952 K
6. Liver: 782 K
7. Cervix: 528 K
8. Oesophagus: 456 K
9. Bladder: 430 K
10. NHL: 386 K

Total: 14.1 million
Cancer Arises From Gene Mutations

### Germline mutations
- Parent
- Mutations in egg or sperm
- Present in egg or sperm
- Are heritable
- Cause hereditary cancer syndromes

### Child
- All cells affected in offspring

### Somatic mutations
- Somatic mutation (e.g., breast)
- Occur in non-germline tissues
- Are non-heritable
- Later onset
CANCER GENES FALL INTO 2 CATEGORIES

1). Tumor Suppressor Genes
   - p53, BRAC-1, BRAC-2

2). Proto-oncogenes
   - HER2, BRAF, KRAS
TUMOR SUPPRESSOR GENES Example p53 gene

- Normal cell
- Excessive DNA damage
- Cell suicide (Apoptosis)

p53 protein
PROTO-ONCOGENES GENES Example HER2

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2. THIS PROTEIN RECEPTOR IS INVOLVED IN THE GROWTH, REPAIR AND DIVISION OF CELLS IN THE BREAST
DNA DAMAGE

Indirect Route
- radiation
- water
- free radical

Direct Route
- radiation

DAMAGE
OUTCOME OF DNA DAMAGE

Outcomes of DNA Damage

- repair
- mis-repair
- not repaired

- viable cell
- mutation
- cancer
- cell death
ENVIRONMENTAL CAUSES OF DNA DAMAGE
Mutated Cancer Genes

Tumor Suppressor Gene Inactivated = No brake!
Mutated Cancer Genes

Proto-oncogene → Oncogene = Too much gas!
Mutated Proto Oncogenes- HER2
Multiple genes can increase the risk of a single cancer

Multiple cancers can be associated with a single gene
EXOSOMES are 30–100 nm sized vesicles

WHITESIDE TL. TUMOR-DERIVED EXOSOMES AND THEIR ROLE IN CANCER PROGRESSION. ADV CLIN CHEM. 2016;74:103-41.

• Tiny vesicles shuttle proteins and genetic information between both neighboring and distant cells
• Exosomes are “representatives” of the parental cell phenotype and genotype
• Cells differ greatly not only in the quantity but also the quality of exosomes they secrete.
EXTRACELLULAR VESICLES VERSUS EXOSOME

• Debate among scientists what the definition of a exosome.
• Extracellular vesicles includes exosomes, microvesicles, apoptotic vesicles, oncosomes nanovesicles
CARGO OF EXOSOMES


- over 4,000 different proteins
- Heat shock proteins (HSP70, HSP90)
- cell-surface proteins CD9, CD63, CD81 antigens
- mRNA, miRNA, longRNA, DNA
EXOSOMES IN THE ROLE OF TUMOR GROWTH


• Normal human blood has approx 2,000 trillion exosomes, and the blood of cancer patients is approx 4,000 trillion exosomes

• Exosomes from cancer have a higher concentration of microRNA
• Particular miRNA may be oncogenic in one type of cancer, and in other cancer may be tumor suppressor
• Example is miR-29 act as a tumour-suppressor in lung tumor whereas in breast cancer this miRNA has oncogenic functions
Cancer cells secrete exosomes via:

- **Paracrine mode**: Releasing micro RNA on neighboring cells, that induce post transcriptional repression or activate membrane surface receptors to favor growth and invasiveness
- **Endocrine mode**
- **Autocrine mode**
EXOSOMES IN THE ROLE OF TUMOR GROWTH


- 2 Major Roles in cancer formation
  - 1) Inducing the environment for cancer to start
  - 2) Disarming anti-tumor immune response
LIQUID BIOPSY

A new, non-invasive technique that can detect disease biomarkers in:

- Blood
- Urine
- Sputum

LIQUID BIOPSY CAN BE USEFUL WHEN:

- Not enough tissue sample is available
- Not enough tumor tissue is in a sample
- A tumor is hard to reach
- Regular monitoring is needed

LIQUID BIOPSIES CAN BE ANALYZED FOR:

- Presence of cancer cells
- DNA
- Other materials released by cancer cells
DIAGNOSING CANCER WITH EXOSOMES

• Pancreatic cancer can be detected with exosomes
• Multiple methods from Antigen based, Ultracentrifugation and Ultrafiltration, sucrose gradient centrifugation and etc.

• Ovarian cancer can be detected with exosomes expressing CD24 and EpCAM (Epithelial Cell Adhesion Molecule)
• Obtained ascites samples N = 20
• Able to monitor clinical response during therapy
DIAGNOSING CANCER WITH EXOSOMES

• Non small cell lung cancer can be diagnosed with exosomes
• Plasma from 109 patients w/ stage 3a - 4
• Was capable of detecting and phenotyping exosomes in all samples from only 10 µL of unpurified plasma

• Rectal cancer can be diagnosed with exosomes
• N= 24 of advanced rectal cancer
• Exosomes containing miRNAs 486-5p, 181a-5p and 30d-5p
Diagnosing Cancer with Urinary Exosomes


- Study of 15 healthy versus 16 with prostate cancer looking at the urinary exosomes
- 221 proteins were up regulated in exosomes from prostate cancer
- The highest sensitivity, 94%, was observed for transmembrane protein 256 (TM256)
- TM256 and LAMTOR1 protein could be used to augment the sensitivity to 100%.
FORGOING PROSTATE BIOPSY?


• N= 516 and 561 in placebo group.
• Urinary exosomes of PCA3 and T2:ERG RNA were measured before prostate biopsy.
• First-catch urine was collected after Digital rectal exam

• 95 percent sensitivity for detecting aggressive prostate cancer, combining testing of urinary T2:ERG and PCA3
• (Prostate cancer antigen 3, Transmembrane Protease Serine 2 fused with ERG- 50% of all prostate cancer has T2:ERG)
Urinary exosomes of *PCA3* and *T2:ERG* RNA are measured after prostate exam.

Algorithm developed at the University of Michigan that combines urine T2:ERG, prostate cancer antigen 3 (PCA3), and serum PSA to predict the risk of detecting PCa on biopsy.

Specificity of 90% and an 80% sensitivity
DIAGNOSING CANCER WITH EXOSOMES


• Exosome company that is FDA approved
• Urine test to rule out prostate cancer
• Exosomes looking at 3 RNA markers ERG, PCA3, SPDEF (SAM-pointed domain-containing ETS transcription factor)
• If level is less than 15.6 then high grade prostate cancer is ruled out
• Lung cancer test with exosomes but only available for research only
MICRO RNA VS SMALL INTERFERING RNA

Imperfect match  →  Block translation
Near-perfect match  →  Degrade mRNA
DILEMMA of getting siRNA into the cancer cells

Problem scientists have wrestled with for over a decade: *getting siRNA into the right cells.*

Synthetic lipid nanoparticles have been the mainstay delivery device for siRNA, but they can cause a toxic immune response in humans.

Lipid nanoparticles are notorious for congregating in the liver.

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EXOSOMES THE RISING STAR IN DRUG DELIVERY


- Exosomes has recently emerged as a promising drug delivery system
- non immunogenic, high biocompatibility, and high efficacy of delivery
In 2011 a study done at University of Oxford demonstrated that exosomes stuffed full of small interfering RNA (siRNA) reached cells inside the brains of mice.

- Lowered production of BACE1, a protein involved in Alzheimer’s disease.
• In 2017 intravenous injections of small interfering RNA-loaded exosomes suppressed pancreatic cancer in mice better than similar injections of siRNA-loaded lipid nanoparticles, and without any obvious immune reactions.
EXOSOMES - Specific to oncogenic KRAS in pancreatic cancer


• Treatment with small interfering RNA-loaded exosomes suppressed cancer in multiple mouse models of pancreatic cancer and significantly increased their overall survival
• Macrophage-derived exosomes loaded with a potent anticancer agent paclitaxel (PTX) and attach on their surface a PEG-conjugated ligand targeting the Sigma receptor, which is overexpressed by lung cancer cells.
Comparative study with different exosomes carrying doxorubicin (DOX) for pancreatic cancer

KANCHANAPALLY R. ET AL, INT J NANOMEDICINE, 2019 JAN 11;14:531-541

• Macrophage-derived Exosomes loaded with DOX showed the highest anti tumor activity
• Versus pancreatic cancer cells derived exosomes and pancreatic stellate cells derived exosomes loaded with DOX
STUDY OF EXOSOMES ON PROSTATE CANCER IN VIVO

• Cancer cell derived Exosomes can deliver Paclitaxel to the prostate cancer cells through endocytosis and showed increased cytotoxic effect

Phase 1 clinical trials with exosomes

Phase 1 study MESENCHYMAL STEM CELL-DERIVED EXOSOMES with siRNA - pancreatic cancer

CLINICALTRIALS.GOV IDENTIFIER: NCT03608631

Mesenchymal stromal cells-derived exosomes with KrasG12D siRNA in treating participants with pancreatic cancer with KrasG12D mutation that has spread to other places in the body.

- Stage 4 pancreatic cancer cells
- with KrasG12D mutation
- Estimated enrollment of 28
- Estimated completion date is Feb 2019
- No results posted
Dexosomes as a therapeutic cancer vaccine: from bench to bedside.


• Exosomes released from dendritic cells, now referred as dexosomes

• Vaccination with dexosomes, designed to express tumor antigens, is a potent strategy to elicit anti-tumor immune response
Phase 1 study with dexosomes – melanoma


- Advanced stage III/IV melanoma expressing the MAGE-3 antigen was treated with MAGE-3 peptides loaded dexosomes from autologous dendritic cell

- 5 out of the 15 enrolled patients experienced some measure of clinical benefit with respect to the primary tumor or lymph node lesions.
9 completed the therapy, but overall no significant induction of MAGE peptide-specific T-cell responses.
Phase 2 study dexosomes NON-SMALL CELL LUNG CANCER


• Second generation dexosomes cancer vaccination with improved immune stimulatory capacities
• A phase II with 41 patients enrolled non-operable NSCLC is completed but no results posted

(NCT01159288)
Antisense oligonucleotides vs small interfering RNA

RNA interference (RNAi)

- siRNA
- RISC
- Target RNA
- RNA cleavage

Antisense oligonucleotides (ASOs)

- ASO (DNA)
- RNase H1
- Target RNA
- RNA cleavage

Dr Edwin Lee
Phase 1 study exosomes on Glioma

CLINICALTRIALS.GOV IDENTIFIER: NCT01550523

• Exosomes derived from autologous glioma loaded with antisense oligodeoxynucleotide targeting the insulin-like growth factor receptor-1

• N = 13

• No data reported
FUTURE EXOSOMES TO DELIVER CANCER DRUGS

• Looking for new kinds of exosomes from bacteria, fungi, plants, animals and fruit.
• Roche agreed to pay PureTech Health up to $36 million for access to its exosomes extracted from dairy cow milk.
PHASE 1 PLANT EXOSOMES COLON CANCER

CLINICALTRIALS.GOV IDENTIFIER: NCT01294072

• Study investigating the ability of plant exosomes to deliver curcumin to normal and colon cancer tissue
• Not recruiting yet
PHASE 1 GRAPE EXOSOMES Head and neck cancer

CLINICALTRIALS.GOV IDENTIFIER: NCT01668849

- Grape exosomes to prevent oral mucositis associated with chemo radiation treatment of head and neck cancer.
- Also the effect of grape exosomes on the production of cytokines and immune responses to tumor exosomal antigens.
GOOD VERSUS BAD EXOSOMES

• Exosomes from cancer is very different from exosomes from mesenchymal stem cell
VEXOSOMES : EXOSOMES – Packaged with virus


• At Massachusetts General Hospital has packaged an adeno-associated virus gene therapy inside an exosome
• Showed benefits in hearing in a mouse with hereditary deafness
CONCLUSION

• Exosomes can be used to diagnosis cancer
• Treatment of cancer can be personalized with engineered exosomes loaded with drugs, miRNA, siRNA, peptides or others
• Using exosomes in cancer is a game changer.