Exosomal liquid biopsy reveals mRNA and lincRNA biomarkers in early stage breast cancer patient plasma

Sudipto K. Chakrabortty1, Robert R. Kitchen1, Christine M. Cotichina1, Erez Eltan1, Michael D. Valentino1, Vasiati R. Tadigota1, Elena Castellanos-Rizaldos1, Lisa Bedford2, Hidefumi Uchiyama3, Sunita Badda4, Nicholas Colaferina1, Mario Morken1, Miguel Williams1, Sylvie Vincent1, Seth Yu and Johan Skog3

1 IIT Research Institute, Wakehi, MA 2 Kedara Pharmaceuticals International Co. Translation and Research Laboratories, Cambridge, MA 3 Kedara Pharmaceutical Co. Integrated Technology Research Laboratories, Fujisawa, Japan

- Breast cancer is the most prevalent cancer in women: ~250,000 diagnoses per year in the US.
- Early detection is critical but current liquid biopsy approaches such as CTCs and cfDNA struggle to detect rare markers of disease.
- Exosomes reflect active cellular processes thus enabling early cancer detection.
- Exosome-based liquid biopsy is minimally invasive and highly sensitive, especially using techniques to enhance signal-to-noise.
- Able to enrich specific exosomal subpopulations originating from a tumor or any tissue of interest.
- Able to deplete non-relevant, non-cancerous exosomes originating from abundant cell-types such as blood cells.

Technology

- A novel platform for enrichment or depletion of subpopulations of exosomes:
  - **EDDE** (Edisense Depletion and Enrichment)
- A novel platform for performing long RNASeq of exosomes:
  - Highly reproducible workflow (Average R² > 0.99)
  - Wide dynamic range: ~10⁻¹⁸ to 1.8 million molecules of RNA
  - Full transcript coverage of exosomal RNA
- Applied EDDE to Stage I & II ER+/Her2: breast cancer and matched healthy controls using:
  - Plasma depleted of non-relevant blood cell-derived exosomes.
  - Exosomes enriched for those derived from the breast tumor.
- We performed long RNA-sequencing on RNA obtained from matched breast cancer formalin-fixed paraffin-embedded (FFPE) tissue and plasma exosomes (both enriched and depleted).

RNASeq of plasma exosomes is comparable to FFPE tissue

ExoDx Long RNASeq analysis suite

Fig. 1. Mapping statistics of matched plasma exosomes & FFPE tissue

Fig. 2. Similar detection of gene bioties in exosomal RNA and FFPE tissue

High rate of mRNA mapping in both FFPE and plasma, ~50% of transcriptome-mapped reads are mRNA

Enrichment vs Depletion results in different gene signatures & biological pathways

ExoDx Depletion and Enrichment (EDDE)

Cells within both **HEALTHY** and **DISEASE** target tissues will be enriched for exosomes into the blood.

EDDE depletion separates healthy individuals from breast cancer patients

A: all genes

B: plasma result

C: breast cancer genes

Able to separate plasma exosomes from healthy individuals.

**EDDE breast cancer enriched plasma exosomes cluster with their matched FFPE tissue**

In addition, 4 of 5 cancer-related plasma samples cluster next to their matched FFPE.

Healthy tissue and plasma samples cluster separately.

EDDE depletion reveals components of tumor microenvironment

EDDE enrichment captures known breast cancer pathways from the tumor

**Fig. 3A. Global clustering by principle component analysis (PCA): samples cluster by disease status with EDDE depletion.**

**Fig. 4A. Enriched plasma clusters with breast cancer FFPE tissue**

4B. Enrichment correlates more strongly with matched FFPE tissue compared to depleted plasma (shown are data from two representative individuals).