Monitoring Breast Cancer Metastasis and Treatment Efficacy

Daniel T. Chiu
Department of Chemistry
University of Washington
Seattle, WA

Cancer Metastasis and Mortality

Cancer Metastasis is responsible for the majority of mortality (>90% for Breast Cancer)

Our Study: Focused on Prognosis
(Stage IV: The cancer has spread to another organ)
Breast Cancer

Breast is heterogeneous with different treatment options:

Hormonal Therapies (for HR+): e.g. anti-estrogens such as Nolvadex (generic, tamoxifen), and the aromatase inhibitors such as Arimidex (generic, anastrazole), Femara (letrozole), and Aromasin (exemestane).

Biologics: e.g. Herceptin or Lapatinib for Her2+ (~25%)

Chemotherapy and Radiation Therapy.

Breast cancer is a dynamic disease: e.g. Changes in Her2 expression.

Monitoring Cancer: BioMarkers (Peripheral Blood)

Protein BioMarkers (Protein Panel): Subject of intense research in proteomics – promising but with limited success so far (issues w/ standardization, dynamic range, etc.)

DNA BioMarkers: Circulating DNA
Methylated DNA – epigenetic changes

Cell BioMarkers: Circulating Tumor Cells:
Seed of Metastasis

Powerful Imaging Techniques
Circulating & Methylated DNA: A New Biomarker?

**Circulating DNA:**
**Source:** From tumor cells (apoptosis & necrosis)

**What we know:** Correlates with cancer prognosis (ovarian and lung cancer) and potential diagnosis.

**Advantages:**
1) *High sensitivity* provided by PCR amplification.
2) Offers molecular/sequence information (e.g. p53)

**DNA Methylation:**
Measures epigenetic changes (i.e. switches that control gene expression).

Patterns change with cancer (e.g. hypermethylation of CpG island – inactivation of arrays of genes).

---

**Circulating Tumor Cells**

Circulating Tumor Cells in Blood

Neutrophil: 50-70%
Lymphocyte: 25-35%
Eosinophil: ~5%
Monocyte: ~5%
Basophil: ~1%
Red Blood Cells: ~1%

In 1 mL of Blood:
~ 5 Million White Blood Cells

~ 5 Billion RBCs

CTC Cutoffs
Veridex Test (7.5 mL):

<table>
<thead>
<tr>
<th></th>
<th>MBC</th>
<th>MCRC</th>
<th>MPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥5 CTCs</td>
<td>≥3 CTCs</td>
<td>≥5 CTCs</td>
<td></td>
</tr>
</tbody>
</table>

Current Technique for Measuring CTCs

Processing by the CellTracks® AutoPrep® System

Aspirate plasma
Add buffer
Add fluorescent

Aspirate fluid and unlabelled cells

Peroxidase and add staining reagents

Magnetic incubation

Remove reagents
Resuspend target cells in buffer

CTC
Neutrophil

Binds to bound streptavidin
Amplifying volume of iron nano-particles
Our Technique

Correlating CTCs with DNA

Next Step: Correlate CTCs and Circ/Methylated DNA with Patient Prognosis and Treatment Outcome.

The presence of circulating total DNA and methylated genes is associated with circulating tumour cells in blood from breast cancer patients.

[References]
Impact: Economical

**Rheoblast**: Seed Funded Startup to Commercialize the Technology

**PUMA**: A New Substrate for Disposable Biochip

A new USP Class VI-compliant substrate for manufacturing disposable microfluidic devices

Lab on a Chip
Droplet NanoLab for Single-Cell Biology

Acknowledgements

Collaborators:
Daniel Sabath
Qinghua Feng
Gabriele Schuster
Karen Koehler
Viorica Popov

Sponsors:
LSDF
National Institutes of Health
National Science Foundation
KECK Foundation
Sloan Foundation

Jason Kuo
Perry Schiro
Thomas Schneider
Mengxia Zhao
Lai Ying Ng
Yongxi Zhao
David Lim
Patrick Shelby

LSDF

National Institutes of Health
National Science Foundation
KECK Foundation
Sloan Foundation