Microfluidics for the Interrogation of Circulating Biomarkers in Cancer Patients

Shannon Stott, PhD
Assistant Professor, Harvard Medical School
Massachusetts General Cancer Center

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Putting Cancer into Perspective

Number of deaths from cancer each \textbf{day} in the US:

1,600 deaths / day (2016)

Number of deaths from cancer each \textbf{day} in the world:

24,000 deaths / day (2016)
9 out of 10 deaths in cancer is due to metastasis!
Circulating Tumor Cells (CTCs)

One in a billion blood cells is a CTC: Huge technical challenge

Primary tumor

Intravasation

CTC

Extravasation

Metastasis
“Liquid Biopsy” in Targeted Therapy

- Detection of sensitizing EGFR mutations
- Acquisition of drug resistance

Lynch et al., *NEJM* 2004
2007: CTCs are found in ~20% of metastatic cancers using modified FACS and MACS, and filters. Cristofanilli et al., *NEJM* 2004.

CTCs are useful mostly for prognosis.
Hypothesis:

Biology vs. Technology
Pillars coated with an antibody to bind CTCs

2nd Generation Herringbone CTC-Chip Scale-up and Dissemination

Stott et al., PNAS 2010
Patient CTCs come in different size, shape & phenotype.
All cancers and all CTCs

Avoid bias in CTC isolation and improve yield, especially in non-epithelial cancers, or cancers undergoing EMT.

Negative depletion:
Removing the hay to uncover the needle...
CTC-iChip: Negative Selection

Magnetic bead-tagged WBCs

Blood

Hydrodynamic Cell Sorting

Inertial Focusing

Magnetophoresis

CTCs

WBCs

Red Blood Cell (8 Billion / mL)

CTCs (1-100 / mL)

White Blood Cell labeled with anti-CD45 magnetic beads (5 Million / mL)

RBCs, platelets, other blood components.

Ozkumur, Shah et al. Sci Transl Med 2013
“World-to-Chip” Coupling

Processor

CTC-iChip

Automated sample processing
Flowrate: 10-20 mL whole blood/hour
20-30 million cells/second
CTC-iChip enables light microscopy

FNA

CTC

Papanicolaou staining of melanoma CTCs
Lung Cancer: Serial Monitoring of EGFR Genotypes

EGFR activating mutation (Del)

T790M resistance: <1/100 alleles → 1/1

Gefitinib

Maheswaran et al, NEJM 2008

Expt.
Measuring androgen signaling pathways in prostate cancer CTCs

qRT-PCR (Fluidigm)

i-Chip: Culture of patient CTCs
Tumorigenesis in mouse models

*In vitro* expansion of CTCs from patients with hormone-positive breast cancer

*Yu et al., Science* 2014
Clinical response and CTC clusters

[Image of CTC chip with 20 μm scale bar]

- CTC-clusters >3 time points
- CTC-clusters 1-3 time points
- Single CTCs

- Progression-free survival
  - CTC-clusters >3 time points (32.6 d)
  - CTC-clusters 1-3 time points (134.8 d)
  - Single CTCs (160.5 d)

Patients (%)

Time (days)

Aceto N. et al, Cell 2016
CTC-Cluster Transit

- CTC-Clusters (2-100+ cells) believed to occlude 5-10 µm capillaries → cannot contribute to dissemination of tumors to distant organs

Sam Au et al. (PNAS, 2016)
Microfluidic Capillary Constriction

Au et al. (PNAS, 2016)
Reorganization of Clusters at Entrance

• Cell membrane stain
• Complex organization dynamics
Fluidic Microchip for Specific Isolation of Tumor EVs

Microfluidic Extracellular Vesicle Capture

Downstream analysis:
• Digital Droplet PCR
• RNASeq
• Proteomics

Functional Studies
• Co-Culture

Eduardo Reátegui (Nat. Comm, Accepted)

In collaboration with the Breakefield Laboratory
Microfluidic Biomaterial Coating

$L_D > \varepsilon$ flow $L_D$

$\Delta$Temp

Antibody Modification

Capture Efficiency [%]

Antibody Modification

GFP Copy Numbers

CTCs and EVs in Precision Medicine

Blood cells ~10\(^{10}\)
CTCs 0~100

Rare-Cell Isolation

Stott et al., *PNAS* 2010

CTCs count stable in fixed blood

prognosis

expression profiling

ex vivo culture

personalized drug testing

Require live cells!
<4 hours after blood draw
Blood degradation damages target cells & cripples precise microfluidic technologies

- Oxidative bursts
- Extracellular traps
- Hemolysis
- Aggregation
- Clotting
- Activation
- Cytokines

Scale: 10 μm
Challenges of whole blood preservation

Temperature (deg C)

-196

4

24

Solid organs

Whole blood

Fixed blood (dead cells & RNA degradation)

Platelets

RBCs

Stem cells

4-12 hrs

24 hrs

3-5 days

42 days

>years

Keith Wong et al.  (Nat. Comm, In press)
Microfluidics for Liquid Biopsy: An Enabling Technology for Cancer Care

High-Risk & Screening Group → Early Detection → Patient Group → Treatment Selection → Real-time Monitoring