Targeting the reattachment of circulating breast tumor stem cells to reduce metastasis.

Stuart S. Martin, Ph.D.
Associate Professor of Physiology
University of Maryland School of Medicine
The limits of clinical imaging shape our understanding of cancer recurrence
“Dormant” cells produce dynamic membrane protrusions when detached

MCF10A mammary epithelial cells

Frame / 1 sec.
Actin rearrangement in MCF10A cells attached to extracellular matrix
Cytoskeletal support of membrane protrusions


Control

LA

Inhibit actin polymerization
Destroys Filopodia and invadopodia

Phase Contrast  α-tubulin  Actin

Control
Microtentacles promote tumor cell aggregation (Live confocal imaging)

Microtentacles increase in invasive/metastatic breast tumor cell lines

In vivo mechanism for circulating tumor cell attachment matches microtentacles


HT-29 colon carcinoma cells
CalceinAM labeled
Injected into portal vein
Imaged in liver sinusoidal capillaries

Tubulin required for attachment

Actin depolymerization enhances attachment

adherent
Confocal imaging of live tumor cell attachment to endothelial cells
Death from fragmentation

Death from apoptosis

Inhibit microtentacles

Fates of circulating cells?

[1] ADHESION Microtentacles

[2] EXTRAVASATION Actin-dependent

Primary tumor

Metastatic tumor
Current working model of microtentacle (McTN) structure
Microtubule expansion is counteracted by actin cortical contraction

EB1-GFP imaging

ECM-attached cell  Detached cell
Kinesin inhibitors reduce microtentacles

Jennifer Yoon et al., *Breast Cancer Research and Treatment* (2011)

Tetracaine (125µM)
EMT induces microtubule detyrosination and microtubules

Rebecca Whipple et al., (Cancer Research, 2010)
McTNs mediate tumor cell reattachment (matrix/endothelial)
Concurrent upregulation of Twist and Glu-tubulin at invasive tumor fronts

Rebecca Whipple et al., (Cancer Research, 2010)
Microtentacles are increased in cancer stem cell populations
Stem cell subpopulations reattach more efficiently
Microtentacles are observed in mammospheres
Microtentacles extend between cells in mammospheres
Drugs targeting cell division can enhance microtentacles

Parthenolide and Costunolide can selectively target Glu-tubulin

Rebecca Whipple et al., (Breast Cancer Research, 2013)
Parthenolide and Costunolide inhibit tumor cell reattachment
Rebecca Whipple et al., (Breast Cancer Research, 2013)
Microtubule Stabilizers (Paclitaxel, Ixabepilone)

Actin inhibitors (Fasudil - ROCK, Dasatinib - Src)
Chemotherapy before surgery and CTC levels

(from Hekimian et al., 2012)

Neoadjuvant therapy

CTCs decrease
CTCs increase

Cumulative relapse-free survival

$P = 0.028$
$HR = 0.036$
Surgical samples from breast cancer patients (membrane dynamics)
Surgical samples from breast cancer patients (membrane dynamics – 600x)

Patent pending
Drug responses can be measured quickly in patient-derived tumor cells (30 minute Colchicine response)
### Automated measurement of McTN characteristics

**Confocal imaging of patient tumorgraft cells (HCI-001)**

**Automated McTN Analysis (MATLAB)**

<table>
<thead>
<tr>
<th>#McTNs/cell</th>
<th>Avg. McTN length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>8.8</td>
</tr>
<tr>
<td>4</td>
<td>10.2</td>
</tr>
<tr>
<td>6</td>
<td>8.7</td>
</tr>
</tbody>
</table>

**HCI-001 cell population**  
McTN frequency = 34%  
Avg.# McTNs/cell = 9.0  
Avg. McTN length = 9.1µm  

Patent pending
Rebecca Whipple Bettes
Eric Balzer
Agnes Cheung
Jennifer Yoon
Ed Cho
Mike Matrone
Michele Vitolo
Keyata Thompson
Monica Charpentier
Amanda Beggs

Susette Mueller (Georgetown)
Jing Yang (UCSD)
Josef Kas (Leipzig)

UM-College Park
Wolfgang Losert
Eleanor Ory
Ben Shapiro
Zach Cummins

ACEA xCelligence
ACEA Travel Award

R01-CA124704, R01-CA154624 (NCI)
Era of Hope Scholar Award (Department of Defense)

Susan G. Komen for the Cure – KG100240
Department of Defense Breast Cancer Idea Award
Department of Defense Breast Cancer Concept Award
DOD Breast Cancer Predoctoral (Cho)
DOD Breast Cancer Predoctoral (Balzer)
S10-RR022434-01 (NCRR, Xenogen)
FAMRI Clinical Innovator Award
UMB Independent New Investigator Award
K01-CA096555 Howard Temin Career Award (NCI)
Maryland Stem Cell Research Foundation
Maryland Cigarette Restitution Fund