Identifying a Novel Diagnostic and Therapeutic Target for Metastatic Breast Cancer

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Clinical Problem: Breast Cancer

#1 cancer (US women)

Metastatic disease: 5-year survival 25%

#2 cause of cancer-related death
Challenges of Metastasis

**Diagnostic**
Limited radiologic detection of early spreading

**Therapeutic**
Systemic agents have limited long-term success

Key: Circulating Tumor Cells (CTCs)
Overview

• Tubulin-based membrane protrusions (microtentacles) observed in detached cells

• Role for microtentacles in cancer progression and metastasis

• α-tubulin acetylation, microtentacle formation, adhesion, and invasive migration
Response of epithelial cells to detachment – Membrane protrusions
Cytoskeletal support of membrane protrusions


Inhibit actin polymerization
Destroys Filopodia and invadopodia

Inhibition of actin polymerization results in the destruction of filopodia and invadopodia.
Microtentacles increase in invasive/metastatic breast tumor cell lines

Example of a Patients CTC

Patient CTC
Microtentacles promote binding to adjacent cells
Confocal imaging of live tumor cell attachment to endothelial cells
Confocal imaging of CTCs isolated from human breast cancer patients
Even small tumors shed millions of cells into bloodstream.

Fates of circulating cells?

Death from apoptosis

[1] ADHESION
Microtentacles

Death from fragmentation

[2] EXTRA VASATION
Actin-dependent

Target microtentacles

Metastatic tumor
Microtubule stabilization and metastasis

αTubulin Acetylation

Acetylase (αTAT1)

Deacetylases (HDAC6, SIRT2)
Metastatic breast tumor cell lines have high acetylation of α-tubulin


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Metastatic breast tumor cell lines have high acetylation of α-tubulin that is enriched in more numerous McTNs.

Reduction of $\alpha$Tubulin Acetylation

$K40R$ acetylation resistant: ($\downarrow$ Acetyl-Tub)
K40R α-tubulin mutant expression


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K40R α-tubulin mutant decreases acetylation and McTN frequency

Increase in αTubulin Acetylation

Acetylase (αTAT1)

αTAT overexpression: (↑Acetyl-Tub)
Overexpression of αTAT1 increases tubulin acetylation


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αTAT1 significantly increases α-tubulin acetylation and McTNs

α-Tubulin acetylation significantly affects reattachment rates of suspended breast tumor cells
Chemotactic movement of breast tumor cells is affected by reducing acetylated α-tubulin

Chemotactic movement of breast tumor cells is affected by reducing acetylated α-tubulin
Acetylated $\alpha$-tubulin is detected in patient primary and matched metastatic tumors

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High acetylation of α-tubulin in patient primary tumors is linked to the basal-like subtype and an increased risk of disease progression and death.
Conclusions

1. Significant association between breast cancer cell lines and high acetylation of α-tubulin that extends along the length of McTNs.
2. Mutation of the specific lysine 40 acetylation site on α-tubulin as well as enzymatic modulation of this PTM has a significant impact on McTNs frequency and cancer cell attachment.
3. α-tubulin is necessary for migration.
4. Match primary and metastatic tissue arrays show tubulin acetylation is maintained or increased in nodal metastases.
5. Large proteomics study (390 patients) link high tubulin acetylation to the aggressive basal-like subtype.
6. A trend of increased risk of disease progression and death when α-tubulin is high in a patient’s primary tumor.
Department of Physiology

State of Maryland Cigarette Restitution Fund
K01-CA096555 Howard Temin Career Award (NCI)
UMB Independent New Investigator Award (Dean’s Office)
Department of Defense Breast Cancer Concept Award
S10-RR022434-01 (NCRR, Xenogen)
R01-CA124704 (NCI)

Department of Defense Breast Cancer Idea Award
FAMRI Clinical Innovator Award
DOD Breast Cancer Predoctoral (Balzer)
DOD Breast Cancer Predoctoral (Cho)
Maryland Stem Cell Research Foundation
Susan G. Komen for the Cure

Department of Defense Breast Cancer Era of Hope Scholar Award
R01-CA154624 (NCI)
K01-CA166576 (NCI)

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Department of Defense Breast Cancer Era of Hope Scholar Award
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Publications including data from the Xcelligence RTCA DP


