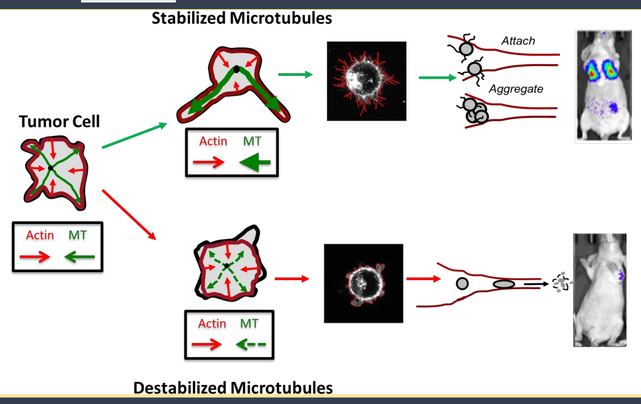
Microtubule disruption reduces tumor cell migration and invasion while microtubule stabilizers increase metastasis phenotypes.

Keyata N. Thompson , Riyan N. Campbell, Julia A. Ju, David A. Annis, Katarina T. Chang, Makenzy L. Mull, Megan Stemberger, Michele I. Vitolo and Stuart S. Martin

[1]Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine. 655 W. Baltimore St. Baltimore, MD 21201. USA. [2]Graduate Program in Molecular Medicine, University of Maryland School of Medicine. 800 W. Baltimore St. Baltimore, MD 21201. USA. [3]Department of Physiology, University of Maryland School of Medicine. 655 W. Baltimore St. Baltimore, MD 21201. USA. [4]Department of Pharmacology, University of Maryland School of Medicine. 655 W. Baltimore St. Baltimore, MD 21201. USA

Premise

95% of research focuses on therapeutics for the primary tumor, while metastasis is responsible for 90% of fatalities. Therefore, it is essential to investigate the cytoskeletal dynamics of CTC and the significant role of therapies as a necessary component to limit metastatic tumor spread. Our working model illustrates the structure and counteracting forces within the cytoskeletal network, but a model of therapeutic options to alter these balancing forces and their predictive outcomes. We have determined through various mechanisms that suspended breast tumor cells stabilize their microtubules, resulting in an increase of McTNs that we believe to aid in attachment and ultimately more metastasis. We believe the stability of microtubules can promote cell-cell adhesion and attachment while destabilization and depolymerizing agents would result in a tense restriction of these structures and subsequent death by fragmentation through narrowing capillaries.



Background

The ability for a cancer cell to survive in the bloodstream and reattachment to endothelial cells is a critical, rate-limiting step in the metastatic cascade. *In vitro* CTC modeling reveals that detached cells form McTNs – dynamic, microtubule-enriched plasma membrane extensions that are antagonized by the actin cytoskeleton (1). Work elucidating these cytoskeletal structures has proven that these protrusions aids in increased homotypic and heterotypic aggregation and reattachment in non-adherent cells. *Moreover, in vivo* cells forming McTNs are more efficiently retained in the microvasculature (2) and correlate to an increase in initial lung trapping and subsequent metastasis.

Cytoskeletal aberrations contribute to many characteristics of aggressive tumors, such as increased cell motility, weakened adhesive contacts, and metastatic dissemination. Our group has found that McTNs are the result of unbalanced forces between the actin cortex and microtubules. Bimolecular evidence suggests that cortical actin provides an internal force while microtubules push outward.

However, microtentacles can form from a disruption of this balance, whether when the actomyosin cortex is weakened or if microtubules are strengthened or stabilized.

Cytoskeletal drugs, including Taxanes and Vinca alkaloids, are chemotherapeutic agents that inhibit proliferating cancer cells by disrupting microtubule dynamics necessary for cell division. While microtubule-targeted drugs are highly successful in primary tumor treatment, their success is limited for metastatic disease. Recent studies show that the neoadjuvant chemotherapy Paclitaxel increases CTC shedding and lung colonization of CTCs in mice. Hence, it is imperative to identify therapies capable of reducing metastasis by directly targeting McTNs on circulating tumor cells.

References

- Matrone MA, Whipple RA, Thompson K, Cho EH, Vitolo MI, Balzer EM, Yoon JR, Ioffe OB, Tuttle KC, Tan M, Martin SS. Metastatic breast tumors express increased tau, which promotes microtentacle formation and the reattachment of detached breast tumor cells. Oncogene. 2010 Jun 3;29(22):3217-27. doi: 10.1038/onc.2010.68.
- Matrone MA, Whipple RA, Balzer EM, Martin SS. Microtentacles tip the balance of cytoskeletal forces in circulating tumor cells. Cancer Res. 2010 Oct 15;70(20):7737-41. doi: 10.1158/0008-5472.CAN-10-1569.

Acknowledgements

University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center

National Cancer Institute

MARTIN LAB

Come visit at ssmartinlab.org!

Results

1. Phenotypic profile of microtubule targeting drugs independent of viability

A

Drug	Target	McTN Effective Dose	McTN Response	Attachment	Migration	Clustering Efficiency
Parthenolide	NF-κB DeTyr-tub Tublin	10uM	+	+	-	+
Eribulin	Destablizer Tubulin	10uM	+	-	-	-
Vinorelbine	Depolymerizer Microtubule	5-10uM	-	-	-	-
Pacitaxel	Stabilizer Non-Taxane	1.17uM	++	++	++	+++
Ixabepilone	Microtubule Stabilizer	10uM	++	++	N/A	++

B

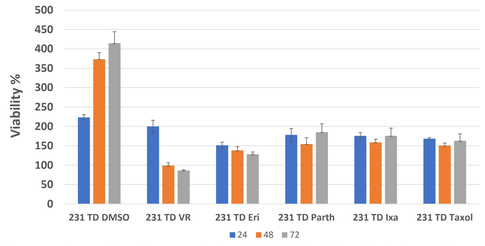
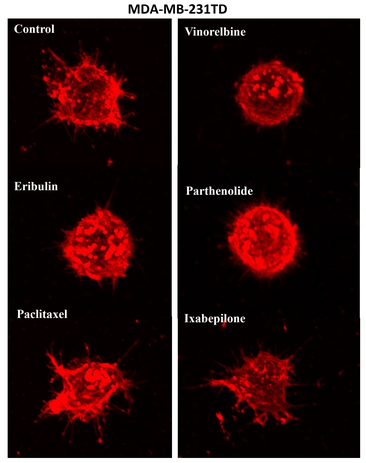


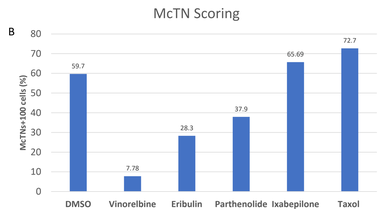
Figure 1: Phenotypic profile of tumor derived MDA-MB-231 cells independent of a loss in viability. **A)**Informative data concerning the phenotypic properties of cells treated with tubulin targeting drugs, including viability, microtentacle formation, reattachment efficiency, migratory properties and cluster formation. Each assay featured in the panel is detailed throughout the poster. **B)** Colorimetric cell viability assay shows that each drug caused minimal toxicity even after 72hrs. Data are shown as mean ± SD, n=3.

2. Microtubule targeting drugs influence microtentacle expression in breast tumor cells. .

A



B



C

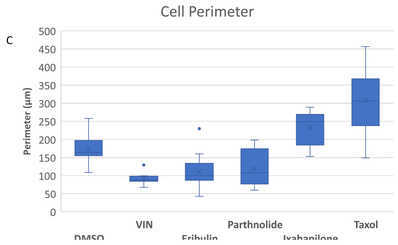
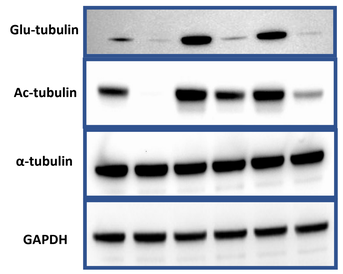


Figure 2: Tubulin targeting drugs effects on microtentacles in breast tumor cells. Microtubule destabilizing and depolymerizing agents show similar effects on microtentacle formations. Moreover, the targeted therapies, Parthenolide which targets NF-κB, shows a similar effect. **A)** MDA-MB-231-TD cells stained with CellMask Orange cell membrane dye, detached and suspended in media containing vehicle (0.1% DMSO, 1h), Vinorelbine (10μM, 1h), Eribulin (10μM, 1h),Parthenolide (25μM, 1h), Ixabepilone (10μM, 1h), and Taxol (1.17μM, 1h). **B)** Vinorelbine, Eribulin and Parthenolide caused a significant decrease in microtentacle frequency (%) compared to vehicle control. Conversely, Ixabepilone and Taxol increase microtentacle frequency. McTN scoring consists of mean values from four independent experiments where 100 cells were blindly counted and averaged. **C)** Analysis measuring the perimeter on individual cells treated with vehicle control DMSO or each drug. Data are shown as mean ± SD, n=3;

3. Microtubule targeting drugs alter the cytoskeletal architecture and molecular expression in breast tumor cells. .

A



B

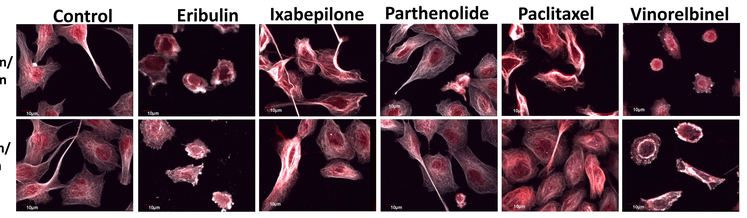


Figure 2: Tubulin Targeting drugs modulate acetylated a- tubulin while altering the filamentous microtubule network. **A)** Western blot analysis of Eribulin (10μM), Ixabepilone (10μM), Ixabepilone (10μM) Parthenolide (10μM), Paclitaxel (10μM) and Vinorelbine (10μM) treated MDA-MB-231-TD cells at 1hr of treatment. Tubulin destabilization and de-polymerization with Eribulin and Vinorelbine were most effective at decreasing acetylated a-tubulin (acetyl-tubulin) whereas tubulin stabilizers Ixabepilone and Paclitaxel increased acetylated a-tubulin (acetyl-tubulin). The NF-kB targeting drug Parthenolide did not result in a significant difference. **B)** Within the 1hr treatment, filamentous alpha tubulin(white) and acetyl and glu-tubulin (red) are altered due to each respective drug treatment. Images taken at 60x magnification. Scale bar = 10μm.

3. Reduction in tumor cell reattachment and migration with microtubule destabilization

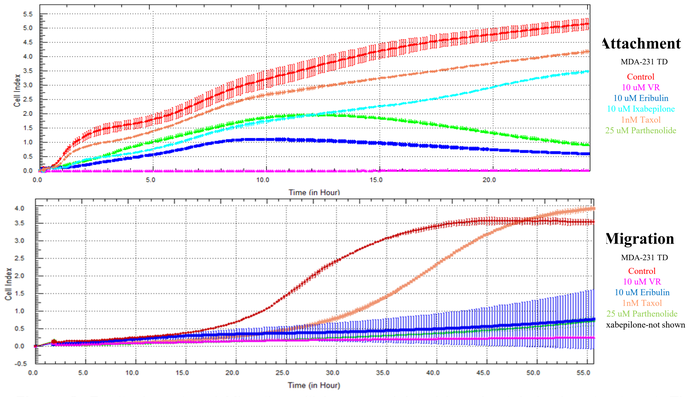
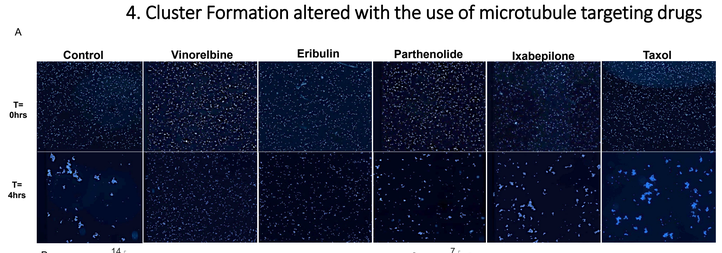


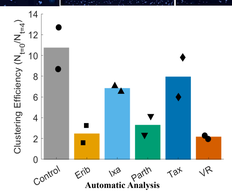
Figure 3: Reattachment and Migration efficiency modulated by microtubule drug treatment. The xCELLigence system was used to measure and quantify all migration and attachment assays. **A)** Reattachment efficiency of the MDA-MB-231-TD cells treated with Vinorelbine (10μM, 1h), Eribulin (10μM, 1h),and Parthenolide (25μM, 1h), was significantly lower than vehicle control treated cells (0.1% DMSO, 1h). Changes in impedance are apparent as early as 1hr and significant differences continue for 24hrs after initial seeding. Surprisingly, cells treated with Ixabepilone (10μM, 1h), and Taxol (1.17μM, 1h) did not significantly increase attachment over time. **B)** Migration was quantified using the same impedance analysis. MDA-MB-231-TD cells treated with both depolymerizing agents and stabilizers show a significant decrease in migration over 55 hrs. Paclitaxel is the only exception. Migration in the Taxol treated condition showed a delay in migration, however, migration shows similar trend with the vehicle control at 30hrs. Data are shown as mean ± SD, n=3;

4. Cluster Formation altered with the use of microtubule targeting drugs

A



B



C

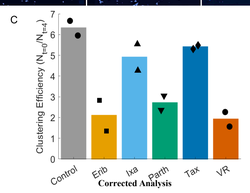
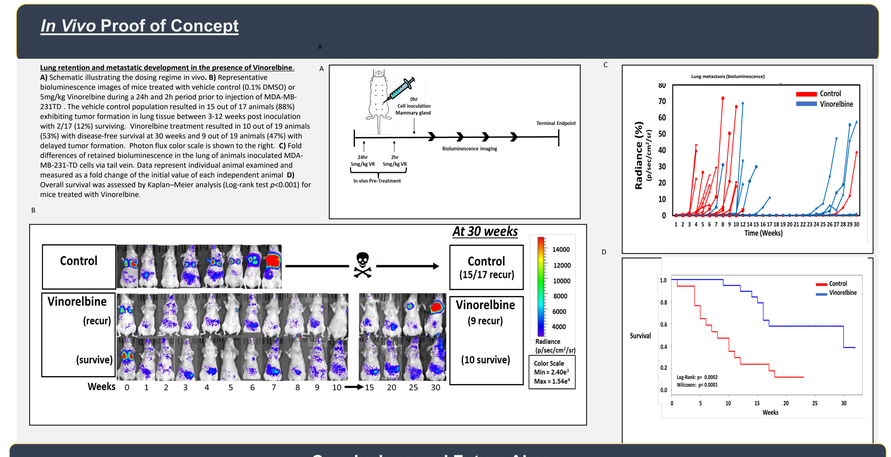


Figure 4: Microtubule depolymerizing treatment decreases tumor cell homotypic aggregation. **A)**Representative Hoechst stained images of MDA-MB-231 TD cells treated with each drug for 1hr in attached conditions and suspended for 4hrs to allow homotypic aggregation. At each respective time point cells were tethered onto TetherChips. Images taken at 4x magnification. Microtubule destabilization, NF-κB targeted therapy and depolymerizing agents show a significant decrease in cluster size **B)** Clustering efficiency analysis on the number of clusters that formed over 4h. Analysis measuring the efficiency of clustering by comparing the number of individual clusters over time. Individual values at t = 0 h were divided by respective experimental final cluster numbers (t = 4 h) for each condition. Graphical representation was generated automatically was by MATLAB without correction. **C)** Graphical representative was normalized to only include a clusters threshold of two nuclei or more. Each data point represents independent cluster formation experiments, n=2.

In Vivo Proof of Concept



Lung retention and metastatic development in the presence of Vinorelbine. **A)** Schematic illustrating the dosing regime in vivo. **B)** Representative bioluminescence images of mice treated with vehicle control (0.1% DMSO) or 5mg/kg Vinorelbine during a 24h and 2h period prior to injection of MDA-MB-231TD . The vehicle control population resulted in 15 out of 17 animals (88%) exhibiting tumor formation in lung tissue between 3-12 weeks post inoculation with 2/17 (12%) surviving. Vinorelbine treatment resulted in 10 out of 19 animals (53%) with disease-free survival at 30 weeks and 9 out of 19 animals (47%) with delayed tumor formation. Photon flux color scale is shown to the right. **C)** Fold differences of retained bioluminescence in the lung of animals inoculated MDA-MB-231-TD cells via tail vein. Data represent individual animal examined and measured as a fold change of the initial value of each independent animal **D)** Overall survival was assessed by Kaplan–Meier analysis (Log-rank test $p<0.001$) for mice treated with Vinorelbine.

Conclusions and Future Aims

- Examine molecular profile of cells treated with microtubule targeted drugs.
- Employing microtubule targeting drugs show a response in McTN formation-next steps will be to investigate the filamentous microtubule network.
- Develop preclinical in vivo models tailored to each microtubule agent.
  - investigative primary tumor formation
  - metastatic recurrence.
- Use preclinical models to investigate increased CTC dissemination after treatment with microtubule targeted agents.
  - CTCs shed from mouse tumors and isolated from blood samples using Vortex technology to examine the presence of McTNs in response to drugs