Microtubule Disruption Reduces Metastasis More Effectively Than Primary Tumor Growth

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Research article

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Abstract

Background: Clinical breast cancer imaging inevitably focuses on tumor growth rather than the metastatic dissemination that is a greater challenge for patient survival. Emerging preclinical evidence indicates that chemotherapy can elevate levels of circulating tumor cells (CTCs) and increase metastatic recurrence. Targeting metastatic phenotypes of CTCs could reveal therapeutic strategies to reduce metastasis that would be overlooked by measurements of tumor growth.

Methods: We investigated how the FDA-approved microtubule-depolymerizing Vinca alkaloid, Vinorelbine, affects three metastatic phenotypes of reattachment, clustering and microtentacles (McTNs). Microfluidic cell tethering technology (TetherChip) was used to measure Vinorelbine effects on McTNs and clustering while xCelligence impedance was used for reattachment assays. Bioluminescence imaging monitored tumor growth and metastasis in mice. ANGLE Parsortix and Vortex Biosciences VTX-1 were used to capture live tumor cells from blood samples for confocal microscopy to rapidly measure tumor cell responses to Vinorelbine.

Results: We demonstrate that a focused (1h) Vinorelbine treatment is sufficient to inhibit reattachment, clustering and McTNs in non-adherent breast tumor cells. Quantitative analysis of treated non-adherent cells reveals that McTNs are significantly lower in number (p= 0.012) and shorter in length (p= 0.000034). Treating mice with Vinorelbine (5mg/kg) for only 24h did not significantly affect primary tumor survival. However, median metastatic tumor survival after injection of circulating tumor cells extended from 8 weeks to 30 weeks after a focused 24h treatment with Vinorelbine. Microtentacle inhibition by Vinorelbine was also detectable within 1h, using live tumor cells isolated from blood samples and analyzed with confocal microscopy for McTNs on TetherChip microfluidic surfaces. As few as 11 tumor cells were sufficient to yield 90% power to detect this 1h Vinorelbine drug response, demonstrating feasibility with the small number of tumor cells available from patient biopsies.

Conclusions: This study establishes a proof-of-concept that targeted microtubule disruption can selectively inhibit metastasis and reveals that existing FDA-approved therapies could have anti-metastatic actions that are currently overlooked when focusing exclusively on tumor growth. Moreover, the anti-metastatic actions of Vinorelbine could be detected in less than one hour using microfluidic TetherChip technology, establishing a new approach for precision medicine aimed at reducing metastasis.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures
Figure 1

Lung retention and metastatic development in the presence of Vinorelbine. A) Representative bioluminescence images of mice treated with vehicle control (0.1% DMSO) or 5mg/kg Vinorelbine during a 24h period prior to injection of MDA-MB-231TD cells that were pre-treated for 1h with the same drug conditions. 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. The 24h window
of 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. B) Fold differences of retained bioluminescence in the lung of animals inoculated MDA-MB-231-TD cells via tail vein. Data represent individual animals examined and measured as a fold change of the initial value for each independent animal. C) Representative images of lung immunohistochemistry for human mitochondria to identify the human tumor cells and hematoxylin and eosin (H&E) staining performed at ethical end-points. Images captured at a magnification of 20× indicate metastatic burden, scale bar, 200µm. D) Overall survival was assessed by Kaplan–Meier analysis (Log-rank test p=0.0002) for mice treated with Vinorelbine.
Figure 1

Primary tumor development uninhibited in the presence of Vinorelbine. A) Representative bioluminescence imaging from mice treated with vehicle control (0.1% DMSO) or 5mg/kg Vinorelbine during a 24h period prior to injection of MDA-MB-231TD cells that were pre-treated for 1h with the same drug conditions. Photon flux color scale is shown to the right. B) Graphical representation of the growth curve of each condition measured as a fold difference of bioluminescence signal over time. To quantitate
values for each time point, the background was subtracted from the peak signal, and the difference was normalized to the initial value (first timepoint) for each animal (n= 5 per group). C) Graphical representation of the tumor size measured by external caliper measurement. Volumetric measurement of xenografted tumors was obtained using the ellipsoid calculation \( V = \frac{xy2}{2} \). D) The probability of overall survival was assessed by Kaplan–Meier analysis (Log-rank test \( p=0.07 \)) for mice treated with Vinorelbine.
Vinorelbine drug response in tumor cells recovered from blood samples. MDA-MB-231-TD cells captured using ANGLE-Parsortix and VTX-1 systems. A) Parsortix schematic of CTC staircase capture method from whole blood. Live DIC images to visualize cell capture and CellMask orange stained cells to visualize McTNs (arrows). B) MDA-MB-231-TD cells expressing GFP (green) spiked into 7.5ml whole-blood were recovered in Parsortix cassette, eluted onto TetherChip slides and imaged live after DNA staining with Hoechst (blue) and CellMask Orange cell membrane dye (red), to visualize McTNs (white arrows). C) Confocal microscopy of MDA-MB-231-TD tumor cells isolated from 7.5ml whole blood using the VTX-1 system and then treated with Vinorelbine (10μM, 1h) or drug vehicle (0.1% DMSO, 1h) before elution onto TetherChip slides, chemical fixation and Hoescht (blue)/WGA (red) staining. Arrows show McTN protrusions on isolated tumor cells. D) McTN analysis of the cell body outline (blue), perimeter (red), and McTNs tips (yellow) of fixed and tethered cells. E) McTN analysis measuring average number McTNs/cell after treatment with vehicle or Vinorelbine. F) Analysis of average distance of McTN tips from cell body boundary for cells treated with vehicle or Vinorelbine. For cell isolation and fixation analysis a total of 78-cells in the vehicle control population and 80-cells for the Vinorelbine treated population was analyzed from 3 independent experimental replicates.
Vinorelbine treatment decreases tumor cell reattachment and homotypic aggregation. A) Reattachment efficiency of the MDA-MB-231-TD cells treated with Vinorelbine (10μM, 1h) is significantly lower than vehicle control treated cells (0.1% DMSO, 1h). Changes in impedance are apparent as early as 1h and significant differences continue for 24h after initial seeding. Representative data from three independent experiments; each performed in quadruplicate. B) Representative phase contrast images of vehicle
control (a-c) and Vinorelbine-treated (d-f) MDA-MB-231-TD cells over time to visualize cell attachment or lack of attachment (rounding). Panels, 4x magnification; Insets, 10x magnification. C) Representative Hoechst stained images of control and Vinorelbine-treated MDA-MB-231-TD cells over time to visualize cluster formation efficiency. Images taken at 10x magnification. Scale bar = 200μm. D) 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.
Figure 1

Vinorelbine decreases microtentacles on breast tumor cells. MDA-MB-231-TD cells stained with CellMask Orange cell membrane dye, detached and suspended in media containing vehicle (0.1% DMSO, 1h) or Vinorelbine (10μM, 1h). A) Representative confocal images (top) and inverted epifluorescence images (bottom) of free floating cells on a low attach plate. Scale bars correspond to 10μm. B) Vinorelbine caused a significant decrease in microtentacle frequency (%) compared to vehicle control. McTN scoring consists of mean values from four independent experiments where 100 cells were blindly counted and averaged. C) Representative raw images of tethered cells and computer determined cell body outline(blue), cell perimeter(red), and McTNs tips(yellow). D) Analysis measuring an average number of McTN tips for cells treated with vehicle, and Vinorelbine (10μM, 1h). McTN number is the number of McTNs per cell. McTN Distance is average distance of McTN tips from cell body boundary for cells treated with vehicle, and Vinorelbine. For live cell image analysis, a population of 25 cells per condition was analyzed from 3 independent experimental replicates.
Figure 1

Vinorelbine increases acetylated α-tubulin while decreasing the filamentous microtubule network. A) Western blot analysis of Vinorelbine (10μM) treated MDA-MB-231-TD cells results in an increase in acetylated α-tubulin (acetyl-tubulin) whereas detyrosinated tubulin (Glu-tubulin) and total-tubulin remain unchanged at 24h and 48h compared with vehicle control (0.1% DMSO) and Staurosporine positive control (1μM). There was a minimal PARP cleavage at 24h. However, by 48hrs, approximately 50% PARP cleavage is observed. B) Within 15min of 10μM Vinorelbine treatment, filamentous tubulin is destroyed (green). Analysis at additional times (data not shown) reveals the continued absence of filamentous
tubulin. Hoechst was used to visualize the nuclei (blue). Images taken at 60x magnification. Scale bar = 20μm.

**Supplementary Files**

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