#2888

Computational Analysis of Microtubule Dynamics for Personalized Prostate Cancer Therapy

A. Matov[1,2], D. Kimovski[2], G. Galletti[1], B. Pera[3], W. Harkcom[1], D. Rickman[1], P. Giannakakou[1]

[1] Weill Cornell Medical College, New York, NY, [2] University for Information Science and Technology St Paul the Apstle, Ohrid, Macedonia, [3] Memorial Sloan-Kettering Cancer Center, New York, NY

Mechanism of action of taxanes in prostate cancer

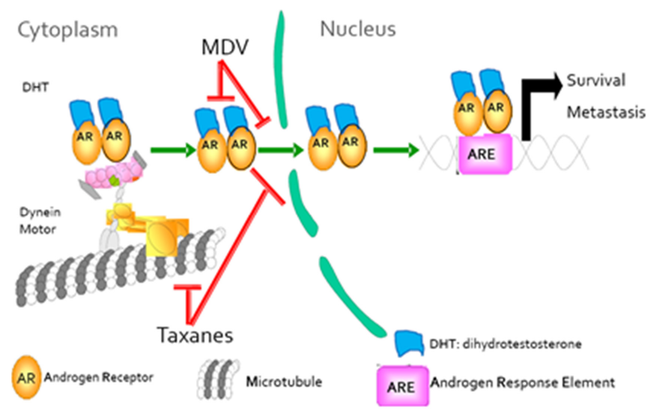

Fig. 1. Proposed model of taxane mechanism of action in prostate cancer. In the model, AR associates with MTs and translocates to the nucleus via the motor protein dynein. Taxanes, which bind to and stabilize MTs perturbing their dynamic properties, inhibit AR from reaching the nucleus and activating target genes.

Thadani-Mulero et al., Androgen receptor on the move: Boarding the microtubule expressway to the nucleus, *Cancer Research,* 2012

Androgen receptor splice variant v567 but not v7 associates with microtubules

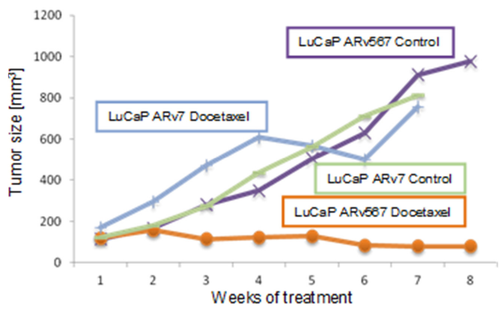

Fig. 2. LuCAP xenografts expressing ARv567, but not AR-V7, are sensitive to Docetaxel treatment. 1x10^6 cells were injected s.c. into castrate athymic nude mice. When tumors reached 100-200 mm^3, they were given a weekly 5 mg/kg dose of Docetaxel. Tumor volume was measured weekly following Docetaxel treatment. Note that the ARv567 expressing xenografts were exquisitely sensitive to Docetaxel treatment for duration of assessment period.

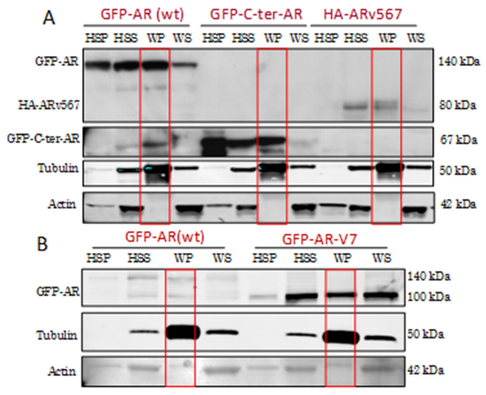

Fig. 3. ARv567 but not ARv7 associates with MTs. MT co-sedimentation assay using whole cell lysate from cells transfected with (**A**) GFP-AR-wt, GFP-C-terminal AR, HA-ARv567 and (**B**) GFP-ARv7 was carried out to test the affinity of these variants for the MT polymer. Note the association of nearly 100% ARv567 with MT polymers (WP). In contrast, only 40% of AR-V7is associated with the MT polymer. Actin was used as a negative control for MT binding.

Thadani-Mulero et al., Androgen receptor splice variants determine taxane sensitivity in prostate cancer, *in preparation*

Imaging and analysis of GFP-labeled microtubule tips

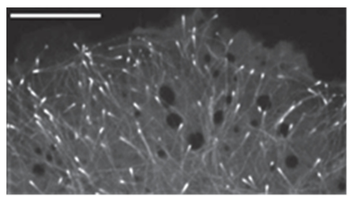

Fig. 4. Spinning disk confocal raw image of a cell stably expressing EB1-EGFP. The bright comets represent clusters of EB1 dimers that bind the tips of MTs during growth. Sequence encoding EGFP-tagged EB1 was introduced into cells by lentivirus mediated viral transduction. Scale bar, 10 µm.

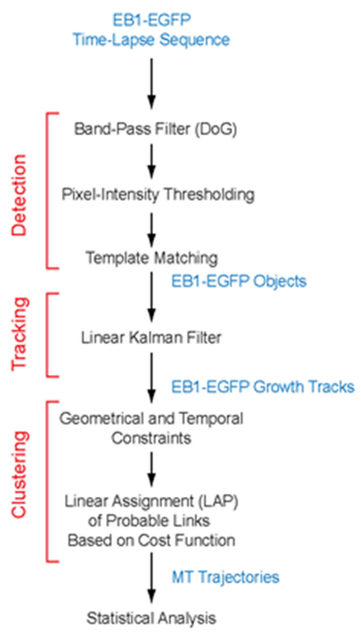

Fig. 5. Flow diagram of EB1-EGFP comet analysis. Detection, tracking, clustering and statistical analysis

Matov et al., Analysis of microtubule dynamic instability using a plus end growth marker, *Nature Methods,* 2010

Strategy for the analysis of MT dynamics

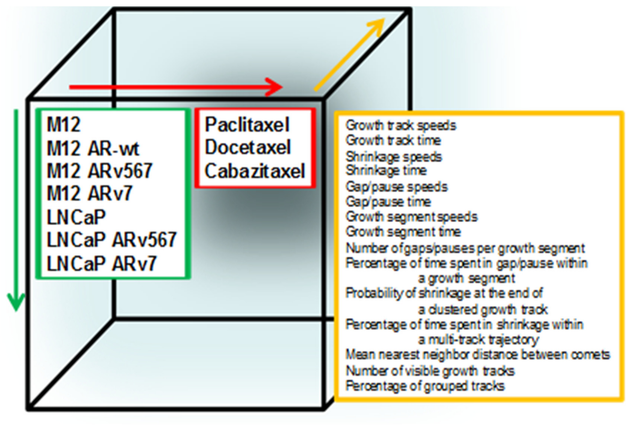

Fig. 6. Strategy for the analysis of MT dynamics. Green box: Cell lines expressing AR variants will be analyzed and inherent MT dynamics will be measured. Red box: Each of the three taxanes will be added to cells of each of the cell lines to measure drug sensitivity for each of the MT dynamics parameters. Yellow box: The list of MT dynamics parameters measured.

Hypothesis

Prostate cancer cells with different microtubule dynamics exhibit differential sensitivity to taxane treatment. Our preliminary data suggest that expression AR splice variants or the presence of TMPRSS2-ERG fusion protein confer taxane resistance. We will examine the impact of these transcription factors on microtubule dynamics and drug sensitivity.

M12 cells expressing ARv7 exhibit altered microtubule dynamics as compared to AR-wt

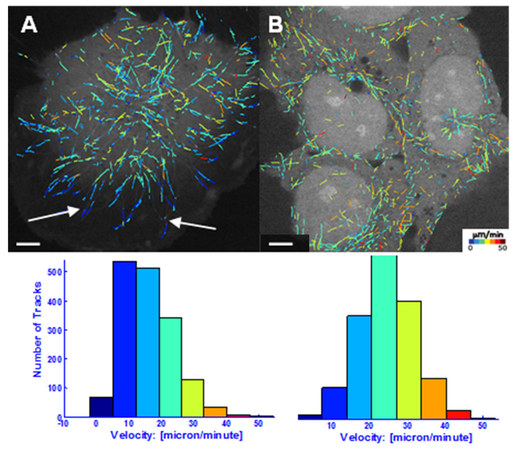

Fig. 7. Tracking of EB1 comets in M12 prostate cells. Colors code for speeds of EB1 comets (i.e. MT growth rates), with warmer colors for higher speed rates and colder colors for lower speed rates. (A) MT growth tracks for PC cells expressing the AR-wt variant. MT tracks growth speeds slow down toward the cell edge (see arrows). (B) MT growth tracks for cells expressing ARv7 variant, which is resistant to Paclitaxel treatment, are significantly faster. Scale bar, 10 µm. Lower panels show corresponding EB1 comet velocity histograms.

DU145 cells expressing the TMPRSS2-ERG fusion protein exhibit taxane resistance

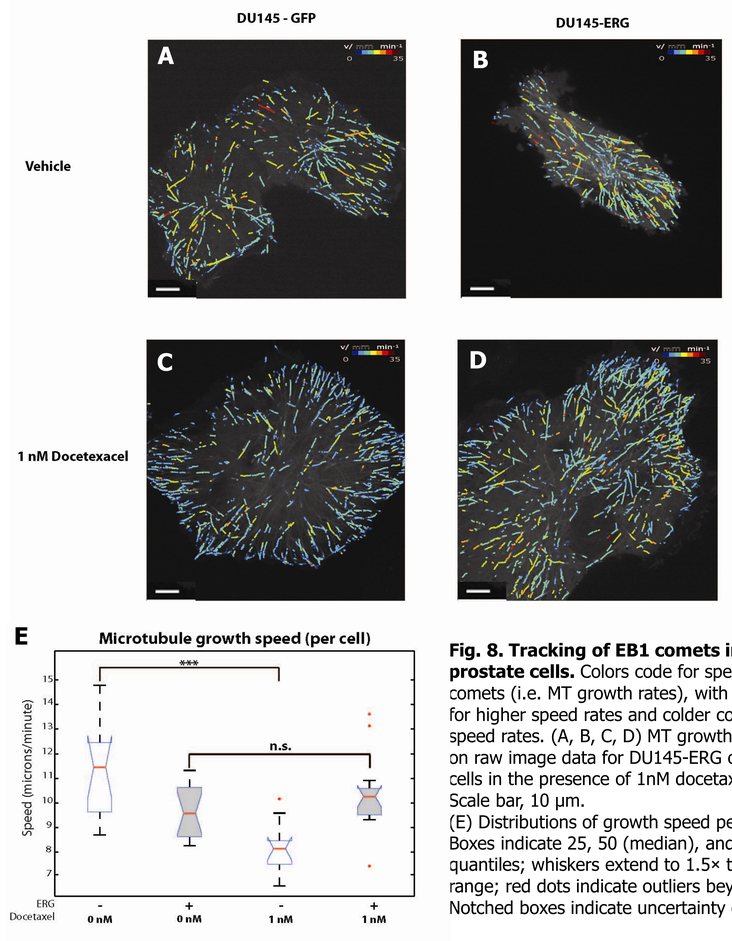

Fig. 8. Tracking of EB1 comets in DU145 prostate cells. Colors code for speeds of EB1 comets (i.e. MT growth rates), with warmer colors for higher speed rates and colder colors for lower speed rates. (A, B, C, D) MT growth tracks overlaid on raw image data for DU145-ERG or DU145-GFP cells in the presence of 1nM docetaxel or vehicle. Scale bar, 10 µm.
(E) Distributions of growth speed per cell means. Boxes indicate 25, 50 (median), and 75% quantiles; whiskers extend to 1.5× the interquartile range; red dots indicate outliers beyond this range. Notched boxes indicate uncertainty of the median.

Galletti et al., TMPRSS2-ERG fusion protein exhibit taxane resistance in prostate cancer, *in preparation*

Conclusion

We use computer vision algorithms to obtain statistically representative results for the effects of ERG or AR on MT homeostasis following treatment with each of the three taxanes. We measure changes in MT behavior as statistically significant shifts in different parameters of MT dynamics measured from >20,000 MTs for each condition. Our preliminary results revealed that the presence of ERG fusion protein and AR variant expression in PC cell lines correlate with changes in MT dynamics.