Mitotic perturbations increase micronuclei formation in breast cancer cells

Winnie Muiru[1], Alisya Anlas[2], Dennis Discher[2]
[1]Bryn Mawr College, Bryn Mawr, Pennsylvania 19010 USA
[2]Mol. Cell Biophysics Lab, University of Pennsylvania, Philadelphia, PA 19104, USA

Introduction: Genomic instability is a hallmark of cancer and contributes to its initiation and progression[1,2]. Among one of the most damaging errors is whole chromosome loss, and this aneuploid state is frequent in solid tumors compared to liquid ones[1]. Chromosomal instability (CIN) is typically a result of errors in DNA replication and mitosis[3]. The monopolar spindle (MPS1) kinase ensures bipolar attachment of chromosomes along the mitotic spindle[4] and has been found to be misregulated in tumors, leading to aneuploidy[4].

The phosphatase and tensin (PTEN) homolog gene is a major lipid phosphatase known for its traditional role in enzymatic activation of the PI3K/AKT signaling pathway and has recently been shown to be necessary for centromere function by interacting with key centromere protein CENP-C[5]. Loss of PTEN can cause CIN via formation of centromere fragments, with aneuploidy in human primary breast cancer[5, 6]. Cytoskeletal motor protein myosin IIA, an ATPase encoded by the myosin heavy chain 9 (MYH9) gene is the main motor protein involved in cytokinesis, and has been implicated in cytokinesis failure[7]. Whereas PTEN is a major tumor suppressor gene (TSG) that is frequently mutated in cancers, MYH9 is more minor and not considered a TSG.

Chromosome missegregation can lead to mispackaging of chromosomes in the form of a micronucleus. Characterization of micronuclei offers insight into understanding the mechanisms of chromosome loss in solid tumors. Here, we pharmacologically inhibit MPS1, PTEN and MYH9 in a human breast cancer cell line to observe CIN in the form of micronuclei formation.

Methods: MCF7 breast cancer cells were treated with an MPS1 inhibitor, a PTEN inhibitor or MPS1 inhibitor blebbistatin (20 uM). Following a 24-hour treatment, the cells were fixed using formaldehyde, permeabilized and blocked, then stained for F-actin. Nuclei were counterstained using Hoechst.
Images were then acquired with fluorescence microscopy using a x20 or x40 magnification air objective to visualize micronuclei and mitotic cells, and quantification was performed using Fiji (Image J).

Results and discussion:

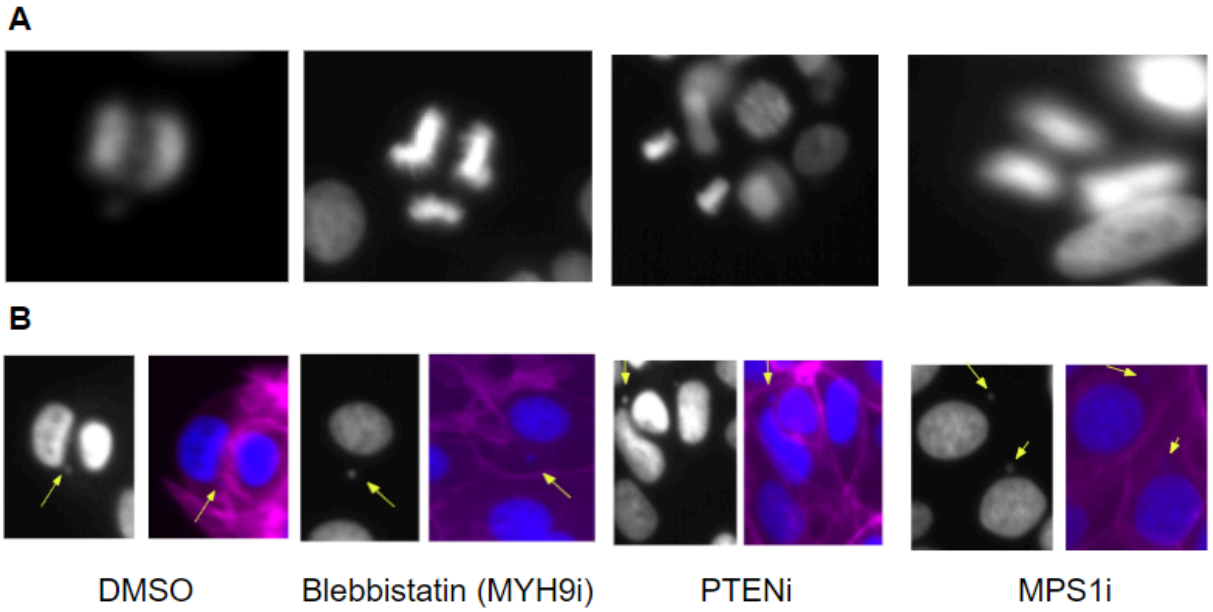

Figure 1. Qualitative analysis of mitotic cells and micronuclei. A) Brightfield 20x images of mitotic cells stained with DAPI to label nuclei. B) Brightfield and merged images of cells containing a micronucleus, with phalloidin probe to label f-actin.

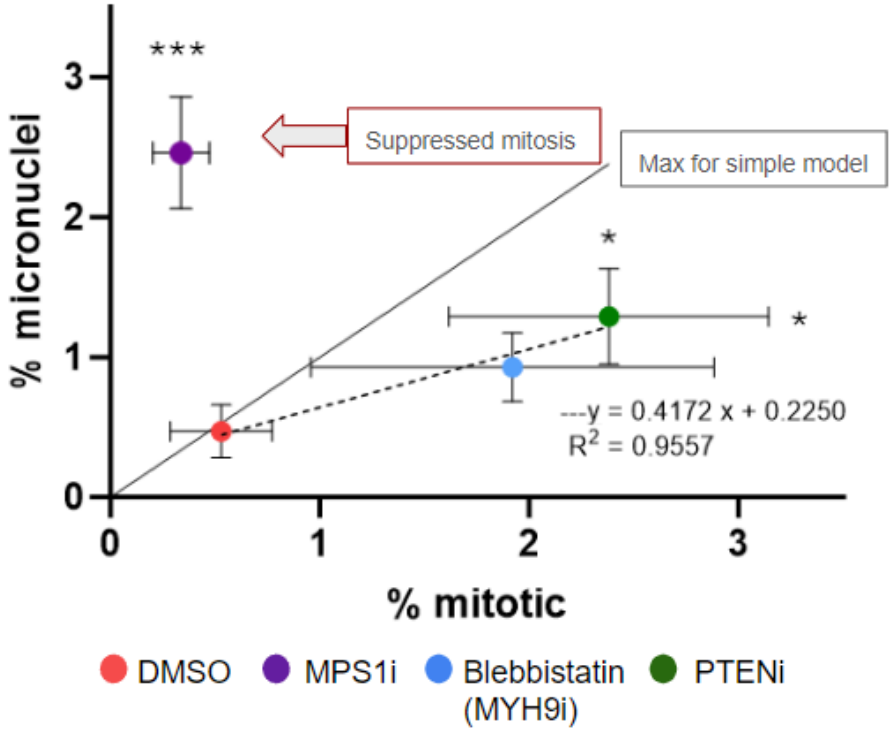

Figure 2. MPS1i and PTENi drive micronuclei formation in standard 2D cultures. Drug treatments MPS1i and PTENi display increased micronuclei ($p<0.001$ and $p<0.05$, respectively), with PTENi also displaying increased mitotic cells.

Our data suggest that inhibiting the SAC leads to increased micronuclei ($p<.001$). Treatment of MCF7 cells with a PTEN inhibitor also led to an increase in micronuclei and mitotic cells ($p<.05$). Treatment with blebbistatin followed the same trend as with PTEN inhibition, though this result was not significant. PTEN is a putative TSG and there's some evidence of MYH9 also being a TSG. Here, we see their inhibition causes an increase in mitotic cells along with increased micronuclei.

Conclusion: When genes involved in mitosis are perturbed, increased micronuclei formation is observed in the case of MPS1 and PTEN inhibition. These results further support other findings in which MPS1 and PTEN are dysregulated in different tumor types[4,5,6]. Myosin IIA links with actin to form the contractile ring during cytokinesis and this motor protein's failure to divide the cytoplasm can lead to multipolar spindle formation, aneuploidy, cell cycle arrest or cell death. Multipolar spindle formation can increase micronuclei since there are more attachment points of spindles to centromeres. Cell cycle arrest or cell death could also explain why there was not a significant increase in micronuclei in the blebbistatin treatment despite studies that show that loss of this gene is associated with increased tumorigenesis[8].

A live-cell method for detecting chromosome loss via endogenous fluorescent tagging of single alleles has been developed and verified in our lab on a variety of solid tumor cell lines. An ongoing project is to validate this tool on the MCF7 cell line. Loss of the signal in previously transformed cells and formation of GFP-positive micronuclei would provide information of at least a key constitutive gene that has been lost. Previously transformed cells that are no longer fluorescent would indicate CIN due to complete loss of the chromosome. This endogenous approach would also help to answer whether this chromosome loss is heritable.

Identification of micronuclei is one step in understanding the effects of CIN, though the exact mechanisms of micronuclei formation such as merotelic attachment or centrosome amplification need to be further investigated in this breast cancer adenocarcinoma line and others.

Impact: Chromosomal instability (CIN) in cancer cells, driven by errors in mitotic processes, is a critical factor in tumor progression and resistance to commonly-used therapeutics. Our research demonstrates that pharmacological inhibition of key mitotic regulators—MPS1, PTEN, and MYH9—increases micronuclei formation in breast cancer cells, revealing their roles in maintaining chromosomal integrity. By identifying specific genes involved in CIN, our study emphasizes the importance of developing targeted therapies that can selectively address these dysregulations and improve patient outcomes in breast cancer.

References

1. Knouse, K. et al. Aneuploidy in Cancer: Seq-ing Answers to Old Questions. *Annu. Rev. Cancer Biol.* **1**,335-54 (2017).
2. Ferguson L. et al. Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. *Semin Cancer Biol*. **35**, S5-S24 (2015).
3. Simi., A. et al. Mechanotransduction, Metastasis and Genomic Instability. *Genomic Instability and Cancer Metastasis*, Cancer Metastasis - Biology and Treatment **20**, DOI 10.1007/978-3-319-12136-9_7 (2015).
4. Liu X, Winey M. The MPS1 family of protein kinases. *Annu Rev Biochem*. **81**, 561-585. (2012).
5. Shen, W. et al. Essential Role for Nuclear PTEN in Maintaining Chromosomal Integrity. *Cell* **128**, 157-170 (2007).
6. Puc, Janusz et al. "Lack of PTEN sequesters CHK1 and initiates genetic instability." *Cancer cell*. **7(2)**, 193-204 (2005).
7. Li Y., Yang W. Myosins as fundamental components during tumorigenesis: diverse and indispensable. Oncotarget **7**, 46785-46812 (2016).
8. Schramek, D. et al. PTEN and myosinIIA are putative tumor suppressors, which in normal cells decreases cell division, though it appears that their inhibition cause an increase in mitotic cell along with increased micronuclei. *Science*. **343**, 309-314 (2014) .
9. Burgess, D. Cytokinesis: New Roles for Myosin. *Cell*. **15(8)**, R310-R311 (2005).
10. Nigg, E., Stearns, T. The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. *Nat Cell Biol.* **13,** 1154–1160 (2011).
11. King, Randall W. "When 2+2=5: the origins and fates of aneuploid and tetraploid cells." *Biochimica et biophysica acta*. **1786(1)**, 4-14 (2008).
12. Kojima, Shihoko, and Daniela Cimini. "Aneuploidy and gene expression: is there dosage compensation?." *Epigenomics.* **11(16)**,1827-1837 (2019).