Investigating the Role of PTEN and Myosin IIa in Regulating Genomic Instability in Lung Cancer and Melanoma Cells

Yariani Vélez Vázquez[1], Alişya A. Anlaş[2 3], and Dennis E. Discher[2 3].

[1]University of Puerto Rico - Cayey, Department of Biology, [2]University of Pennsylvania, Molecular and Cell Biophysics Lab, [3]University of Pennsylvania, Department of Chemical and Biomolecular Engineering.

Introduction

Cancer progression is characterized by the acquisition of traits known as the hallmarks of cancer [1]. Among these, genomic instability (GIN) that results from cell division errors is a critical factor that promotes genetic alterations and cancer progression through losses or gains of chromosomes with key genes. We explored the potential roles of PTEN and Myosin IIa as tumor suppressors in A549 lung adenocarcinoma and B-16 mouse melanoma cell lines and investigated how perturbing these might lead to GIN. Specifically, Myosin IIa drives the contraction of the actomyosin cytoskeleton, which is essential for proper cell division, while PTEN is involved in cell growth and proliferation given its role as a PI3K/AKT downregulator. Thus, we examined how blocking PTEN or Myosin IIa can quickly undermine division and lead to chromosome mis-segregation, which is evident as a 'micronucleus' containing a chromosome that is lost or gained by one of the daughter cells. We were further interested in using Palbociclib to inhibit CDK4/6, involved in cell cycle entry, and in using Reversine to inhibit the spindle assembly checkpoint (SAC). For each of the four drugs used, we hypothesized the cells will experience an increase in GIN, as evidenced by a higher incidence of micronuclei formation and a perturbation to mitotic cell counts.

Materials and methods

A549 cells were treated with drugs reversine [1μM] (SAC inhibitor), VO-OHpic [50μM] (PTEN inhibitor), blebbistatin [20μM] (Myosin II ATPase inhibitor), and DMSO [50μM] as a vehicle treatment in four separate wells, followed by a 24-hour treatment and no washout. In a separate six-well plate, the cells were treated with Palbociclib [10μM], a known CDK4/6 inhibitor used for breast cancer treatments, and DMSO [10μM], then left for a 48-hour treatment. Meanwhile, the B-16 cells were only treated with 1μM reversine, along

with 1µM DMSO in a separate well, following the same protocols used on the A549 cells, treating them for 24 hours. After the treatments, both cell lines in the three six-well plates were fixed, permeabilized, and then stained with 750µL DAPI and 400µL Phalloidin to label the nuclei and the actin filaments of the cells, respectively. Finally, they were imaged into 9 frames of two fields of view (DAPI and Deep Red channels) per well/drug, followed by a qualitative and quantitative analysis using the software Fiji ImageJ.

Results, discussion, and conclusions

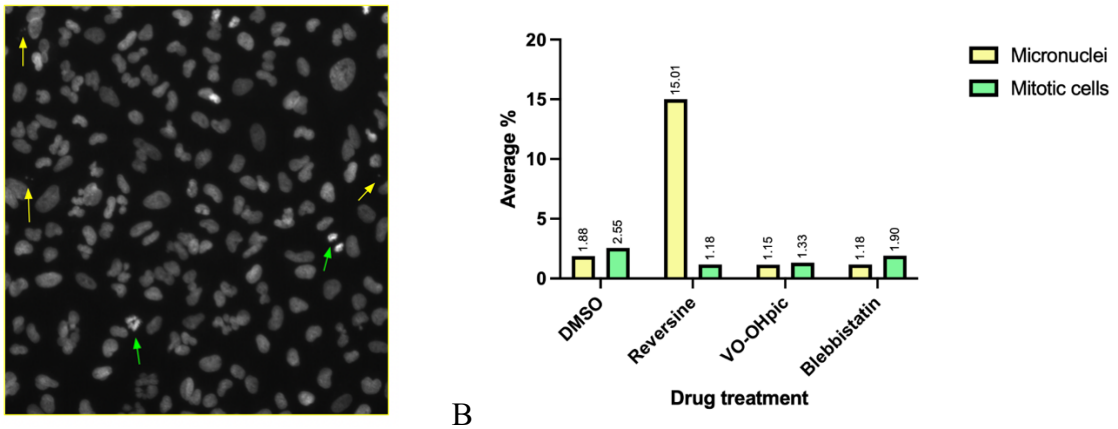

Figure 1. A549 cells after drug treatments (20x) (zoomed) on DAPI channel. Yellow arrows point at micronuclei, green arrows point at mitotic cells. (A) DMSO control. (B) Average percentage of micronuclei and mitotic cells in A549 cells post-drug treatments.

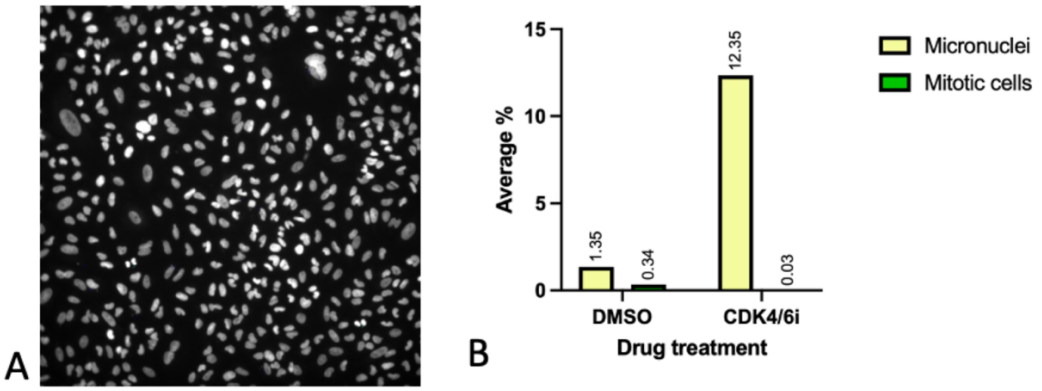

Figure 2. A549 cells after CDK4/6i treatment (20x) (zoomed) on DAPI channel. (A) DMSO control. (B) Average percentage of micronuclei and mitotic cells in A549 cells post-CDK4/6i treatment.

Results, discussion & conclusions

Perturbing tumor suppressors using reversine, CDK4/6i, VO-OHpic, and blebbistatin on A549 and B-16 cells demonstrates that disrupting tumor regulatory pathways can lead to GIN, as evidenced by altered micronuclei formation and mitosis. Reversine, a known inhibitor of the mitotic spindle assembly checkpoint (SAC), greatly increased micronuclei while suppressing mitosis. These findings align with existing literature, which indicates that inhibitors of the SAC can cause delays in mitosis and increase GIN [3]. By selectively inducing chromosome missegregation, these results highlight the importance of the MPS1/SAC in maintaining chromosomal stability.

CDK4/6 inhibitors are used to treat metastatic breast cancer, by targeting CDKs 4 and 6, which are crucial for cell cycle progression from G1 to S phase [7]. By inhibiting CDK4/6, cell proliferation was effectively halted, as indicated by the absence of successful mitotic cells, suggesting the possible decline of tumor progression in A549 cells. Nonetheless, the presence of micronuclei indicates the persistence of GIN without ongoing cell division. Thus, while CDK4/6 inhibitors can slow down tumor growth in A549 cells by preventing cell division, they may not completely prevent genetic abnormalities that could drive cancer progression.

VO-OHpic and Blebbistatin did not significantly differ from the control in terms both micronuclei formation and mitosis. The percentage of micronuclei and micronuclei were both slightly lower compared to the DMSO control, suggesting that inhibiting PTEN and Myosin IIa with these treatments may not directly induce GIN but could influence in other ways.

Inhibiting tumor suppressor pathways and cell cycle regulators leads to GIN, highlighting their role in maintaining chromosomal stability. Future directions for this study include incorporating other approaches to induce GIN, such as constricting cells via squashing, altering the stiffness of the microenvironment with methylcellulose, and using CRISPR/Cas9 gene editing technology to knockout genes involved in tumor progression/suppression, to further investigate their specific roles.

References

[1] Hanahan, Douglas; Weinberg, Robert A. Hallmarks of Cancer: The Next Generation. Cell 144. p. 646-674. (2011). DOI 10.1016/j.cell.2011.02.013

[2] Schramek, Daniel, et al. Direct in Vivo RNAi Screen Unveils Myosin IIa as a Tumor Suppressor of Squamous Cell Carcinomas. SCIENCE vol 343, p. 309-312. (2014).

[3] Santaguida S, Amon A. Short- and long-term effects of chromosome mis-segregation and aneuploidy. Nat Rev Mol Cell Biol. 2015 Aug;16(8):473-85. doi: 10.1038/nrm4025. Erratum in: Nat Rev Mol Cell Biol. 2015 Sep;16(9):576. PMID: 26204159.

[4] Cohen-Sharir, Y., McFarland, J.M., Abdusamad, M. et al. Aneuploidy renders cancer cells vulnerable to mitotic checkpoint inhibition. Nature 590, 486–491 (2021). https://doi.org/10.1038/s41586-020-03114-6

[5] Brito C, Sousa S. Non-Muscle Myosin 2A (NM2A): Structure, Regulation and Function. Cells. 2020; 9(7):1590. https://doi.org/10.3390/cells9071590

[6] Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, Yin Y. Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell. 2007 Jan 12;128(1):157-70. doi: 10.1016/j.cell.2006.11.042. PMID: 17218262.

[7] O'Leary, B., Finn, R. & Turner, N. Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol 13, 417–430 (2016). https://doi.org/10.1038/nrclinonc.2016.26