Inhibition of p38 kinase in cancer cells with Hybrid EMT induces Sensitivity to TGF-β Inhibitor And Disrupts Plasticity & Stemness

Kelsey Medina[1], Micaela Morgado[2], Petra Den Hollander[2], Sendurai A. Mani[2]

[1]The University of Texas Health Science Center at Houston McGovern Medical School

[2]The University of Texas MD Anderson Cancer Center Department of Translational Molecular Pathology

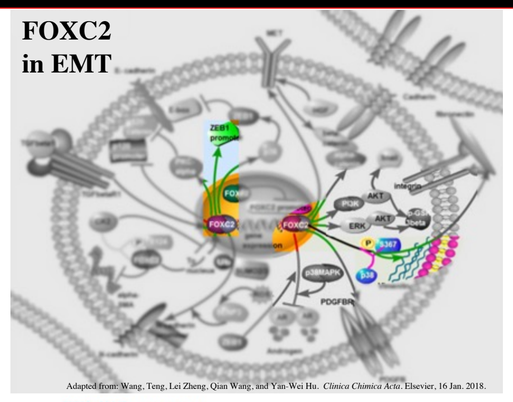
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

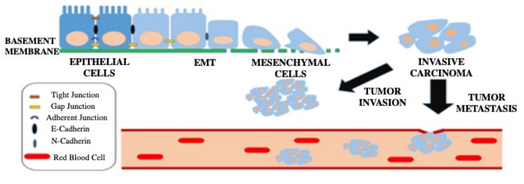
ABSTRACT

- Epithelial to Mesenchymal Transition (EMT) is an embryonic program which is critical for the development of embryo. Reactivation of EMT endows cancer cells with migratory and stem cell properties, increased metastatic capacity and resistance to chemotherapeutics[1].
- FOXC2, a fork head box containing transcription factor, plays a critical role in EMT through various pathways.
- Targeting FOXC2 signaling may render them sensitive to treatments, however since it is a transcription factor it is difficult to target.
- We previously discovered that p38 kinase phosphorylates FOXC2 at the serine 367 position. This phosphorylation leads to the stabilization of FOXC2 rendering it active and able to transform epithelial cells to gain mesenchymal-like and cancer stem cell properties and making them more metastatic and resistant[2].
- We focused on inhibiting p38 using the small molecule inhibitor SB203580 leading to FOXC2 protein degradation and inhibition of EMT and stem cell properties[3].
- D2A1 and 4T1 mouse syngeneic breast cancer cell lines are known to express epithelial and mesenchymal markers and display hybrid phenotype. Hybrid phenotype is associated with high levels of stem cell properties[4].
- Unpublished observations in our lab have shown that treatment of hybrid cancer cells with either TGF-β1 or TGF-β Inhibitor (SB 431532) did not seem to have an effect on inducing a mesenchymal or epithelial state, respectively.
- These cancer cells with hybrid phenotypes are harder to treat due to their plasticity, therefore it is important to try and bring them to a more mesenchymal or epithelial phenotype, so that they will be easier to target. We hypothesized that inhibiting p38 will push them to an epithelial state and make them sensitive to TGFβ1 or TGFβ Inhibitor.

INTRODUCTION

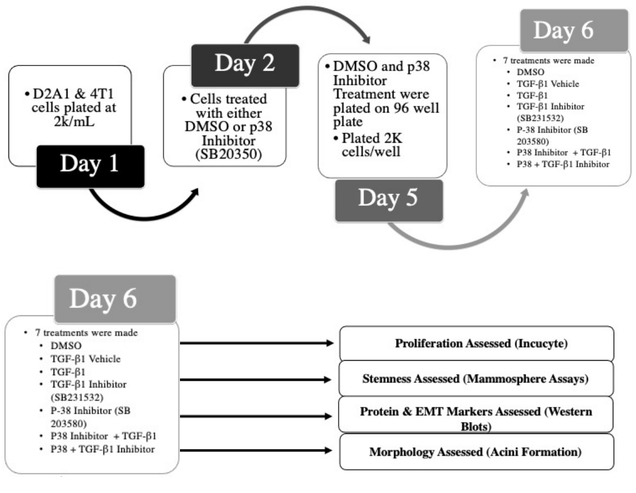


Adapted from: Wang, Teng, Lei Zheng, Qian Wang, and Yan-Wei Hu. *Clinica Chimica Acta*. Elsevier, 16 Jan. 2018.



Adapted from: Li, Mingzhe, Xin Bu, Bolei Cai, Ping Liang, Kai Li, Xuan Qu, and Liangliang Shen. *Oncology Reports*. Spandidos Publications, 01 Feb. 2019.

METHODS



RESULTS

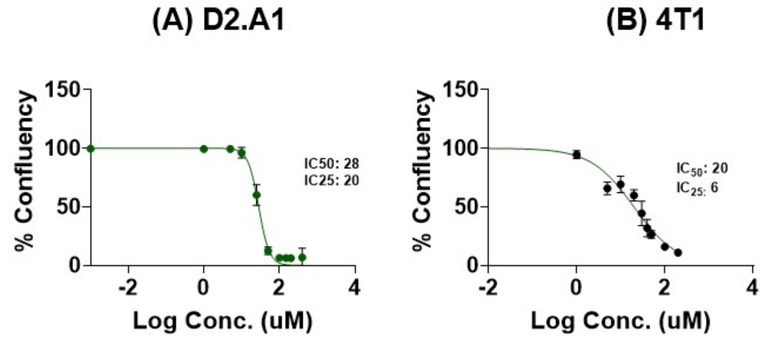


Figure 1: Dose-response curve of p38 in (A) D2. A1 and (B) 4T1 cells
Dose-response curve for the effects of p38 inhibitor SB203580 on both cell lines shown as a percent of confluency. The values are expressed as a mean of four determinations, with error bars representing 95% CI.

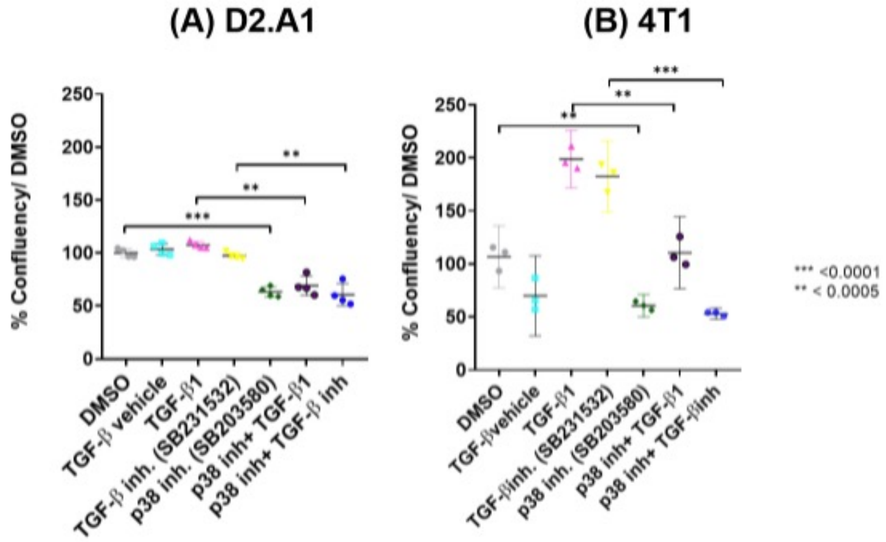


Figure 2: p38 drives cell growth inhibition in (A) D2. A1 and (B) 4T1 cells
Cell lines were pre-treated with the p38 inhibitor SB203580 (SB) using IC25 for 72 hrs., then plated in quadruplicates in 96-well plate, allowed to recover for 24 hrs. and treated with p38 alone or in combination as indicated in the figure. Single treatments, DMSO (.01%), TGF-β (5ng/mL), SB231532 (5 ng/mL) were included as well. Plate was put in incucyte and percent confluency was measured.

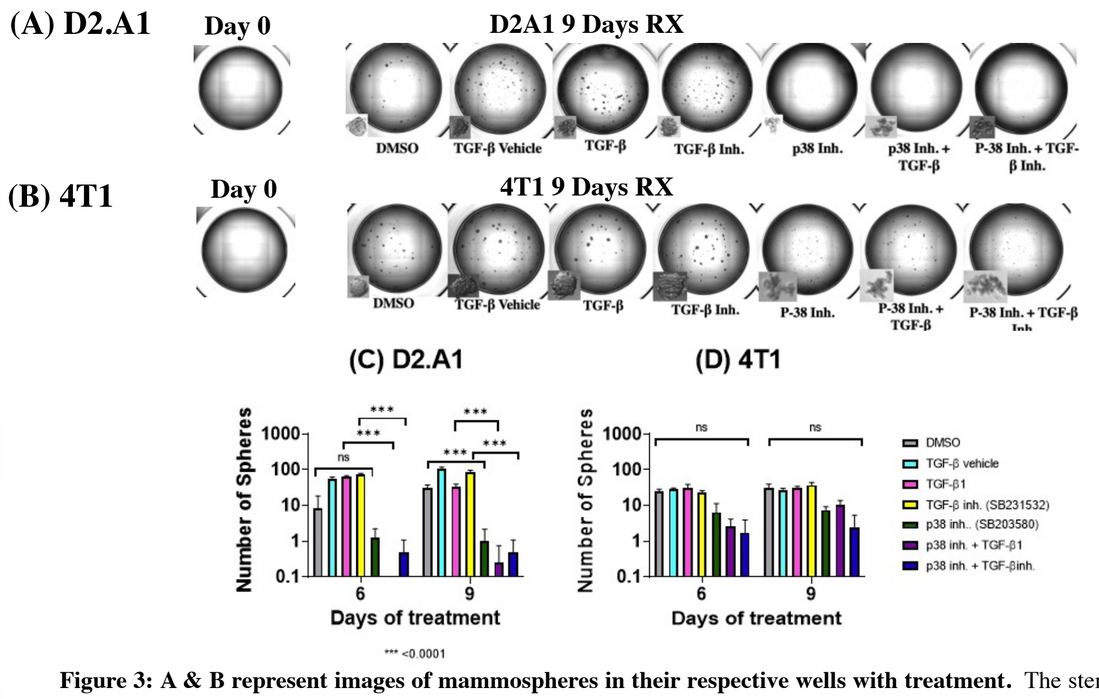


Figure 3: A & B represent images of mammospheres in their respective wells with treatment. The stemness of D2A1 & 4T1. Stemness was drastically affected with the addition of the p38 Inhibitor as seen in the images above. The Vehicle & TGF-β1 or TGF-β Inhibitor alone seemed to form large spheres, however once treated with p38 Inhibitor, there was a drastic decrease in sphere size and count.

P38 drives cell growth inhibition in (C) D2.A1 and (D) 4T1 cells
Cell lines were pre-treated with the p38 inhibitor SB203580 (SB) using IC25 for 72hrs., then plated in quadruplicates in 96-well plate using mammospheres media and low attachment plates, allowed to recover for 24hrs. And treated with p38 alone or in combination as indicated in figure. Single treatments, DMSO (.01%), TGF-β (5ng/mL), SB231532 (5 ng/mL) were included as well. Mammospheres were allowed to form for up to 9 days. Spheres were counted as depicted above.

CONCLUSION

- p38 Inhibitor reduces the formation of spheres due to the inhibition of stemness and plasticity. They are in a chaotic state when treated with p38 compared to sphere formation with the vehicles.
- p38 Inhibition alone and followed in combination with TGF-β1 or TGF-β Inhibitor shows a dramatic decrease in cell proliferation when compared to DMSO and TGF-β1 or TGF-β Inhibitor alone.
- Preliminary data depicts that there is an inhibition on invasiveness seen in D2A1 cell line. Protrusions are reduced after treatment of p38 Inh.
- P38 appears to be the major driver in regulating plasticity of metastatic cancer cells.
- This will prove to be vital in the clinical setting as we can use the cancer cell plasticity as a marker to manipulate them to our advantage and transition them into a more stable state rendering them sensitive to standard of care and inhibit metastasis.

FUTURE DIRECTIONS

- Expand to other hybrid breast cancer cell lines to verify the effect of p38 inhibition on plasticity.
- Check for epithelial & mesenchymal markers via western blots.
- Conduct immunofluorescence staining of epithelial & mesenchymal markers.
- Perform acini formation assay assessing the cell morphology after treatment.

ACKNOWLEDGEMENTS

This project was sponsored by the PCCSM program. Thank you for allowing me to take part in this amazing organization, and a big thank you to the Mani Lab Faculty & members for making it a memorable summer!

REFERENCES

1. Mani SA;Guo W;Liao MJ;Eaton EN;Ayyanan A;Zhou AY;Brooks M;Reinhard F;Zhang CC;Shipitsin M;Campbell LL;Polyak K;Brisken C;Yang J;Weinberg RA;. "The Epithelial-mesenchymal Transition Generates Cells with Properties of Stem Cells." *Cell*. U.S. National Library of Medicine, 16 May 2008.
2. Werden SJ;Sphyris N;Sarkar TR;Paranjape AN;LaBaff AM;Taube JH;Hollier BG;Ramirez-Peña EQ;Soundararajan R;den Hollander P;Powell E;Echeverria GV;Miura N;Chang JT;Piwnica-Worms H;Rosen JM;Mani SA; *Phosphorylation of serine 367 of FOXC2 by p38 regulates ZEB1 and breast cancer metastasis, without impacting primary tumor growth*)
3. Martínez-Limón, Adrián, Manel Joaquin, María Caballero, Francesc Posas, and Eulàlia De Nadal. "The P38 Pathway: From Biology to Cancer Therapy." *International Journal of Molecular Sciences*. MDPI, 11 Mar. 2020.
4. Jolly, Mohit Kumar, Sendurai A. Mani, and Herbert Levine. "Hybrid Epithelial/mesenchymal Phenotype(s): The 'fittest' for Metastasis?" *Biochimica Et Biophysica Acta (BBA) - Reviews on Cancer*. Elsevier, 08 July 2018.