Mimicking Vimentin Phosphorylation Results in Multinucleation and Loss of Stemness in Aggressive Breast Cancer Cells

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Background

Epithelial-mesenchymal transition (EMT) is a critical step in cancer metastasis. During EMT, epithelial cancer cells lose their cell polarity and adhesion to acquire migratory and invasive properties. EMT generates cancer stem-like cells (CSCs) that are involved in chemoresistance and cancer recurrence. Vimentin is a mesenchymal marker, which is upregulated during EMT, that functionally increases motility and migratory properties. Vimentin's regulation is tightly controlled through phosphorylation by multiple different kinases. The small molecule compound, FiVe1 increases the phosphorylation of vimentin most strikingly at serine-56, a site important for cell division. When the vimentin phosphorylation process is dysregulated by FiVe1, cells that have undergone EMT become multinucleated resulting in a loss of stemness and decreased metastasis *in vivo*.

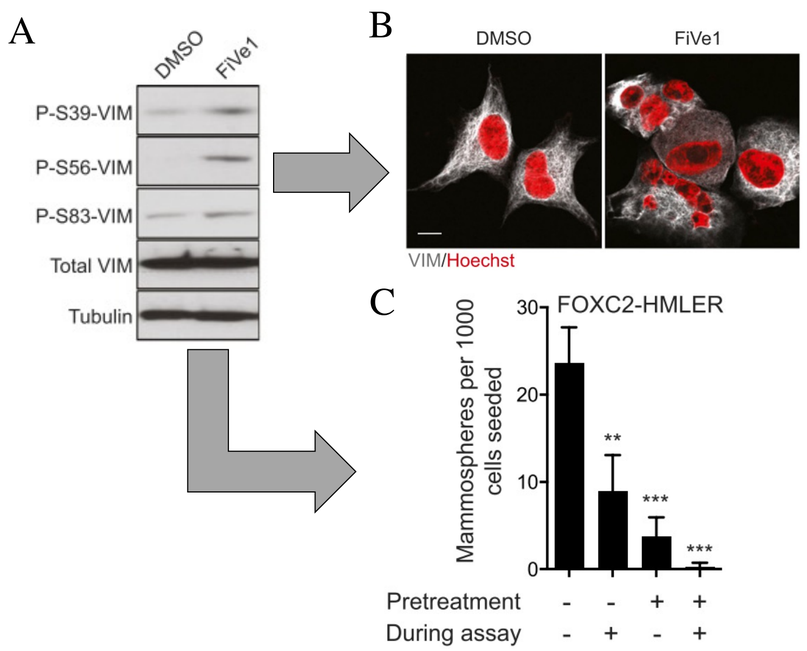

Figure 1. FiVe1 induces increased phosphorylation leading to multinucleation and stemness inhibition A) Western blot analysis of different vimentin phosphosites. B) Multinucleation images following FiVe1 treatment. C) Quantified mammosphere formation following FiVe1. From Bollong et al. PNAS 2017.

Hypothesis

Mimicking vimentin phosphorylation with a glutamic substitution at serine-56 will lead to increased multinucleation and decreased mammosphere formation.

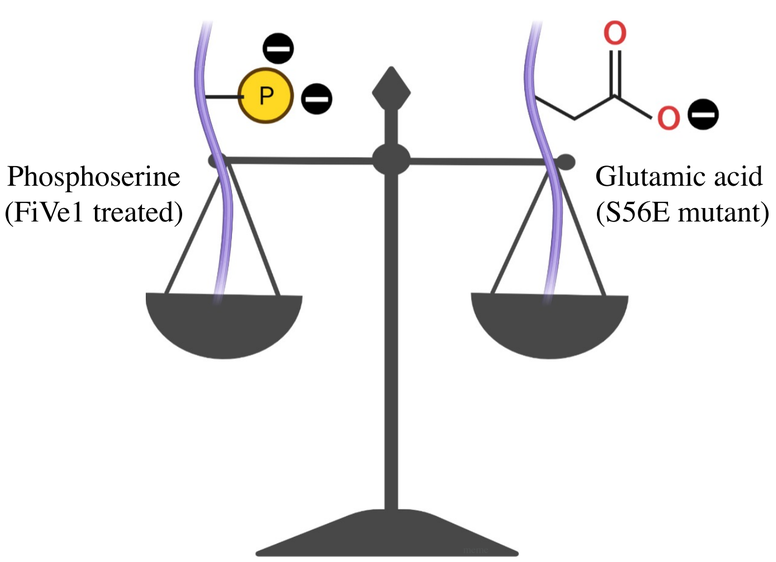

Figure 2. Depiction of vimentin phosphomimic mutation.

Methods

- 4T1 transduction with vimentin wild type and phospho-mimetic (VIM-S56E) doxycycline inducible vectors.
- Western blot verification of doxycycline induced expression of HA-vimentin.
- Cells were imaged and counted for multinucleation by immunofluorescence with anti-HA primary antibody with goat anti-rabbit-488 secondary and Hoechst dye.
- CSCs were quantified through mammosphere suspension assay under serum free conditions with and without doxycycline

Results

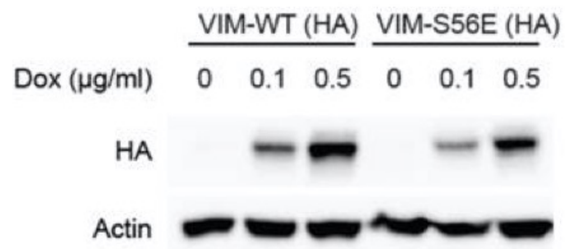

Figure 3. HA-VIM expression increased in a doxycycline dose dependent manner. Transduced 4T1 cells were treated with varying concentrations of doxycycline as indicated. HA and actin was detected by western blot.

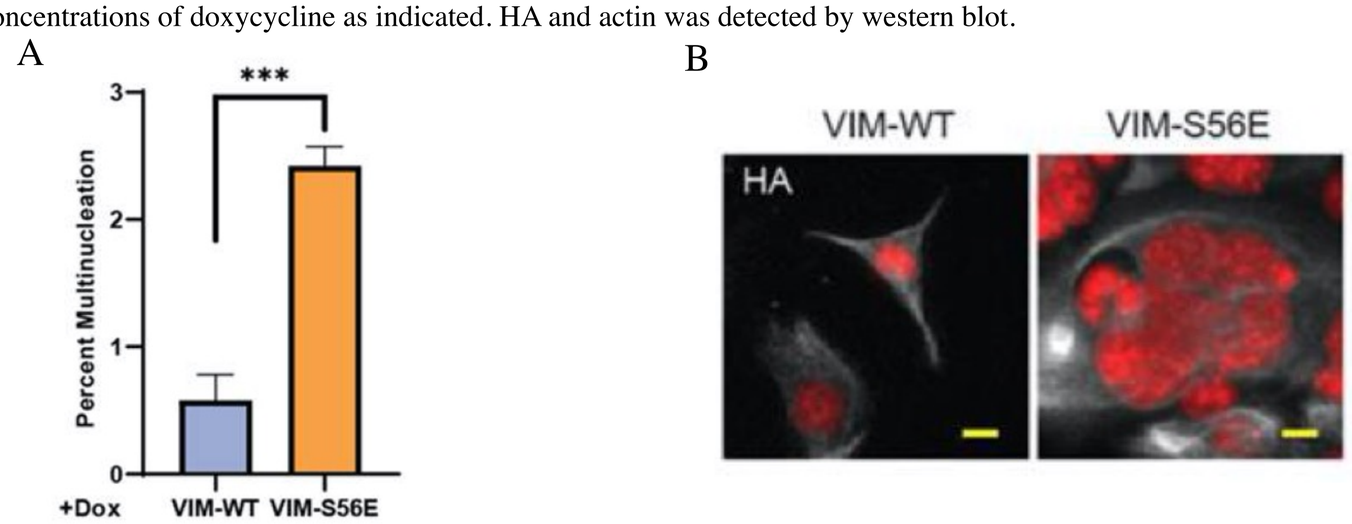

Figure 4. 4T1 murine breast cancer cells mimicking vimentin serine-56 phosphorylation resulted in an increase of multinucleation relative to cells expressing WT vimentin. A) Quantification of multinucleation (n > 3 nuclei per cell) and B) florescent images of multinucleation for VIM-S56E and VIM-WT. Anti-HA was used to stain HA-VIM and Hoechst was used to stain nuclei.

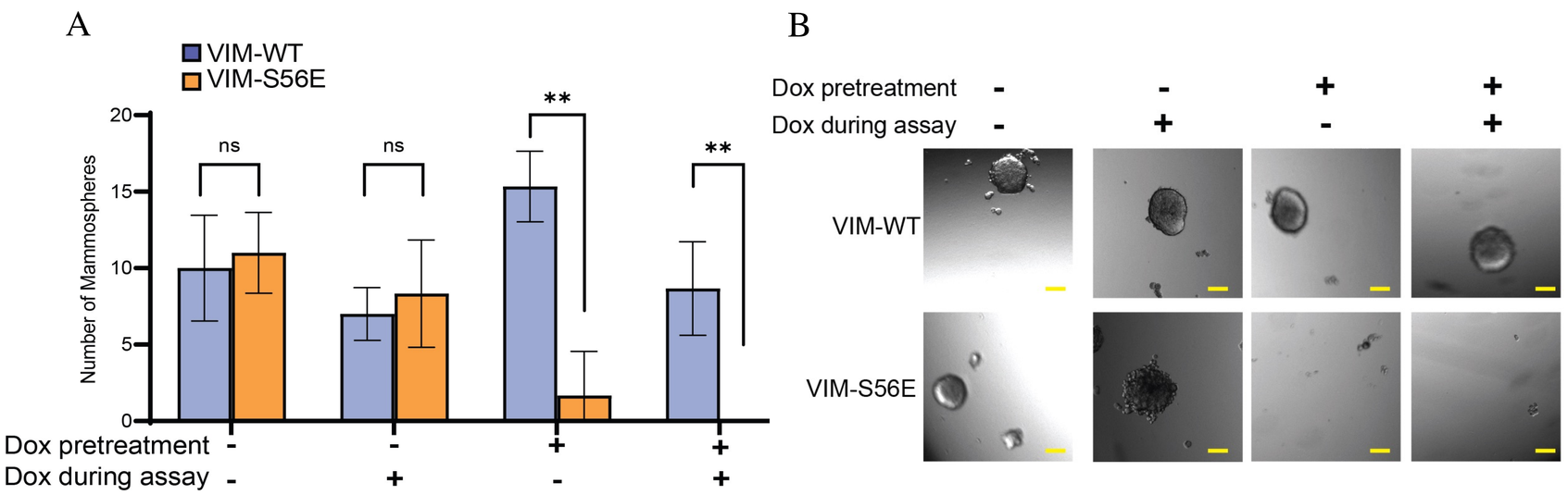

Figure 5. 4T1 murine breast cancer cells mimicking phosphorylation of vimentin at serine-56 (VIM-S56E) resulted in a decrease of multinucleation relative to cells expressing WT vimentin (VIM-WT). A) Quantification of mammosphere formation (greater than 80 μm in diameter) and B) images of mammosphere formation for VIM-S56E and VIM-WT under indicated conditions. Scale is 100 μm.

Conclusion

- Vimentin phospho-mimetic mutation for serine-56 results in multinucleation and inhibition of stemness in 4T1 cells.
- Serine-56 hyperphosphorylation in vimentin is the mechanism behind the ability of FiVe1 to disrupt EMT-enriched carcinoma cells.
- The increased phosphorylation of vimentin by FiVe1 at serine-56 leads to a loss of stemness properties.
- In future works, we plan to conduct mouse studies for gauging the impact of this mutation on metastasis.

References

1) Mani, SA et al. Cell 133;2008;704–715
2) Bollong, M et al. PNAS 114;2017;9903-9912

Acknowledgement

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