

Assaying the Effect of Knock-down of Ubiquitin-Specific Proteases in Malignant Pleural Mesothelioma

Margarito Hernandez Fuentes, Biomedical Engineering

Mentor: Dr. Christopher Plaisier, Assistant Professor

School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ

Introduction

Malignant Pleural Mesothelioma (MPM) is a rare but aggressive cancer residing in the pleural space of the lungs and is typically caused by exposure to asbestos fibers. Treatment options for MPM consists of surgical resection, radiotherapy and chemotherapy. Even with treatment, MPM is commonly diagnosed at a late stage with a median survival rate of less than one year after diagnosis.

Ubiquitination is a post-translational modification where ubiquitin become attached to lysine residues. Ubiquitin can be detached by a large family of deubiquitinating enzymes (DUBs). DUBs provides a mean of modulating regulatory proteins that support tumor progression.

Objective

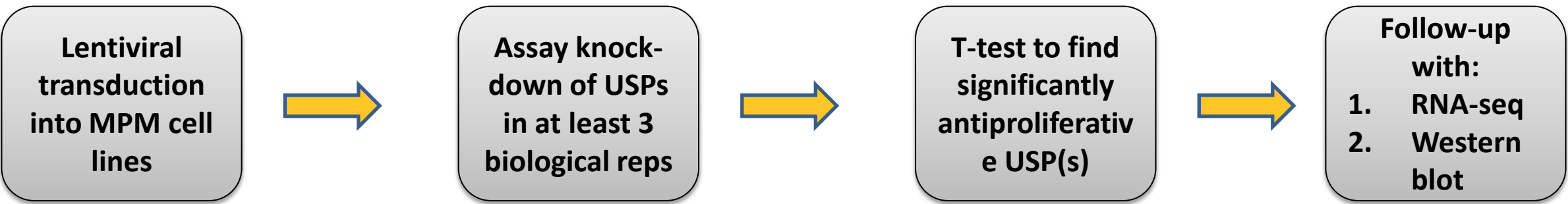
Through a CRSPR/Cas9 knock-out growth screen, the Plaisier Lab determined that USPs, a class of DUBs, were enriched in the significantly antiproliferative gene knockouts. Furthermore, PR-619 was found to be significantly antiproliferative across multiple primary cell lines of pleural mesothelioma.

We chose to identify the specific USP driving this antiproliferative effect using knock-downs of each USP independently. We used lentiviral transduction, which has a higher efficiency, to reveal the USP(s) that would be the most beneficial antiproliferative drug target in pleural mesothelioma.

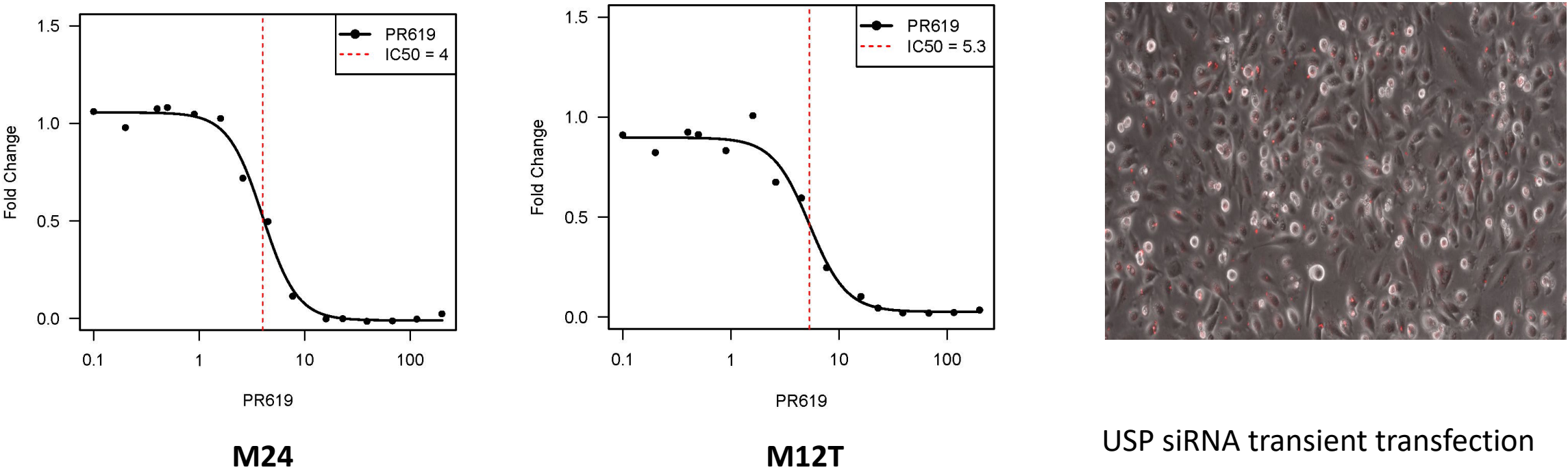
PR-619

Targeting DUBs in cancer reveal an important role in tumorigenesis, making them an attractive therapeutic agent. PR-619 is a DUB inhibitor that has shown to inhibit cell adhesions and proliferation by inducing G2/M cell cycle arrest. Several studies have reported PR-619 as an effective anti-proliferative agent in cancers like urothelial carcinoma and oesophageal squamous cell carcinoma; however, little research has been done on PR-619 and its effect on mesothelioma cell lines. The Plaisier lab was able to determine PR-619 as an effective tumor inhibition tool on malignant pleural mesothelioma. Using a knock-down approach, we can identify which USP is most responsible for this anti-proliferative effect on MPM.

shRNA knockdown of USPs:

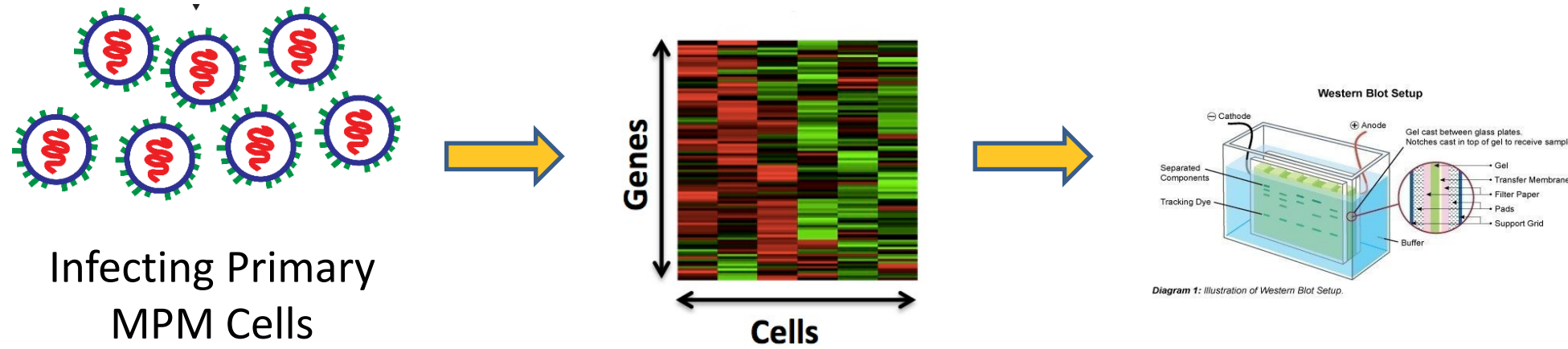


Experimental Analysis



Experimental Design

The Plaisier lab selected a list of USPs that have shown an average JACKS essentiality score of less than -1.0. The shRNA oligos will be ligated into a pLKO.1. The resulting plasmid will then be transfected to produce knock-down lentiviruses that will be transduced into the MPM cell lines. Cell proliferation will be quantified using fluorometric CyQuant direct cell proliferation assay.



Future Directions

Come up with concrete experimental plan for the list of ubiquitin-Specific proteases so representative sequence can target all family members into pLKO.1. vector.

References

1. Mott, Frank E. "Mesothelioma: a review." *The Ochsner journal* vol. 12,1 (2012): 70-9. [Accessed 13 March 2021].
2. Robinson B. M. (2012). Malignant pleural mesothelioma: an epidemiological perspective. *Annals of cardiothoracic surgery*, 1(4), 491–496. <https://doi.org/10.3978/j.issn.2225-319X.2012.11.04>
3. Zhou, Yudong et al. "Ubiquitin-specific protease 4 promotes glioblastoma multiforme via activating ERK pathway." *OncoTargets and therapy* vol. 12 1825-1839. cc
4. Mesothelioma Hope Team, & by: (2021, October 5). Pleural mesothelioma - symptoms, causes, & treatment. MesotheliomaHope.com. Retrieved November 9, 2021, from <https://www.mesotheliomahope.com/mesothelioma/pleura/>
5. Addgene.org. 2021. *Addgene: Protocol - pLKO.1 - TRC Cloning Vector*. [online] Available at: <<http://www.addgene.org/protocols/plko/#E>> [Accessed 10 March 2021].
6. Xu, H., Liu, YL., Yang, Ym. *et al.* Knock-down of ubiquitin-specific protease 22 by micro-RNA interference inhibits colorectal cancer growth. *Int J Colorectal Dis* **27**, 21–30 (2012). <https://doi.org/10.1007/s00384-011-1275-8>
7. Milone, M.C., O'Doherty, U. Clinical use of lentiviral vectors. *Leukemia* **32**, 1529–1541 (2018). <https://doi.org/10.1038/s41375-018-0106-0>

Acknowledgments

I would like to thank members of the Plaisier lab for their support and guidance, specifically my graduate mentor Sierra Wilferd and my PI Dr. Christopher Plaisier. I would also like to thank the FURI program for their generous support.