Lysosomal transmembrane protein TMEM106B alters TFEB signaling and the tumor immune microenvironment

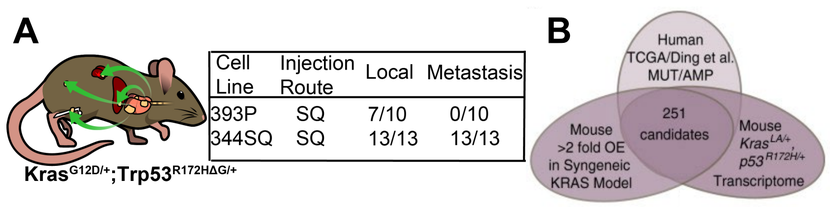
Amanda Warner, Samrat T. Kundu, Rakhee Bajaj, Bertha L. Rodriguez, Don L. Gibbons

University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences
Department of Thoracic Head & Neck Medical Oncology; Contact info: **awarner2@mdanderson.org**

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center | UTHealth Houston
Graduate School of Biomedical Sciences

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer related deaths particularly due to its high propensity to metastasize[1]. Our lab performed an *in vivo* screen[2] and identified transmembrane lysosomal protein 106B, TMEM106B, as a novel driver of NSCLC metastasis. TMEM106B activates transcription factor EB (TFEB) and lysosomal exocytosis which releases cathepsins into the tumor microenvironment (TME) that promote metastasis[3]. Beyond cathepsin release, the mechanism of how TMEM106B alters cell biology and the TME to promote metastasis remains unclear. TFEB is necessary for lysosomal exocytosis[4], autophagy[4], invasion in prostate cancer[5], and activates immune gene transcription[6,7]. *We hypothesize that (1) TMEM106B mediated TFEB activation upregulates autophagy to promote metastasis and (2) TMEM106B is dependent on TFEB to regulate lysosomal exocytosis and secretion in NSCLC which alter the tumor immune microenvironment (TIME) to become pro-metastatic*. It will be crucial to know how TMEM106B mechanistically alters cellular biology and the TIME as this will provide new therapeutic targets and strategies to limit NSCLC metastasis.



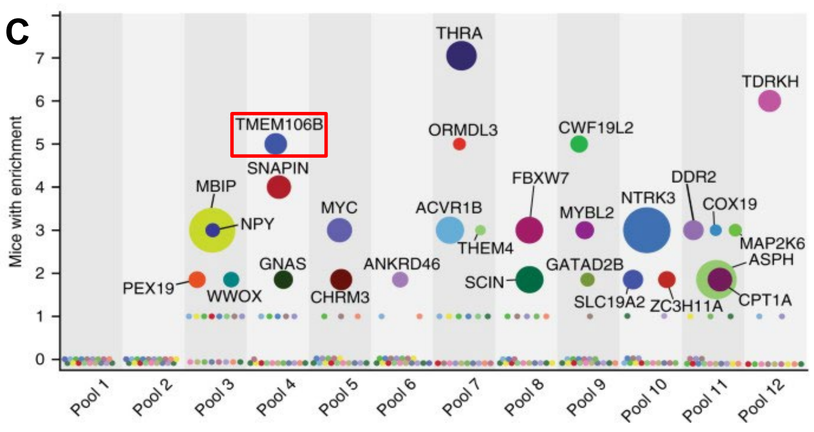


Figure 1: TMEM106B identified as a metastasis driver in NSCLC from *in vivo* murine screen. (A) Cell lines generated from KP murine GEM tumors. **(B)** Gene list for *in vivo* screen[2]. **(C)** TMEM106B was present in >2 lung metastasis lesions per five individual mice[2].

Results

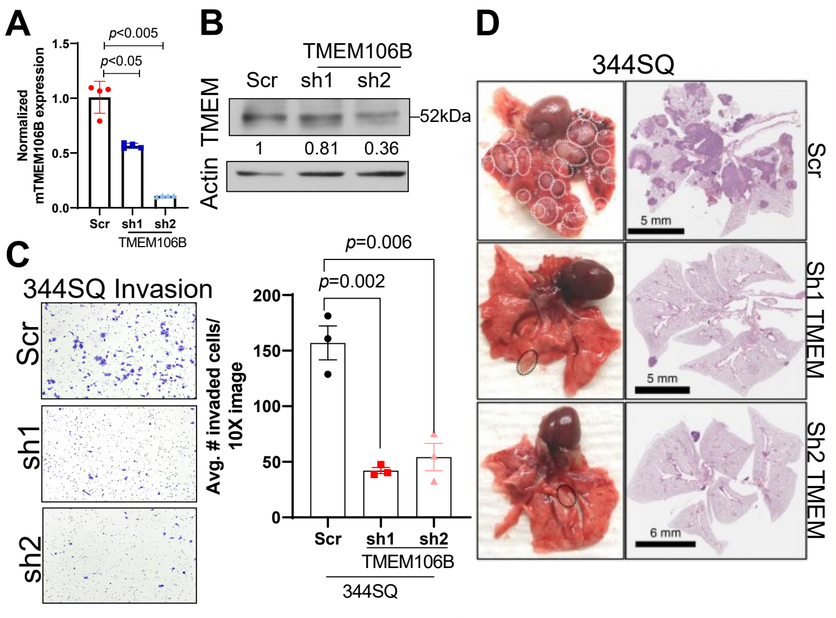


Figure 2: TMEM106B is necessary for invasion and metastasis. (A, B) Knock-down of TMEM106B by RNA and protein. **(C)** TMEM106B is necessary for invasion *in vitro*. **(D)** TMEM106B is necessary for murine NSCLC metastasis to lungs[3].

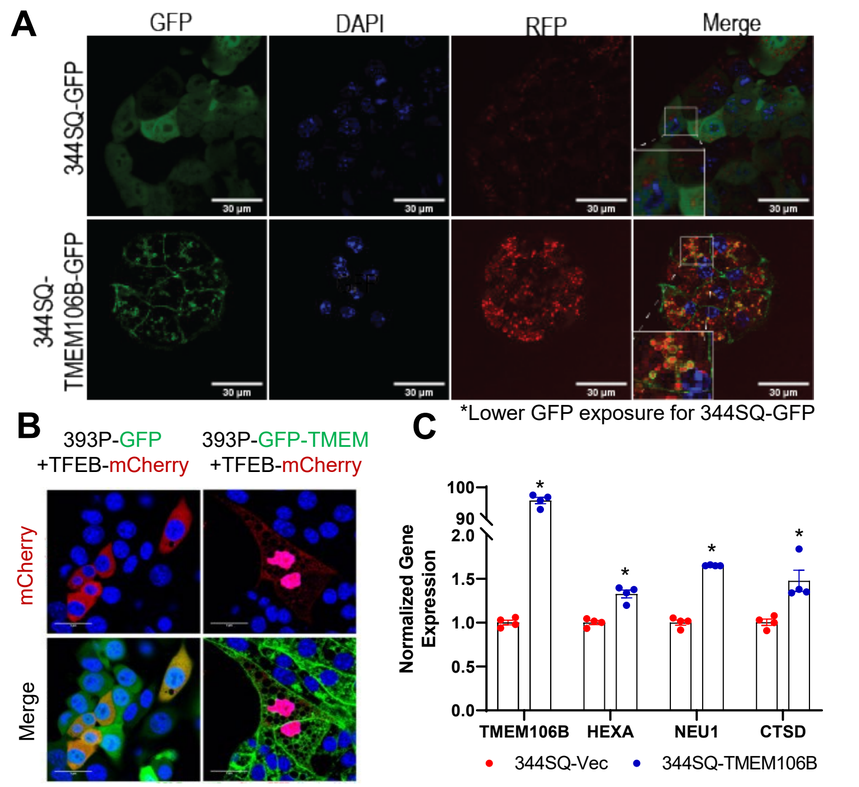


Figure 3: TMEM106B induces lysosomal biogenesis and TFEB activation. (A) Lysotracker dye (RFP) identifying lysosomes. **(B)** TMEM106B enhances TFEB nuclear translocation[3] and **(C)** elevates TFEB target gene expression.

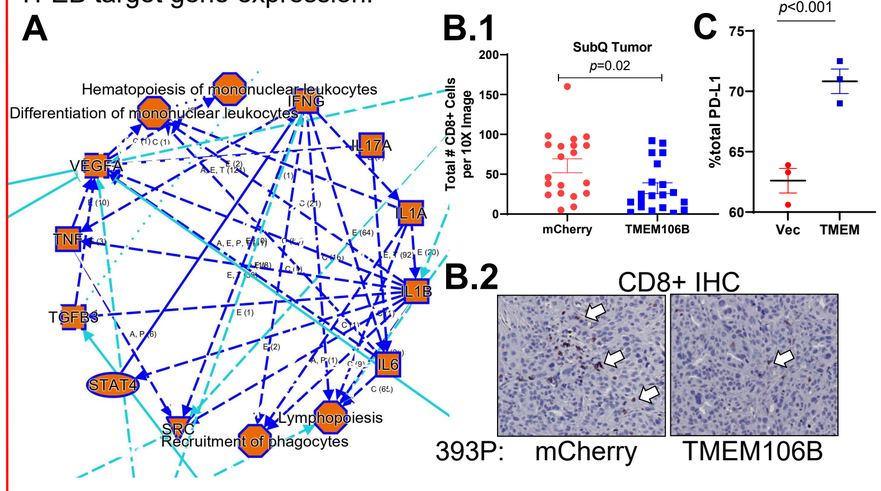


Figure 4: TMEM106B predicted to affect transcriptional regulation of immune signaling genes. (A) Ingenuity Pathway Analysis (IPA) on genes (FC>1, p<0.05) from microarray on TMEM106B subQ tumors which **(B)** have less CD8+ T-cells by IHC. **(C)** TMEM106B enhances surface PD-L1 upon co-culture with activated naïve immune cells.

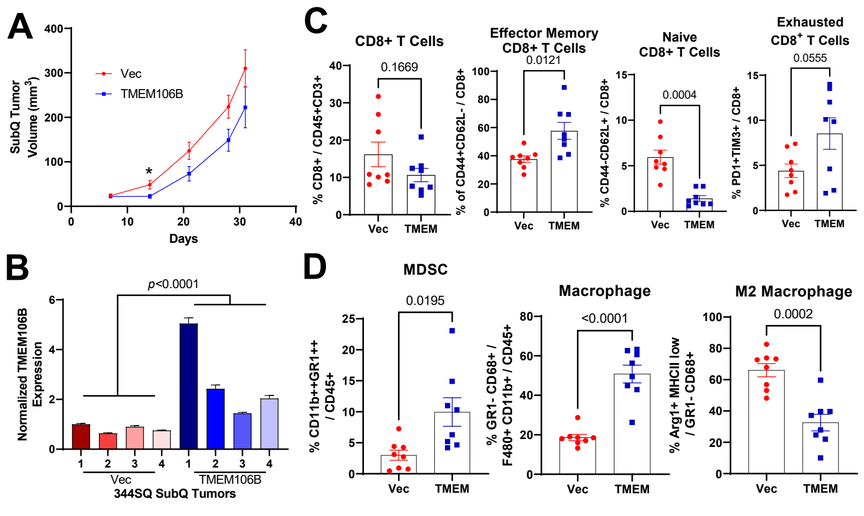


Figure 5: TMEM106B overexpression alters the TIME to be immunosuppressive. (A) subQ tumor volume unchanged. **(B)** *Tmem106B* tumor RNA expression. **(C)** T- lymphocyte and **(D)** myeloid population percentage changes within 344SQ tumors.

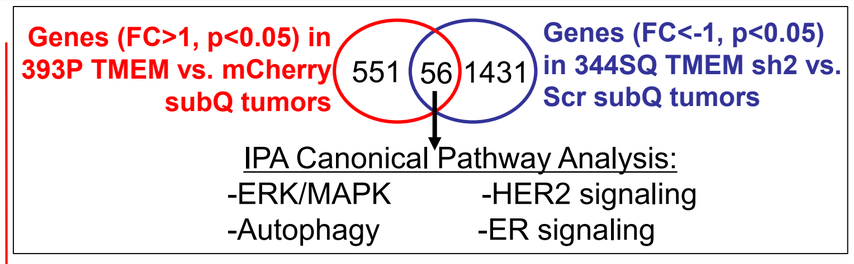


Figure 6: Autophagy predicted to be dependent on TMEM106B in murine tumors. Canonical Pathways by Ingenuity Pathway Analysis (IPA) on microarray. Venn Diagram by Venny2.1, FC=Fold Change.

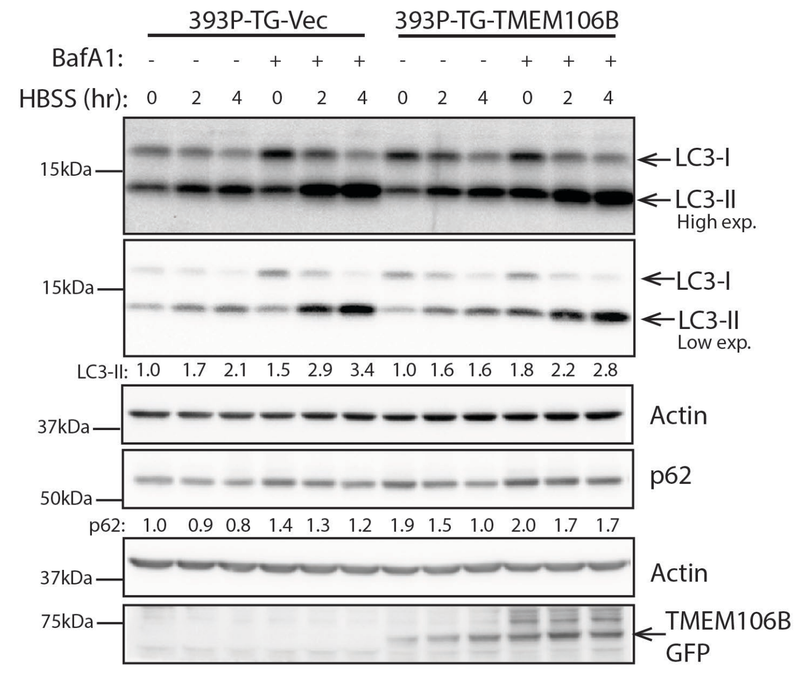


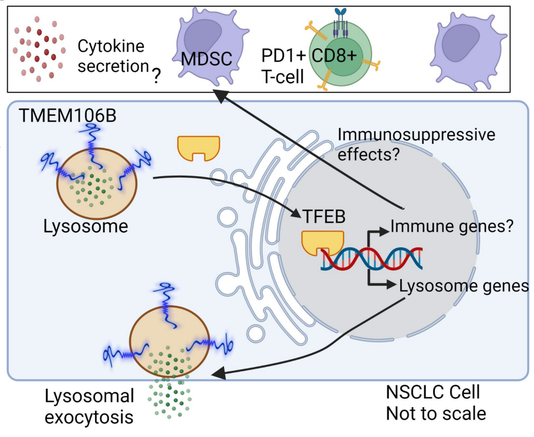
Figure 7: TMEM106B is not sufficient to alter autophagy flux. Autophagy flux measured by starving cells in HBSS +/- BafilomycinA1, which inhibits lysosome acidification and autophagosome degradation, and measuring LC3-II and p62, each normalized to actin.

Conclusions

- TMEM106B is necessary for NSCLC invasion and metastasis.
- TMEM106B induces TFEB activation which enhances lysosomal biogenesis but not autophagosome number or flux *in vitro*.
- TMEM106B tumors have less total CD8+ T-cells, more exhausted T-cells, and altered myeloid cell populations suggesting TMEM106B could contribute to immunosuppression.

Future Directions

- Perform qPCR immune gene screen and cytokine array to validate immune genes/signaling pathways regulated by TMEM106B as identified by microarray.
- Determine contribution of TFEB as a downstream target of TMEM106B mediated alterations on TIME to affect metastasis (Image by BioRender).



References and Funding

1) Siegel, R. L., et al., *CA Cancer J Clin.*, 2022
2) Grzeskowiak, C. L., et al., *Nat. Commun.*, 2018
3) Kundu, S. T., et al., *Nat. Commun.*, 2018
4) Medina, D. L., et al., *Dev Cell.*, 2011
5) Zhu, X., et al., *Front. Oncol.*, 2021
6) El-Houjeiri, L., et al., *Cell Rep.*, 2019
7) Palmieri, M., et al., *Hum Mol Genet.*, 2011

This work was funded by D.L.G. grants: NIH R37 (CA214609); CPRIT MIRA (RP160562-P3); SPORE (2P50CA070907-21A).