

Determining glioblastoma proteome changes in response to lateral ventricle neural stem cells

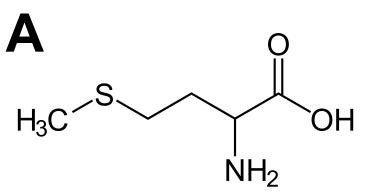
Background

Glioblastoma

- Glioblastoma (GBM) is the most common and malignant primary tumor in adults.
- GBM tumors located near the lateral ventricle display a more aggressive recurrence pattern, negatively impacting patient survival.
 - Suggests involvement of 0 subventricular zone neurogenic niche in GBM malignancy.

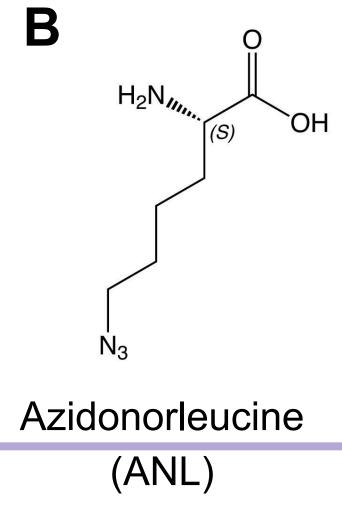
Methionyl-tRNA synthetase (MetRS)

• Mutant MetRS L274G (MetRS*) allows for incorporation of azide-tagged methionine analog azidonorleucine (ANL) into newly formed proteins.



Methionine

Figure 1. Methionine (A) can be substituted with azidonorleucine (B)² in cells expressing mutant MetRS for cell-specific nascent protein labeling.

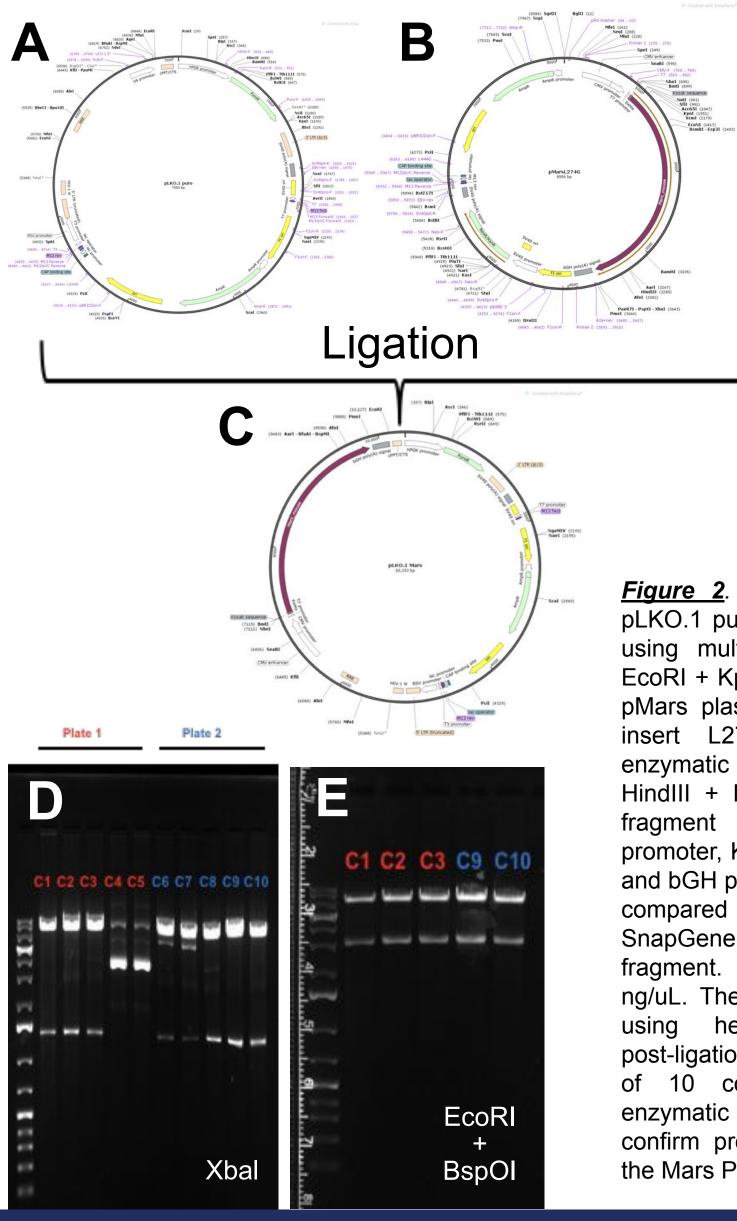


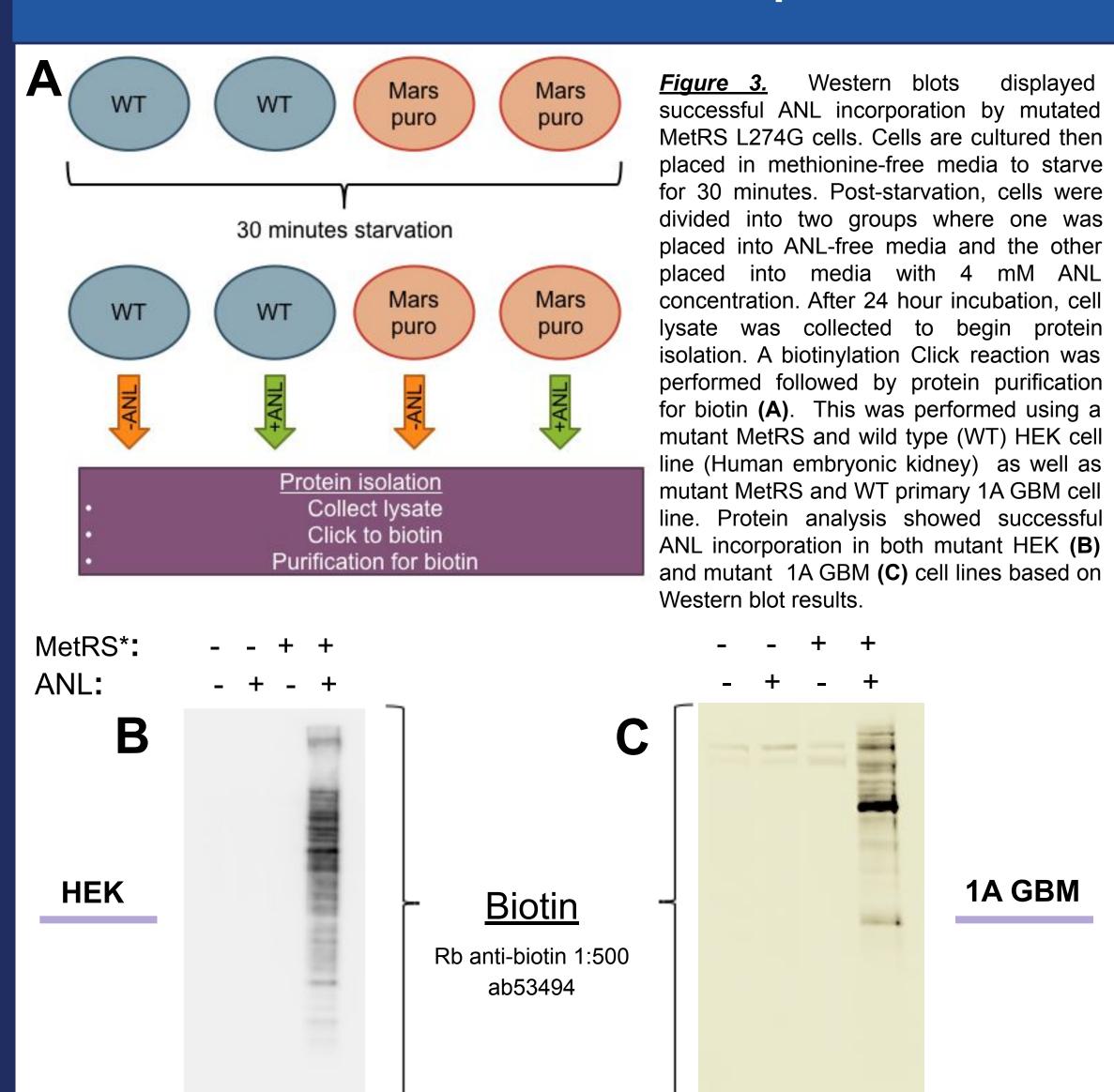
Objectives

- To optimize a tool to determine cell-specific proteomic changes of GBM cells in response to neural stem cell proximity.
- To define the intercellular communication between neural stem cells and GBM cells.

Methods

- Molecular cloning of MetRS* into lentiviral backbone with puromycin selection
- Confirmation of ANL incorporation into multiple MetRS mutant cell lines using western blot and silver stain
 - HEK
 - 1A
- GBM
- (patient-derived primary GBM line)
- Lentivirus production from cloned plasmid Quantification and concentration
 - Titration
- Lentivirus validation using puromycin selection
- Co-culture proteomic analysis





Lauren Whaley¹, Emily S Norton², Natanael Zarco², Hugo Guerrero-Cazares² ¹ University of North Florida, Jacksonville FL, 32224; ² Department of Neurosurgery, Mayo Clinic, Jacksonville FL, 32034, USA

Cloning MetRS* into lentiviral plasmid

Sample	Concentration (ng/uL)	
LKO.1 puro	6.0	
Mars L274G	10.3	
Isolated Mars fragment:		

• KTII FcoRI

Figure 2. The sequential organization of the pLKO.1 puro backbone plasmid (A) was verified using multiple enzymatic restriction reactions: EcoRI + KpnI, BshTI + NcoI, NdeI + BamHI. The pMars plasmid (B) carrying the mutant MetRS L274G was verified using multiple enzymatic restriction reactions: BgIII + BamHI, HindIII + Notl, XhoI + Sall. PCR of the Mars fragment consisted of CMV enhancer, CMV promoter, Kozak sequence, 6X His, Mars L274G, and bGH polyA signal. The 35x cycle product was compared to a simulated agarose gel created by SnapGene software to ensure 3676 bp length of fragment. Isolated PCR product yielded 37.8 ng/uL. The Mars puro plasmid (C) was formed heatshock ligation. Two separate post-ligation cultures were plated to select a total colonies. After MiniPrep, additional enzymatic restriction reactions were used to confirm proper sequencing and organization of the Mars Puro plasmid (D, E).

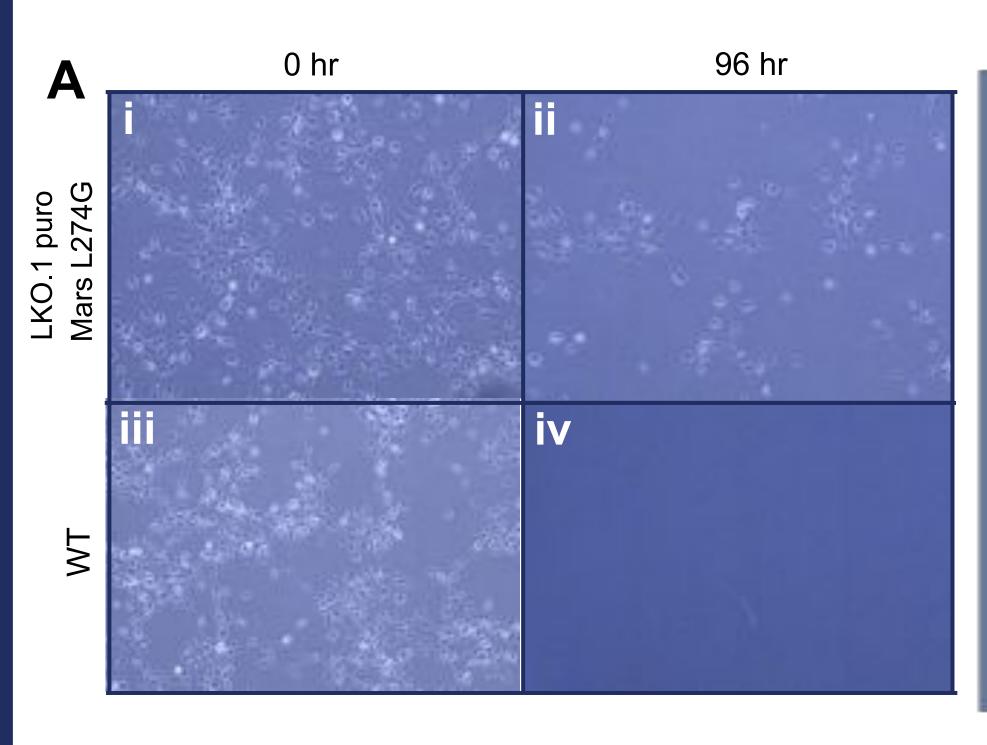
Confirmation of ANL incorporation

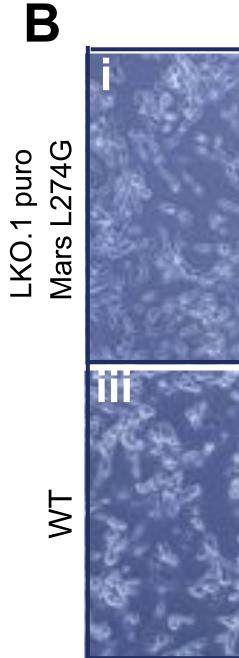
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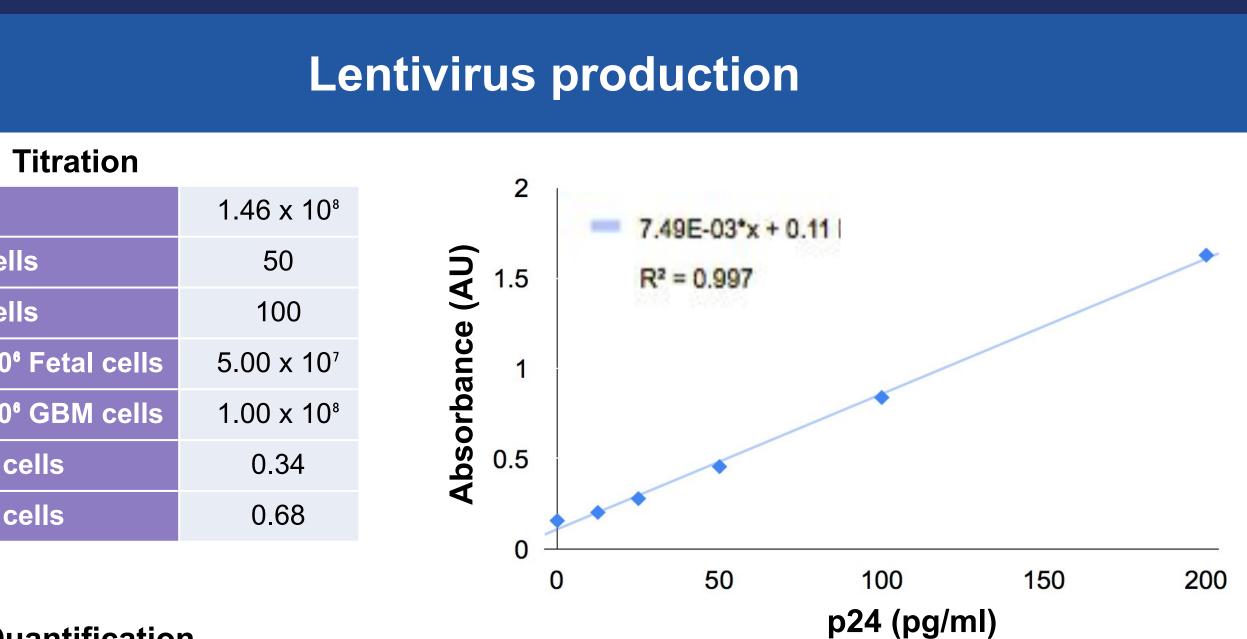
MOI Fetal cells
MOI GBM cells
LPS for 1x10 ⁶ Feta
LPS for 1x10 ⁶ GB
uL for Fetal cells
uL for GBM cells
Quanti
Average

LPS/uL

Average	0.9885	
pg/mL in well (1:1 ⁶ dilution)	117.17	
pg/mL virus	1.17 x 10 ⁸	
Adj. conc virus pg/mL	1.17 x 10 ⁷	
LPS/mL	1.46 x 10 ¹¹	
Approx. IFU/mL	1.46 x 10°	
Puromycin selection		







ification

Figure 4. The Mars puro plasmid was used to create a lentivirus (LV) for stable integration of puromycin resistance and MetRS* L274G insert into primary cell genome. The psPAX2 plasmid was used for LV packaging and pMD2.G was used for LV enveloping. After the second harvest of virus, the LV was concentrated then titrated to find optimized quantity needed to transduce 1A GBM cell line and hfNSC cell line (human fetal neural stem cell), F50.

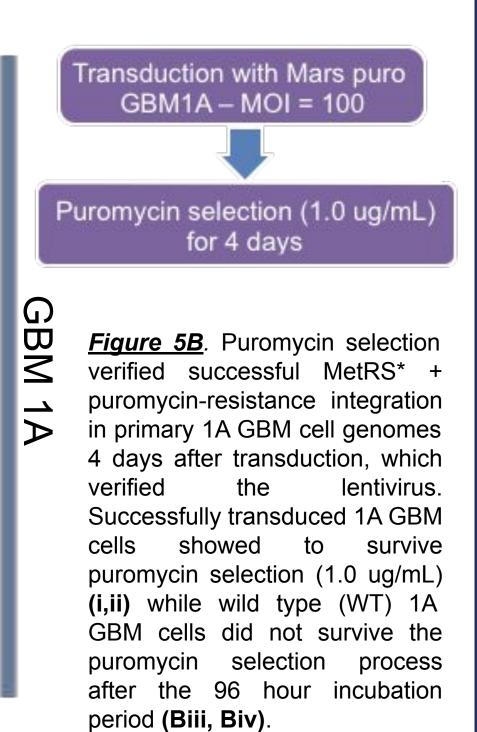
of transduced cells

Transduction with Mars puro F50 - MOI = 50Puromycin selection (1.0 ug/mL)

for 4 days

Figure 5A. Puromycin selection verified successful MetRS* + puromycin-resistance integration in hfNSC cell genomes 4 days C \mathbf{O} after transduction, which verified lentivirus. Successfully transduced F50 cells showed to survive puromycin selection (1.0 ug/mL) (Ai,Aii) while wild type (WT) F50 cells did not survive the puromycin selection process after the 96 hour incubation period (Aiii, Aiv).

0 hr 96 hr



Proteomic changes in GBM cells co-cultured with neural stem cells

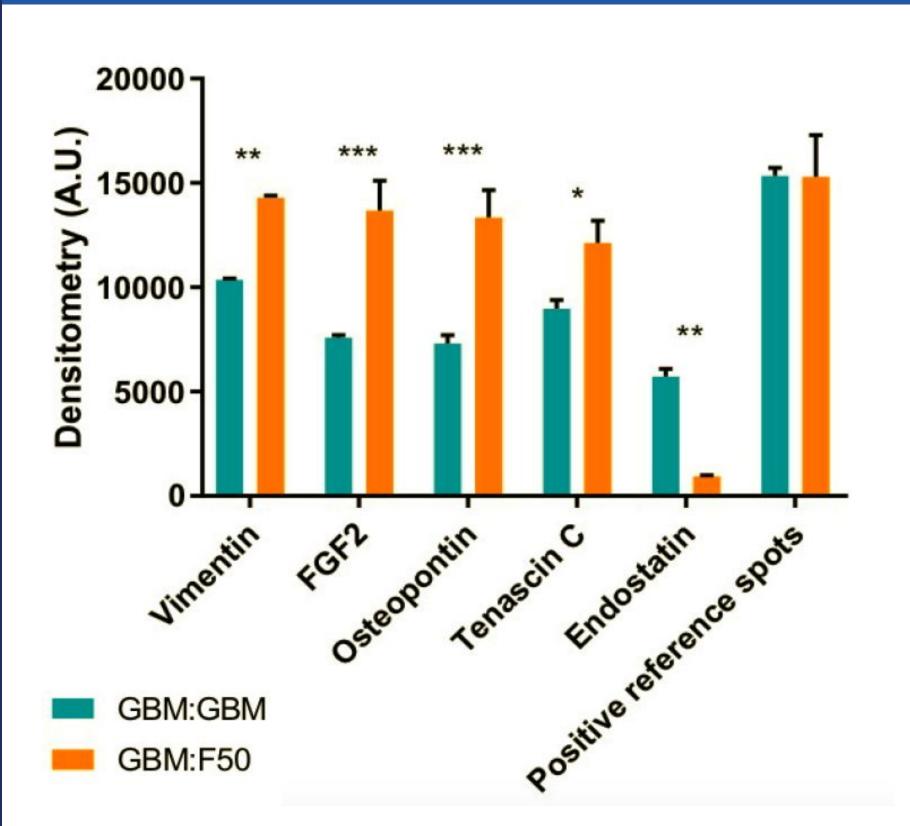


Figure 6. Co-Culture Oncology XL Proteome Profiler array results suggest an upregulation in malignancy promoting proteins. Co-cultures of F50 and 1A GBM were utilized to simulate the environment of glioblastoma neighboring neural stem cells.

Vimentin (upregulation): Component of intermediate filament cytoskeleton. Contributes towards processes including migration, metastasis, and cholesterol signaling. Usually overexpressed in cancers (cell invasion, metastatic tumor spread) and best known in cancers as a marker of cellular epithelial mesenchymal transition (EMT).

FGF2 (upregulation): Commonly expressed in malignant tumors. This protein can promote cell motility, proliferation, increase tumor angiogenesis and inhibit apoptosis.

Osteopontin (upregulation): Typically mediates normal physiological function including cell adhesion, migration, and tissue repair. Specifically in glioblastoma, this protein can act as a chemokine by recruiting macrophages to the GBM tumor site. Also mediates crosstalk between GBM cells.

Tenascin C (upregulation): Extracellular matrix glycoprotein. An upregulation of this protein has been seen as a biomarker for a high grade glioma, Diffuse intrinsic pontine glioma(DIPG).¹

Endostatin (downregulation): Specifically inhibits endothelial proliferation while significantly inhibits angiogenesis and tumor growth.

Conclusions

- MetRS* metabolic labeling can be successfully cloned into a lentivirus and utilized as a tool for cell-specific proteomics with the use of ANL.
- GBM cells within close proximity of neural stem cells show an increase of proteins representative of malignant cancer spread.

References

- Qi, J., Esfahani, D.R., Huang, T. *et al.* Tenascin-C expression contributes to pediatric brainstem glioma tumor phenotype and represents a novel biomarker of disease. acta neuropathol commun 7, 75 (2019). https://doi.org/10.1186/s40478-019-0727-1 Link, A. James; Vink, Mandy K. S.; Tirrell, David A. Journal of the American Chemical Society (2004), 126(34), 10598-10602.
- DOI:10.1021/ja047629c; Sinnaeve J, Mobley BC, Ihrie RA. Space invaders: brain tumor exploitation of the stem cell niche. Am J Pathol. 2018;188(1):29-38. doi: 10.1016/j.ajpath.2017.08.029.