The Role of UBQLN in Survival Under Nutrient Deprivation

Rachel L. Eckert[1], Alexis A. Vega[2], Parag P. Shah[1], and Levi J. Beverly[1]
[1]Brown Cancer Center, [2]Department of Biochemistry and Molecular Genetics
University of Louisville, Louisville, Ky

Tumorigenesis and Therapeutic Outcomes Laboratory (ToTOL)

We take a "ToTOL" approach to Cancer Research!

Introduction

According to the CDC, lung cancer is the third most common cancer in the US however, more people will die from lung cancer than any other type of cancer. Lung cancer also has the lowest five-year survival rate (18.6%) compared to other leading cancers, such as colorectal (64.5%), breast (89.6%), and prostate (98.2%). Lung cancer, much like many solid tumors, require large amounts of nutrients. Certain nutrients are considered essential, meaning cells are incapable of synthesizing *de novo.* Preliminary data from the lab has shown methionine (Met), an essential amino acid, is critical for tumor survival and proliferation. Interestingly, a CRISPR/Cas9 screen identified Ubiquilin 1(UBQLN1) as one of the genes to show increased lethality under Met deprivation. Ubiquilins are a family of proteins that include UBQLN1-4 and UBQLNL. They are associated with the ubiquitin-proteasome system in which UBQLN interacts with polyubiquitinated proteins to deliver them to the proteasome for degradation. Preliminary data from the Beverly lab also suggests that the knockdown of UBQLN1/2 increases tumor progression in lung cancer. Given methionine availability is crucial for tumor survival, we hypothesize that as UBQLN1/2 KO cells require more Met, deprivation would lead to an increase in cell death.

Methods

- Alamar Blue assay: Seeded 1,000 cells per well in Met complete or restricted media and read fluorescence once per day for 5 days.
- AnnexinV stain: Seeded 2x10^5 cells per well in Met complete or restricted media. Collected cells after 4 days and stained with AnnexinV and PI.
- Clonogenic assay: Seeded 1,000 cells per well in Met complete or restricted media. Cells grew for 10 days, replacing the media every 3 days.
- Western blot analysis: Seeded 1x10^6 cells in 10cm plates in Met complete media and 2x10^6 cells in Met restricted media. Cells were collected after 3 days.

Results

GENE ID	Function
ARRDC4	Arrestin Domain Containing 4 (Endocytosis of activated GPCRs)
GALNT1	Glycosyl Transferase Enzyme (O-glycosylation)
EPSTI1	Epithelial Stromal Interaction (Tumor Invasion and Metastasis)
ABI1	Abelson Interactor 1 (actin polymerization)
UBQLN4	Regulates degradation of specific substrates via proteasomal pathway
★ UBQLN1	Regulates degradation of specific substrates via proteasomal pathway
SLC7A7	Sodium dependent neutral amino acid transporter
SLC7A5	Sodium independent neutral amino acid transporter

Figure 1: Preliminary CRISPR/Cas9 screen

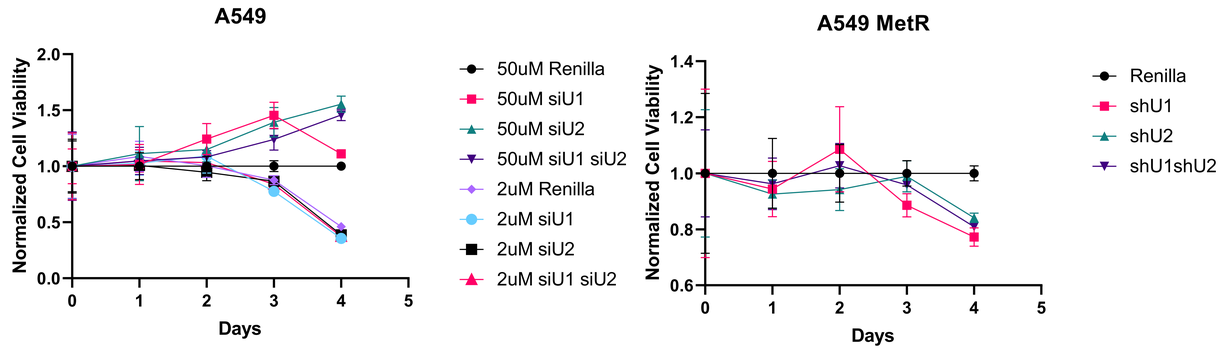

Figure 2: Alamar Blue assay of shUBQLN and Renilla on A549 UBQLN knockdown cells over 5 days of Met compete or restricted media.

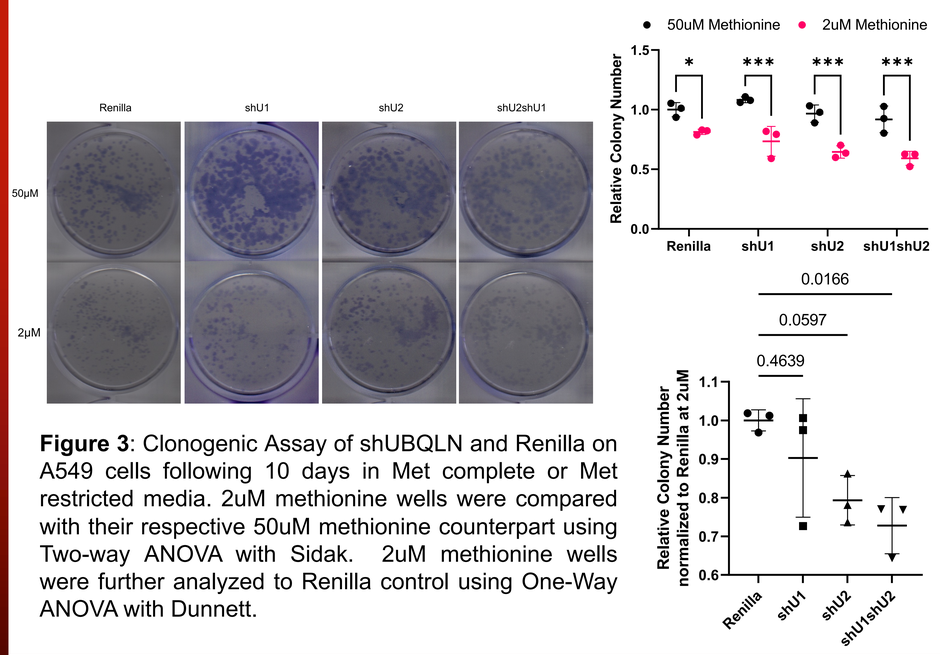

Figure 3: Clonogenic Assay of shUBQLN and Renilla on A549 cells following 10 days in Met complete or Met restricted media. 2uM methionine wells were compared with their respective 50uM methionine counterpart using Two-way ANOVA with Sidak. 2uM methionine wells were further analyzed to Renilla control using One-Way ANOVA with Dunnett.

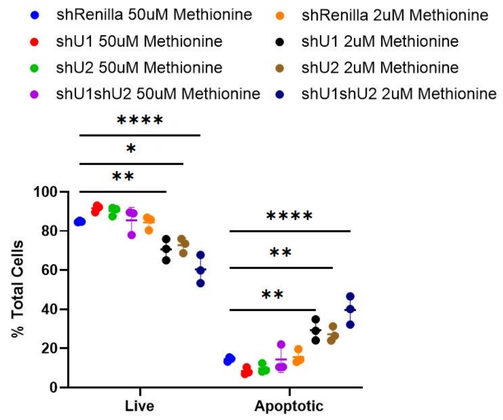

Figure 4: Annexin V stain of shUBQLN and Renilla on A549 cells following 4 days of Met complete or Met restricted media. Analysis was performed using a 2-way ANOVA with Dunnett.

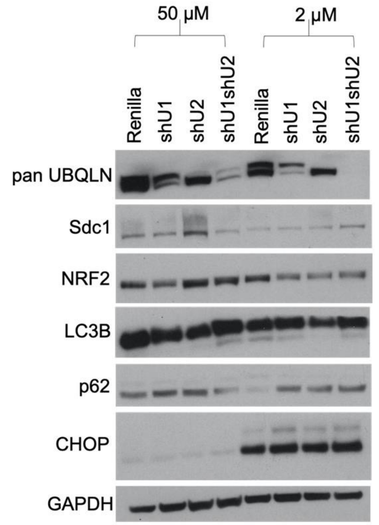

Figure 5: Western Blot analysis of shUBQLN and Renilla on A549 cells following 3 days of Met complete or restricted media.

Conclusion

- Results suggest the knockdown of UBQLN1/2 increases sensitivity of Met restriction: increasing cell apoptosis in Annexin V
- Western blot data suggests a decrease in autophagy as seen in increased p62
- Future studies: Further investigate the interaction between Sdc1 and UBQLN (specifically at the STI regions) as seen in Western blot data.
- Repeat experiments and investigate how the overexpression of UBQLN affects cells under nutrient deprivation.

Acknowledgements

This research was funded by the National Cancer Institute grant R25 C-A134283 and the University of Louisville Cancer Education Program.