2024-08-11

Exploring the Mechanism of Increased Survival in RB1-mutant Glioblastoma Multiforme

Lucia Wesemann

HONORS THESIS

EXPLORING THE MECHANISM OF INCREASED SURVIVAL IN *RB1*-MUTANT GLIOBLASTOMA MULTIFORME

by
LUCIA J. WESEMANN

Submitted to Brigham Young University in partial fulfillment
of graduation requirements for University Honors

Department of Biology
Brigham Young University
August 2024

Advisor: Samuel H. Payne

Faculty Reader: Matthew H. Bailey

Honors Coordinator: Steven L. Peck

ABSTRACT

EXPLORING THE MECHANISM OF INCREASED SURVIVAL IN *RB1*-MUTANT GLIOBLASTOMA MULTIFORME

Lucia J. Wesemann
Department of Biology
Bachelor of Science

Glioblastoma Multiforme (GBM) is an incredibly invasive and particularly lethal central nervous system cancer. Recent work has shown that GBM patients with mutated *RB1* have greater overall survival. A proposed mechanism for the improved prognosis of this molecular subgroup is mainly supported by previous research that is not specific to GBM. I utilize the Clinical Proteomic Tumor Analysis Consortium (CPTAC) dataset to interrogate this mechanism using GBM-specific data. The mechanism is largely not supported by the CPTAC GBM dataset, and the trend of significantly improved overall survival in *RB1*-mutant GBM patients is not validated here. This study highlights the need for additional experimentation and offers suggestions for future research.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my thesis committee for their support and guidance. Dr. Samuel Payne in particular provided incredible mentorship throughout the entire process and taught me about the importance of storytelling in science. My parents, siblings and friends never failed to lift me up when I was discouraged about my project. Hunter Lindsay provided invaluable feedback on my writing, and John Caelin Campbell offered suggestions to improve my presentation. Additionally, I would like to acknowledge Jac Taylor and Sierra Moore, my partners for the BIO 462 final project that, in part, inspired my Honors thesis. I used code that we wrote as a group in my analysis, especially in visualizing my data. This work is dedicated to the memory of Sydney Lynn Poulsen.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Glioblastoma Multiforme (GBM) is the most aggressive and most common cancer of the central nervous system. The prognosis of this disease is dismal, with a 5-year survival of 5.3% (Brennan et al., 2013) and a median overall survival of around 14 months in patients undergoing treatment (Delgado-López et al., 2016). Over the last three decades, there has been minimal progress made in improving the survival rate, despite the fact that this disease was the highest funded intracranial malignancy by the American National Institutes of Health during the same time period (Delgado-López et al., 2016). Most ongoing clinical trials have not shown potential to augment current treatment strategies (Schaff and Mellinghoff, 2023), emphasizing the need for an even deeper understanding of disease etiology and progression.

GBM has been classified into distinct morphologic, mutation-based, transcriptional, proteogenomic, metabolomic, and immune subtypes, though no specific treatment program has yet been found to impact survival in any one subtype over another (Verhaak et al., 2010; Rutledge et al., 2013; Wang et al., 2021). That said, hypermethylation of the MGMT promoter is a predictive biomarker of sensitivity to temozolomide, the standard-of-care alkylating agent, as well as response to radiotherapy (Stupp et al., 2005; Rivera et al., 2010). Though no novel therapy has yielded an improvement in subtype-specific prognosis, certain molecular abnormalities have been demonstrated to correlate with disease progression and patient survival. Mutation of the *RB1* gene is one such abnormality that is significantly associated with improved progression-free and overall survival (Dono et al., 2021).

RB1 was the first tumor suppressor described and is best known for its role as a negative regulator of the cell cycle (Chinnam and Goodrich, 2011). In its active state, pRB, the protein product of the *RB1* gene, sequesters the E2F transcription factor family, which controls a genetic program necessary to drive the cell cycle forward from G1 through S-phase. In response to growth signals, pRB is phosphorylated and thereby inactivated by cyclin-dependent kinase complexes, ultimately allowing for the transcription of a myriad of genes required for cell cycle progression. This canonical "RB pathway" has been well-characterized. It has become increasingly apparent that pRB has a massive range of functions independent of this pathway. With an estimated 100-300 biologically relevant binding partners, pRB is incredibly versatile and likely exerts its tumor suppressive effects through multiple avenues (Dyson, 2016). For example, pRB is capable of regulating a wide variety of protein complexes that participate in DNA replication, DNA repair, and mitosis, in addition to transcription of cell cycle genes (Chinnam and Goodrich, 2011).

The effects of pRB loss on patient outcomes are diverse. *RB1* alteration is associated with significantly worse overall survival in several cancers, including high-grade neuroendocrine cervical carcinoma, non-small cell lung cancer, and advanced prostate cancer (Flores Legarreta et al., 2023; Bhateja et al., 2019; Abida et al., 2019). On the other hand, pRB loss—coupled with disruption in the homologous recombination DNA repair pathway—is associated with improved response to treatment and overall survival in high-grade serous ovarian cancer (Garsed et al., 2018). This highlights the need for cancer type-specific investigation into the mechanisms of pRB-mediated tumor suppression.

The reason for increased survival in GBM patients with mutated *RB1* is unknown, but hints of a possible mechanism exist in the literature. The 2021 study that revealed the increased overall survival in *RB1*-mutant patients also outlined a potential mechanism that might explain this phenomenon. GBM tumors harboring an *RB1* mutation are correlated with an enrichment in tumor-infiltrating lymphocytes, agents of the immune system that are capable of recognizing and killing cancer cells (Rutledge et al., 2013). In addition, expression of *CD3G*, a gene that encodes the T-cell surface marker CD3, is significantly higher in *RB1*-mutated tumors than *RB1*-WT tumors (Rutledge et al., 2013). However, although TILs are correlated with improved prognosis in other cancer types, the abundance of TILs in GBM tumors has no significant impact on overall survival (Rutledge et al., 2013).

Additionally, pRB loss has been demonstrated to elicit the DNA damage response in human cell lines and in mice (Tort et al., 2006). The DNA damage response represents a complex network that serves to repair DNA lesions, stall the progression of the cell cycle, and, if necessary, trigger apoptosis (Giglia-Mari et al., 2011). p53 is a crucial mediator of this response, particularly in cell-cycle arrest, and is frequently mutated in *RB1*-mutant GBM tumors (Giglia-Maria et al., 2011; Dono et al., 2021). Taken together, these data suggest that *RB1* loss leads to DNA damage, which is less likely to be resolved in tumors that also lack p53 function. An accumulation of DNA damage, coupled with DNA repair inability, presents a higher probability of generating neoantigens—mutant tumor proteins that the immune system recognizes as foreign—and eliciting an immune response that may neutralize the malignancy (Fang et al., 2022). While this mechanism for increased survival in *RB1*-mutant GBM patients is supported by the fact that *RB1*-

mutant GBM tumors are associated with higher TIL count, there are few other GBM-specific studies available to bolster this claim.

I examined the GBM cohort collected and analyzed by the Clinical Proteomic Tumor Analysis Consortium (CPTAC) (Wang et al., 2021) in an effort to provide GBM-specific evidence in favor of or against the proposed mechanism for increased survival in *RB1*-mutant GBM patients. The findings presented here both validate previous reports on the *RB1*-mutant GBM subtype and offer new insights into the proteogenomic landscape of these particular tumors. This work confirms the need for further experimentation to clarify the potential reasons behind the improved prognosis of *RB1*-mutant GBM patients.

METHODS

Data accession

Data used in this publication were generated by the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (CPTAC). Clinical, proteomic, transcriptomic, phosphoproteomic, and mutation data were accessed from all ten of the public tumor cohorts in the CPTAC dataset. Data were accessed through the "cptac" package in Python (Lindgren et al., 2021).

Data accessibility

All codes for the analysis and visualization can be found at this link: https://doi.org/10.5281/zenodo.13298892

Percentage of RB1-*mutated tumors and top ten most frequently mutated genes*

For each of the ten publicly available CPTAC datasets, all tumors were included in this analysis. If a tumor had more than one *RB1* mutation, it was only counted once. The percentage of tumors with mutated *RB1* was calculated by dividing the number of *RB1*-mutated tumors by the total number of tumor samples. No mutation type was excluded from this analysis. The most frequently mutated genes were also derived from the somatic mutation dataset for each cancer type. In this analysis, every mutation was tallied regardless of whether a tumor had multiple mutations in the same gene.

Lollipop plot

Mutplot, a free online tool (https://bioinformaticstools.shinyapps.io/lollipop/), was utilized to visualize *RB1* mutations (Zhang et al., 2019). No mutations were excluded from this analysis. Though there are ten tumors with mutated *RB1*, one tumor bears two *RB1* mutations, both of which were plotted. Any characters after an "*" were removed prior to importing the data into Mutplot.

Survival analysis

Overall survival was defined as the time in months from initial diagnosis until death or last available follow-up. Survival was reported in days and converted to months for the analysis. One month was defined as 30 days. The lifelines Python package was imported to visualize the survival curves using the Kaplan-Meier method. A log-rank test was

employed to assess the statistical significance of the difference between the *RB1*-wildtype and the *RB1*-mutant groups. Patients with insufficient data were automatically excluded from this analysis.

Other analyses

Fisher's exact test from the SciPy Python library was employed to assess the statistical significance of *RB1* and *TP53* co-mutation. In this analysis, patients with multiple mutations in either gene were only counted once. The Kruskal-Wallis test was utilized in the analysis of non-silent somatic mutations. All mutation types were included in this analysis except for silent mutations. No non-silent somatic mutation in the CPTAC GBM cohort was excluded, even if patients possessed multiple mutations in the same gene. Welch's t-test was used to assess the significance of the differences in mean protein and transcript abundances of TIL markers CD39, CD8A, CD3E, and CD4 among *RB1*-mutant tumor samples, *RB1*-wildtype tumor samples, and non-tumor samples. Welch's t-test was also used to investigate the differences in mean protein abundances of H2AFX and RPA2 across the same groups. No tumors were excluded; all available values were included in every analysis. Across all statistical tests, significance was defined as a p-value of less than 0.05.

RESULTS

Among all ten publicly available CPTAC datasets – breast cancer, clear cell renal cell carcinoma, colorectal cancer, endometrial carcinoma (uterine), glioblastoma, head and neck squamous cell carcinoma, lung squamous cell carcinoma, lung adenocarcinoma, high grade serous ovarian cancer, and pancreatic ductal adenocarcinoma – GBM has the

highest percentage of tumors harboring at least one mutation in the *RB1* gene at 9.5% (Figure 1a). In addition, GBM is the only cancer type in the cohort for which *RB1* is one of the ten most frequently mutated genes. Notably, *TP53* is the most frequently mutated gene in the CPTAC GBM cohort (Figure 1b), validating the same finding in The Cancer Genome Atlas (TCGA) GBM cohort (Brennan, et al., 2013).

A

Cancer type	Mutated RB1
GBM	9.5%
LUAD	7.2%
EN	5.2%
COL	4.5%
LSCC	4.4%
OV	3.6%
HN	1.8%
BRCA	1.6%
PD	.07%
CCRCC	0%

B

	BRCA	CCRCC	COL	EN	GBM	HN	LSCC	LUAD	OV	PD
1	TTN	VHL	TTN	PTEN	TP53	TP53	TTN	TTN	TTN	KRAS
2	TP53	PBRM1	APC	CCDC168	PTEN	TTN	TP53	RYR2	MUC17	TP53
3	MUC4	TTN	TP53	MUC16	NF1	CSMD3	CSMD3	MUC16	TP53	CDKN2A
4	MUC16	KDM5C	PLEC	ARID1A	EGFR	NOTCH1	RYR2	USH2A	MUC4	SMAD4
5	PIK3CA	BAP1	TCF7L2	PIK3CA	RYR2	FAT1	USH2A	CSMD3	MUC12	TTN
6	FLG	SETD2	NRXN1	FSIP2	MUC16	MUC16	ZFHX4	TP53	AHNAK	MUC4
7	PCLO	MUC16	DMD	SYNE1	MUC17	LRP1B	MUC16	LRP1B	SPATA31D1	CTNNA2
8	HYDIN	CSMD3	SYNE1	NEB	RB1	CDKN2A	SYNE1	EGFR	NF1	RYR2
9	RYR1	CUBN	RIMS2	USH2A	PIK3CA	DNAH5	XIRP2	ZFHX4	LPA	MUC16
10	ZNF717	MUC5AC	TRIP12	CSMD3	HCN1	RYR2	LRP1B	CSMD1	ARHGAP5	FMN2

C

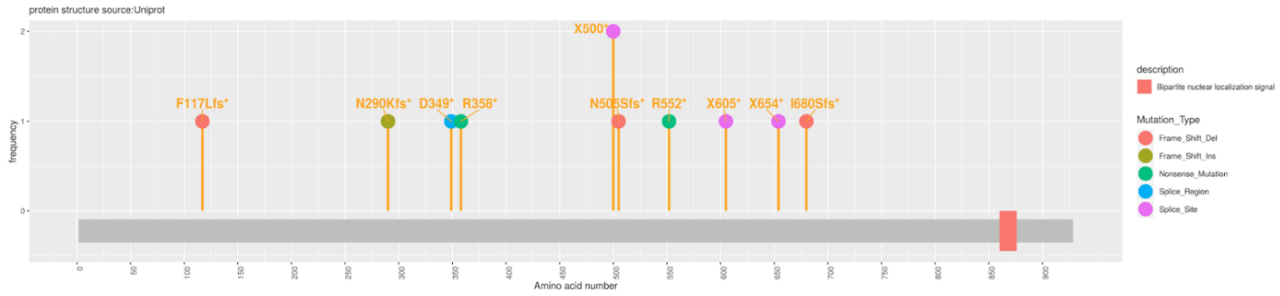

Figure 1: Characterizing the *RB1* mutations present in the CPTAC GBM cohort. **A.** Percentage of patients harboring at least one *RB1* mutation in each publicly available CPTAC cancer dataset. **B.** The top 10 genes with the most mutations in each CPTAC cancer dataset, not accounting for multiple mutations within the same patient. **C.** Lollipop plot representing the location and type of all *RB1* mutations in the CPTAC GBM cohort.

The proposed mechanism for increased survival in *RB1*-mutant patients relies on the assumption that the function of the pRB protein is impaired by alterations to the *RB1* gene. A gene may be mutated and still encode a viable protein product that retains both form and function, due to the redundancy of the genetic code. Thus, it is crucial to

investigate every *RB1* mutation to infer its particular effect on the pRB protein. Figure 1c is a visualization of the location and nature of each of the eleven total *RB1* mutations in the GBM cohort (one of the tumors bears two *RB1* mutations). The most common mutation types (5 out of 11, or 45%) are splice region or splice site mutations, which occur at the boundary of an intron and an exon. These mutations may interrupt intron excision and exon splicing, leading to a nonfunctional protein product. In addition, there are three frameshift deletions, two nonsense mutations, and one frameshift insertion. All six of these mutations result in the premature termination of transcription, ultimately leading to the truncation of the pRB protein product. Notably, all eleven mutations occur upstream of the bipartite nuclear localization signal that facilitates the transport of pRB from the cytoplasm to the nucleus (Zacksenhaus et al., 1993). Thus, it is unlikely that any mutated pRB in the CPTAC GBM cohort is capable of localizing to the nucleus or repressing the activity of the E2F transcription factor family. Furthermore, none of these truncated *RB1* protein products are likely to possess any functional capabilities that non-mutated pRB performs outside the nucleus. More analysis is needed to confirm the effect of these mutations on pRB function outside of the canonical RB pathway.

A

	TP53-WT	*TP53*-mutant
RB1-WT	81	24
RB1-mutant	2	8

Odds ratio: 13.5
P-value: 0.0005123

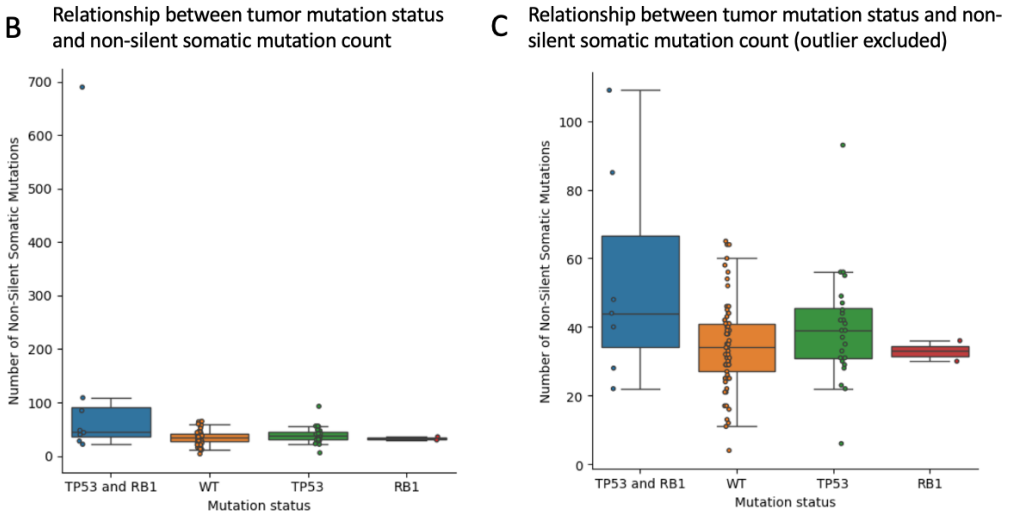

Figure 2: *RB1* and *TP53* co-mutation. A. Contingency table showing the distribution of *TP53* and *RB1* mutations in the CPTAC GBM cohort. The p-value shown is from a Fisher's exact test. **B.** Bar chart showing the relationship between mutation status and number of non-silent somatic mutations. p = 0.20 (Kruskal-Wallis test) **B** Same plot as B with outlier removed. p = 0.35 (Kruskal-Wallis test).

RB1 and *TP53* are frequently co-mutated across many different cancer types (Cai et al., 2022). This trend is seen in the CPTAC GBM cohort: 80% of patients with an *RB1* mutation also have at least one mutation in the *TP53* gene (Figure 2a). The association between *RB1* mutation and *TP53* mutation is statistically significant (Figure 2a), consistent with the same analysis in the UTHealth GBM cohort (Dono et al., 2021). As pRB and p53 are both involved in the cellular response to DNA damage (Harrington et al., 1998; Williams & Schumacher, 2016), loss of one or both proteins can lead to greater genome instability in tumor cells. In urothelial bladder cancer, for example, tumors with concurrent *RB1* and *TP53* genomic alterations have a significantly greater tumor mutational burden (number of non-silent somatic mutations per megabase of exome

DNA) than tumors with neither alteration (Manzano et al., 2021). I investigated whether the mutation status of *RB1* and *TP53* have an effect on the number of non-silent somatic mutations in the CPTAC GBM cohort. There is no significant correlation between the number of non-silent somatic mutations and mutation status of *TP53* or *RB1* (Figure 2c). This remains the case after excluding an extreme outlier with nearly 700 somatic non-silent mutations. In this particular cohort, *RB1* and *TP53* co-mutation does not have an impact on the overall mutational burden of GBM tumors.

Tumor-infiltrating lymphocytes (TILs) are significantly associated with *RB1*-mutant GBM tumors, and it is proposed that the greater abundance of TILs in these particular tumors could account for the increase in overall survival. I interrogated the proteomic and transcriptomic data available in the CPTAC GBM dataset in order to assess the relationship between *RB1* mutation status and protein abundance and expression of TIL biomarkers CD39, CD4, CD3E, and CD8A. There is no significant difference in the protein abundances of CD39, CD4, CD3E, or CD8A between *RB1*-mutant and *RB1*-wildtype GBM tumors in the CPTAC cohort (Figure 3a). The expression levels for all TIL biomarkers show the same trend (Figure 3b). This is not consistent with previous findings in which *RB1* mutation was significantly correlated with the presence of lymphocytes in GBM tumors ($p = 0.04$) (Rutledge et al., 2013). That said, the same study reported no significant difference in *RB1* status between tumors with lymphocytes present in the majority (>=50%) of tumor tissue and those with lymphocytes present in <50% of tissue combined with tumors with no lymphocytes ($p = 0.22$). Notably, the TIL transcript abundances in tumors are significantly higher than in normal samples,

regardless of *RB1* mutation status, for all biomarkers except CD8A. This trend is not seen at the protein level.

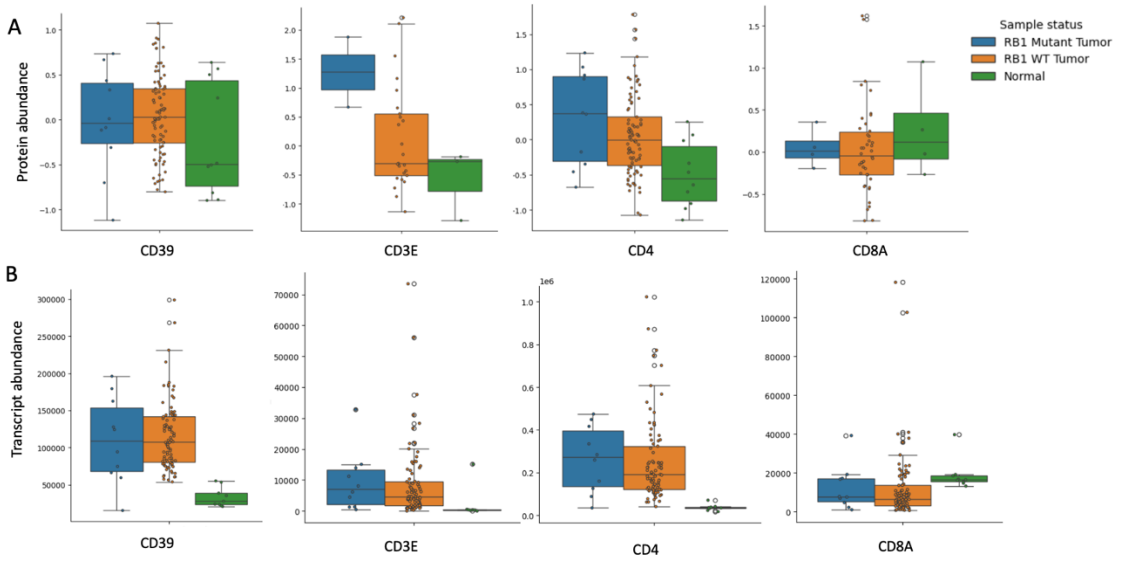

Figure 3: Protein abundance and expression of biomarkers for tumor-infiltrating lymphocytes by tumor mutation status. A. Bar charts showing the protein abundances of various biomarkers of tumor-infiltrating lymphocytes, separated by sample status (*RB1* mutant, *RB1* wildtype, or normal). **B.** Bar charts showing the mRNA transcript abundances of various biomarkers of tumor-infiltrating lymphocytes, also separated by sample status.

The mechanism proposed by Dono et al. (2021) suggests an increase in replication stress and DNA damage, coupled with DNA repair inability, in *RB1*-mutant GBM tumors. While this suggestion is supported by data in other cancer types, it has not been confirmed in GBM specifically. I assessed the protein levels of two biomarkers of replication stress in the CPTAC GBM cohort. Though there are three biomarkers that are frequently used to detect replication stress, two of them—γ-H2AX and pRPA2—have been reported to reliably assess oncogene-induced replication stress specifically (Meessen et al., 2022). H2AFX is not significantly more abundant in *RB1*-mutant GBM tumors as compared to *RB1*-wildtype tumors ($p = 0.43$), but both *RB1*-mutant tumors and *RB1*-wildtype tumors possess a greater abundance of H2AFX than normal samples ($p =$

.0080 and p = .0016, respectively) (Figure 4a). In *RB1*-mutant tumors, RPA2 abundance is significantly higher than both *RB1*-wildtype and normal samples (p = 0.013 and p = 0.00016, respectively) (Figure 4b). RPA2 protein is also significantly more abundant in *RB1*-wildtype tumors as compared to normal samples (p = 0.0011).

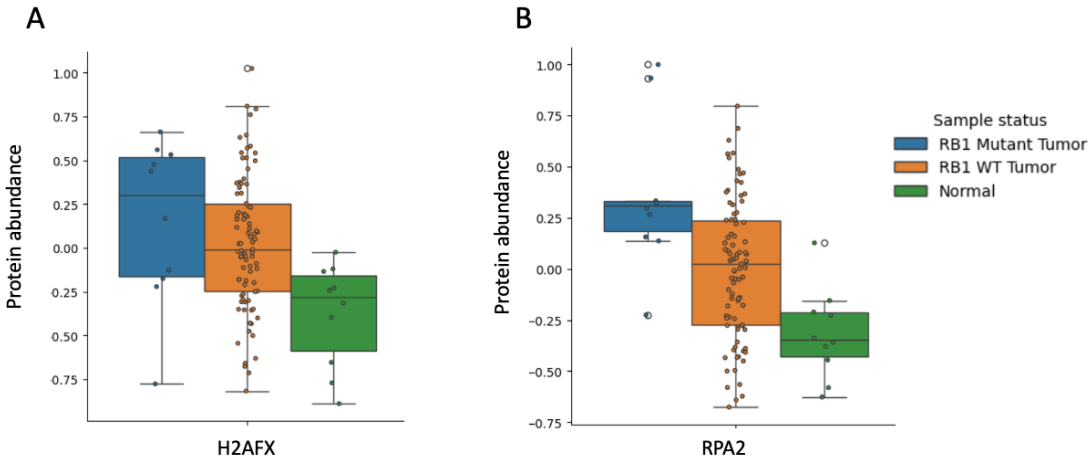

Figure 4: Protein abundance of biomarkers for replication stress by tumor mutation status. A. Bar chart showing the effect of *RB1* mutation on the protein abundance of H2AFX. Kruskal-Wallace p-value = 0.0040. Welch's t test, *RB1*-mutant vs. *RB1*-wildtype p-value = 0.44. Welch's t test, *RB1*-mutant vs. normal p-value = 0.0080. Welch's t test, *RB1*-wildtype vs. normal p-value = 0.0016. **B.** Bar chart showing the effect of *RB1* mutation on the protein abundance of RPA2. Kruskal-Wallace p-value = 0.00025. Welch's t test, *RB1*-mutant vs. *RB1*-wildtype p-value = 0.013. Welch's t test, *RB1*-mutant vs. normal p-value = 0.00016. Welch's t test, *RB1*-wildtype vs. normal p-value = 0.0011.

Finally, I conducted a Kaplan-Meier survival analysis to determine whether the increased overall survival in *RB1*-mutant GBM patients is validated in the CPTAC cohort. There is no significant difference in overall survival between patients with wildtype *RB1* and those with mutated *RB1* (p = 0.94). The median overall survival for *RB1*-mutant GBM patients in the CPTAC cohort is 11.83 months, and the median overall survival for *RB1*-wildtype patients is 11.63 months. The total median overall survival is 11.67 months.

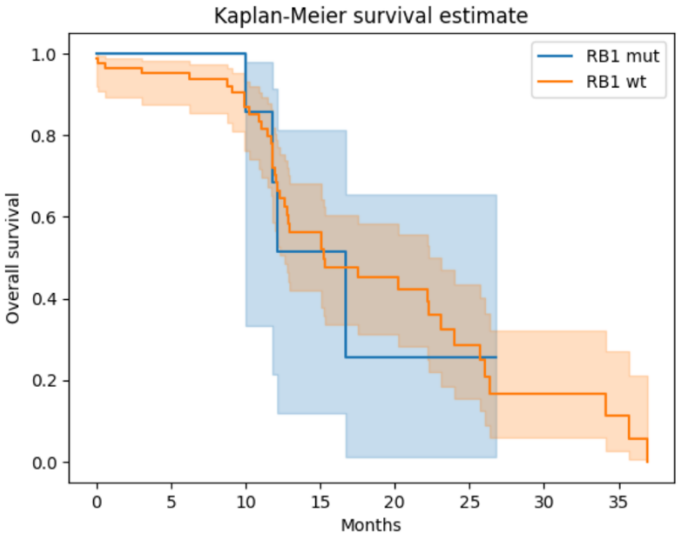

Figure 5: Survival probability based on *RB1* mutation status. Kaplan-Meier survival estimate of *RB1*-mutant and *RB1*-wildtype patients in the CPTAC GBM cohort. Log-rank test p-value = 0.94.

DISCUSSION

The percentage of CPTAC GBM tumors harboring at least one *RB1* mutation validates reports from other datasets (Dono et al., 2021; Brennan et al., 2013). Additionally, the high prevalence of *TP53* mutation within the *RB1*-mutated cohort has been previously described (Zhang et al., 2019). Other data from this study are at odds with prior investigations and the proposed mechanism for improved prognosis in *RB1*-mutant GBM tumors. Perhaps most importantly, the trend of increased overall survival in *RB1*-mutant patients was not observed in the CPTAC GBM cohort. The small number of *RB1*-mutant patients for which survival data were available could account for this discrepancy (n=9), though the notably high p-value (p=0.94) suggests that observed similarity in means may not be attributed to the small sample size alone. In addition, there were no significant differences in either the protein abundances or the transcript abundances of any of the four TIL biomarkers assessed. This finding conflicts with previous work that revealed a statistically significant correlation between *RB1* mutation

and presence of TILs, a study that investigated both histology and *CD3G* expression (Rutledge et al., 2013). Finally, the proposed mechanism for increased overall survival in *RB1*-mutant GBM patients, in which an increase in DNA damage and replication stress were key components, was largely not supported by the CPTAC GBM dataset. The somatic non-silent mutation count did not show significant correlation with the mutation status of *TP53* or *RB1*, and there was no significant difference in the abundance of H2AX. However, *RB1*-mutant tumors did show an increase in RPA2, which does support the hypothesis that *RB1* loss promotes replication stress in GBM tumors.

The limitations of this study include the prominent difference in sample size between *RB1*-mutant and *RB1*-wildtype tumors (n=10, n=95, respectively) and the retrospective design. In addition, the non-silent somatic mutation counts were not corrected for the number of base pairs sequenced per patient. Copy number variation, a phenomenon that affects protein abundance, was not assessed. Tumor purity was also not accounted for, and there were very few *RB1*-mutant samples with available proteomic data for CD3E and CD8A. An immune cell killing assay should be performed to quantify the efficacy of the TILs present in GBM tumors to eliminate cancerous cells. Importantly, I analyzed the protein abundances of non-phosphorylated H2AX and RPA2, whereas both biomarkers possess specific phosphosites that were not available in the CPTAC GBM dataset. Thus, neither biomarker is a completely reliable proxy for the levels of oncogene-induced replication stress in GBM tumor samples. These data were primarily included to motivate a future targeted assay. An *in vitro* assessment of γ-H2AX and pRPA2 levels in *RB1*-mutant GBM tumor cells should be performed for a more accurate quantification of the replication stress within this molecular subtype.

As is the case in most other cancer types, the heterogeneity of glioblastoma poses a challenge in treatment. In this new era of precision medicine, it is crucial to deepen our understanding of the molecular peculiarities within disease subtypes to identify more tumor-specific treatment programs. Hopefully, the data reported here inspire others to continue to shed light on the mechanism behind the increased overall survival in GBM patients harboring an *RB1* mutation. Elucidating the potential reasons for this anomaly could reveal promising therapeutic strategies that increase longevity and quality of life for GBM patients.

REFERENCES

Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, Cieslik M, Benelli M, Robinson D, Van Allen EM, Sboner A, et al. (2019). Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci U S A.* 116(23):11428-11436.

Bhateja P, Chiu M, Wildey G, Lipka MB, Fu P, Yang MCL, Ardeshir-Larijani F, Sharma N, Dowlati A. (2019). Retinoblastoma mutation predicts poor outcomes in advanced non-small cell lung cancer. *Cancer Med*. 8(4), 1459-1466.

Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, et al. (2013). The somatic genomic landscape of glioblastoma. *Cell* 155(2), 462-477.

Cai L, DeBerardinis RJ, Xiao G, Minna JD, Xie Y. (2022). A Pan-Cancer Assessment of *RB1*/*TP53* Co-Mutations. *Cancers (Basel)* 14(17)

Chinnam M, Goodrich DW. (2011). RB1, development, and cancer. *Curr Top Dev Biol*. 94, 129-169.

Delgado-López, PD, Corrales-García, EM (2016). Survival in glioblastoma: a review on the impact of treatment modalities. *Clinical and Translational Oncology* 18, 1062–1071

Dono A, Ramesh AV, Wang E, Shah M, Tandon N, Ballester LY, Esquenazi Y. (2021). The role of *RB1* alteration and 4q12 amplification in IDH-WT glioblastoma. *Neurooncol Adv.* 3(1)

Dyson NJ. (2016). RB1: a prototype tumor suppressor and an enigma. *Genes Dev.* 30(13), 1492-1502.

Fang X, Guo Z, Liang J, Wen J, Liu Y, Guan X, Li H. (2022). Neoantigens and their potential applications in tumor immunotherapy. *Oncol Lett.* 23(3), 88

Flores Legarreta A, Salvo G, Gonzales NR, Chisholm G, Hillman RT, Frumovitz M. (2023). *RB1* alteration and poor prognosis in women with high-grade neuroendocrine carcinoma of the uterine cervix: a NeCTuR study. *J Gynecol Oncol.* 34(4)

Garsed DW, Alsop K, Fereday S, Emmanuel C, Kennedy CJ, Etemadmoghadam D, Gao B, Gebski V, Garès V, Christie EL, Wouters MCA, Milne K, et al. (2018). Homologous Recombination DNA Repair Pathway Disruption and Retinoblastoma Protein Loss Are Associated with Exceptional Survival in High-Grade Serous Ovarian Cancer. *Clin Cancer Res.* 24(3), 569-580.

Giglia-Mari G, Zotter A, Vermeulen W. (2011). DNA damage response. *Cold Spring Harb Perspect Biol.* 3(1).

Harrington EA, Bruce JL, Harlow E, Dyson N. (1998). pRB plays an essential role in cell cycle arrest induced by DNA damage. *Proc Natl Acad Sci U S A.* 95(20), 11945-50.

Lakin ND, Jackson SP. (1999). Regulation of p53 in response to DNA damage. *Oncogene.* 18(53), 7644-55.

Lindgren CM, Adams DW, Kimball B, Boekweg H, Tayler S, Pugh SL, Payne SH. Simplified and Unified Access to Cancer Proteogenomic Data. J Proteome Res. 2021 Apr 2;20(4):1902-1910. doi: 10.1021/acs.jproteome.0c00919. Epub 2021 Feb 9. PMID: 33560848; PMCID: PMC8022323.

Manzano RG, Catalan-Latorre A, Brugarolas A.(2021). RB1 and TP53 co-mutations correlate strongly with genomic biomarkers of response to immunity checkpoint inhibitors in urothelial bladder cancer. *BMC Cancer.* 21(1), 432.

Marshall AE, Roes MV, Passos DT, DeWeerd MC, Chaikovsky AC, Sage J, Howlett CJ, Dick FA. *RB1* Deletion in Retinoblastoma Protein Pathway-Disrupted Cells Results in DNA Damage and Cancer Progression. Mol Cell Biol. 2019 Jul 29;39(16):e00105-19. doi: 10.1128/MCB.00105-19. PMID: 31138663; PMCID: PMC6664603.

Rivera AL, Pelloski CE, Gilbert MR, Colman H, De La Cruz C, Sulman EP, Bekele BN, Aldape KD. (2010). MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. *Neuro Oncology* 12(2), 116-121.

Rutledge WC, Kong J, Gao J, Gutman DA, Cooper LA, Appin C, Park Y, Scarpace L, Mikkelsen T, Cohen ML, Aldape KD, et al. (2013). Tumor-infiltrating lymphocytes in glioblastoma are associated with specific genomic alterations and related to transcriptional class. *Clin Cancer Res.* 19(18), 4951-4960.

Schaff LR, Mellinghoff IK. (2023). Glioblastoma and Other Primary Brain Malignancies in Adults: A Review. *JAMA* 329(7), 574–587.

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, et al. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 352(10), 987-96.

Tort F, Bartkova J, Sehested M, Orntoft T, Lukas J, Bartek J. (2006). Retinoblastoma pathway defects show differential ability to activate the constitutive DNA damage response in human tumorigenesis. *Cancer Res.* 66(21), 10258-63.

Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, et al. (2010). Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 17(1), 98-110.

Williams AB, Schumacher B. (2016). p53 in the DNA-Damage-Repair Process. *Cold Spring Harb Perspect Med*. 6(5).

Wang LB, Karpova A, Gritsenko MA, Kyle JE, Cao S, Li Y, Rykunov D, Colaprico A, Rothstein JH, Hong R, Stathias V, Cornwell M, Petralia F, Wu Y, Reva B, Krug K, Pugliese P, Kawaler E, Olsen LK, et al. (2021). Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell* 39(4), 509-528.

Zacksenhaus E, Bremner R, Phillips RA, Gallie BL. (1993). A bipartite nuclear localization signal in the retinoblastoma gene product and its importance for biological activity. *Mol Cell Biol.* 13(8), 4588-99.

Zhang M, Yang D, Gold B. Origin of mutations in genes associated with human glioblastoma multiforme cancer: random polymerase errors versus deamination. Heliyon. 2019 Mar 7;5(3):e01265. doi: 10.1016/j.heliyon.2019.e01265. PMID: 30899826; PMCID: PMC6407082.

Zhang W, Wang C, Zhang X. Mutplot: An easy-to-use online tool for plotting complex mutation data with flexibility. PLoS One. 2019 May 15;14(5):e0215838. doi: 10.1371/journal.pone.0215838. PMID: 31091262; PMCID: PMC6519802.