RRN3P2, RPL7AP64, ACTR3C, & RPL7AP30 as Novel Prognostic Biomarkers for Glioblastoma (GBM): A Bioinformatics-Based Analysis

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RRN3P2, RPL7AP64, ACTR3C, & RPL7AP30 as Novel Prognostic Biomarkers for Glioblastoma (GBM): A Bioinformatics-Based Analysis

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Abstract: The World Health Organization (WHO) notes gliomas as the most frequent intrinsic tumors of the CNS and classifies gliomas into multiple histological types. Among these, the most invasive type of brain cancer histology is Glioblastoma Multiforme (GBM). GBM is an extremely fast-growing type of malignant brain tumor seen in more than 15% of all patients with primary malignant brain tumors. The treatment of GBM poses a substantial clinical challenge due to tumor heterogeneity, ineffectiveness of surgery/radiotherapy, and chemoresistance. Prognostic biomarkers will enable clinicians to identify patients with a more aggressive tumor evolution. To identify novel biomarkers in GBM, gene expression datasets of metastatic brain cancer were obtained from The Cancer Genome Atlas (TCGA), a publicly-available collection of genomic data relating to the molecular and clinical basis of cancer. Genes from patient files were retrieved and analyzed for oncogenic capacity, metastatic contribution, and potential for prognostic signature. Using the differentially expressed genes RRN3P2, RPL7AP64, ACTR3C, and RPL7AP30, survival curves were generated using Kaplan Meier test to explore the prognostic value of the select risk signature. Further, immune infiltration analyses were performed to assess the immune infiltrates of six tumor-immune infiltrating cells (TIIC) subsets. Gene Ontology and KEGG were used to identify the correlated molecular pathways of the target genes and present possible therapeutic direction. This study explores the prognostic value of RRN3P2, RPL7AP64, ACTR3C, and RPL7AP30 genes in GBM, and provides new insight into the therapeutic value of the correlated molecular pathways of the set of genes. Further, this model is expected to provide guidance for clinical evaluation of the prognosis of GBM patients with overexpression of the aforementioned genes.

Summary: The treatment of GBM poses a substantial clinical challenge due to tumor heterogeneity, ineffectiveness of surgery/radiotherapy, chemoresistance, and high rate of recurrence. Prognostic biomarkers enable clinicians to identify patients with a more aggressive tumor evolution and offer treatments accordingly. To identify novel biomarkers in GBM, gene expression datasets of metastatic brain cancer were obtained from publicly-available genomic datasets. Validation of the prognostic signature was performed using survival analysis and through analyzing various gene expression datasets. Here we present high expression of RRN3P2, RPL7AP64, ACTR3C, and RPL7AP30 as having significant prognostic value in GBM, as well as the correlated molecular pathways as potential therapeutic targets.

Introduction: The World Health Organization (WHO) notes gliomas as the most frequent intrinsic tumors of the CNS and classifies gliomas into multiple histological types (Wesseling et. al, 2018). Among these, the most invasive type of brain cancer histology is Glioblastoma Multiforme (GBM). The cause of most cases of GBM is unknown. Genetic disorders, such as neurofibromatosis, and previous radiation therapy have resulted in development of GBM in some patients, although disproportionately (Hanif et. al, 2017). There is uncertainty regarding whether other well-known carcinogens in other forms of cancer are linked to GBM. GBM has seen very little clinical progress over the last few decades, in part due to lack of effective prognosis and drug delivery strategies (Hanif et. al, 2017).

GBM has classically been defined by its hallmark histomorphological "multiform" features. These include its cellular tumor (CT), infiltrating tumor (IT), and microvascular proliferation (MVP) regions, as well as characteristic hypoxia where tumor cells palisade around necrosis (PAN). This diverse combination of histomorphological features, observed disproportionately across patients, establishes a heterogeneous tumor microenvironment with various molecular attributes that contribute to aggressive tumor evolution and resistance to current treatments (Lam et. al, 2022).

Glioblastomas represent more than 15% of all primary malignant brain tumors, and can either originate from healthy brain tissue or develop from an existing low-grade astrocytoma. Diagnosis is typically made by a CT scan, MRI scan, or tissue biopsy. Current treatments for GBM include surgery, often followed by chemotherapy and radiotherapy (Davis et. al, 2016). Commonly used therapeutics include temozolomide, bevacizumab, carmustine, among others. However, even with the aforementioned therapeutic interventions, including surgical removal of the tumor, overall survival is dismal, and only extended by a few months. Additionally, GBM patients almost always experience a recurrence. While survival without treatments is typically three months, patients who undergo treatment see a typical survival duration of 12-15 months (Hanif et. al, 2017).

A poor prognosis is almost always seen in GBM patients. Clearly, the treatment of GBM poses a substantial clinical challenge due to intra-tumoral heterogeneity, ineffectiveness of surgery/radiotherapy, chemoresistance, and possibility of recurrence. These factors make the prognosis of GBM dismal, with a 5-year survival rate of 5% (Hanif et. al, 2017). However, the expression of various molecular markers have been linked to either better or worse survival outcomes in cohorts of GBM patients. These markers may present crucial prognostic value, and may help clinicians identify patients with a more aggressive tumor evolution and offer such patients a certain type of treatment accordingly (Śledzińska et. al, 2021). Therefore, the search for new prognostic biomarkers and therapeutic targets for GBM is an urgent clinical need.

The RRN3P2 gene is involved with rRNA synthesis and transcription initiation, and has been linked to breast cancer as a prognostic biomarker, as part of a 15-pseudogene risk signature (Tan et. al, 2020). The ribosomal protein L7A family of genes, involved in rRNA processing and peptide chain elongation during the translation stage of protein synthesis, has been linked to association with survival outcomes in patients with breast cancer (Kim et. al, 2021). This family of genes includes RPL7AP64 and RPL7AP30. Expression of the ACTR3C gene has not previously been linked to survival outcomes in cancer patients. However, none of the aforementioned genes have been explored for prognostic value in GBM.

The Cancer Genome Atlas (TCGA) is a publicly-available dataset, funded by the National Institute of Health, containing the molecular and clinical characteristics of over 20,000 primary cancer and matched normal samples spanning 33 cancer types. At the time of this study, the TCGA included RNA-sequencing data and clinicopathological data of a cohort of 599 GBM patients. RNA-sequencing data from patient samples in TCGA has been used in numerous studies to identify novel prognostic biomarkers in various cancer types (He et. al, 2020).

This study aims to explore the prognostic value of a set of genes selected from GBM patient cohorts of the Cancer Genome Atlas (TCGA) dataset, namely the RRN3P2, RPL7AP64, RPL7AP30, and ACTR3C genes. A risk signature was generated by performing survival analysis with the Kaplan-Meier test, and pathway analysis was confirmed using Krypto Encyclopedia for Genes & Genomes (Kanehisa et. al, 2000) and Gene Ontology (Ashburner et. al, 2000). Further, the constructed signature was used to explore the association between the immune microenvironment and the set of selected genes through the TIMER database (Taiwan et. al, 2020). This study comprehensively explores the correlation of the selected genes with the tumor microenvironment, prognosis of GBM patients, and therapeutic efficacy.

Methods:

Genomic Data Acquisition

Glioblastoma Multiforme (GBM) patient datasets, with gene expression profiles and various clinicopathological information, were obtained from the publicly-available Cancer Genome Atlas (TCGA). TCGA has molecularly characterized over 20,000 primary cancer samples spanning 33 cancer types, as of March 2022. Datasets that profile GBM were retrieved from the TCGA-GBM project and analyzed for oncogenic capacity, metastatic contribution, and potential for prognostic signature through the Gene Expression Profiling Analysis (GEPIA) database. GEPIA is an interactive resource for researchers to analyze RNA-seq expression data of many tumor/normal samples from the TCGA and GTEx projects using a standard processing pipeline, and provides a diverse range of gene expression and patient survival analyses (Tang et. al, 2019).

RNA Sequence Analysis & Identification of DEGs

The Gene Expression Profiling Analysis (GEPIA) database (Tang et al.) was used to perform Differentially-Expressed Gene (DEG) Analysis between the primary tumor tissue of 599 GBM patients and the paired adjacent non-malignant tissue. DEGs were identified using the built-in LIMMA package, and must have adjusted P value < 0.05 and $|\log2\text{foldchange (FC)}| > 1.2$.

KEGG/GO Pathway Enrichment Analysis

The Gene Ontology (GO) database clarifies the function of genes through describing cellular components (CC), molecular function (MF), and biological processes (BP). The Kyoto Encyclopedia of Genes & Genomes (KEGG) database provides data for understanding high-level functions/utilities of biological metabolic pathways. Both databases were used to understand molecular pathways correlated with the select gene signature.

Survival Analysis for Prognostic Signature

To analyze the prognostic value of the selected gene signature in patients with GBM, Kaplan-Meier survival curves were generated using expression data from GBM-cohorts in the TCGA study. Statistical significance was confirmed using P values less than 0.05 and Hazard Ratios.

Immune Infiltration Analysis

The Tumor Immune Estimation Resource (TIMER) was used to analyze the immune infiltration of six TIIC subsets, including CD4+ T lymphocytes, CD8+ T lymphocytes, B lymphocytes, neutrophils, dendritic cells, & macrophages, through the "CIBERSORT-Abs" function. Observations were validated through Spearman's coefficient (Rho factor) and P values < 0.05.

Results:

RNA Sequence Analysis & Identification of DEGs

DEGs were screened from 599 patient samples of primary tumor tissues of the TCGA dataset using the LIMMA package within the GEPIA database. A total of 3,195 DEGs were identified using the built-in LIMMA package, fitting the adjusted P value < 0.05, overexpressed chromosomal distribution, and $|\log2\text{foldchange (FC)}| > 1.2$. Expression data of four key genes (RRN3P2, RPL7AP64, ACTR3C, and RPL7AP30) was retrieved with the purpose of further exploring the prognostic value of the aforementioned risk signature.

Survival Analysis for Prognostic Signature

GEPIA-generated Kaplan-Meier survival curves showed significant prognostic value of the selected genes. As shown in Figure 1, all survival curves showed statistical significance in results as indicated by p values < 0.05 and hazard ratios. The survival curves show that (Figure 1: A) high expression of RRN3P2 gene correlates with worse survival outcomes, (Figure 1: B) high expression of RPL7AP64 gene correlates with worse survival outcomes, and (Figure 1: C) high expression of ACTR3C gene correlates with worse survival outcomes. However, (Figure 1: D) high expression of the RPL7AP30 gene correlated with relatively better survival outcomes among patients, as indicated by the probability of survival.

Legend for Figure 1: A) Survival outcomes in patients with high/low expression of RRN3P2. B) Survival outcomes in patients with high/low expression of RPL7AP64. C) Survival outcomes in patients with high/low expression of ACTR3C. D) Survival outcomes in patients with high/low expression of RPL7AP30.

Immune Infiltration Analysis

Immune infiltration analyses was performed through the TIMER database with CD8+ T lymphocytes (Figure 2: A), neutrophils (Figure 2: B), CD4+ T lymphocytes (Figure 2: C), macrophages (Figure 2: D), memory B lymphocytes (Figure 2: E), and dendritic cells (Figure 2: F). Analysis presents high expression of RRN3P2 as having positive correlation with only the macrophage TIIC subset (Figure 2: D), with statistical significance as determined by (p value < 0.05) and an in-range Spearman's coefficient value (Rho factor). No other TIIC subset showed statistical significance in any of the three other genes (ACTR3C, RPL7AP30, or RPL7AP64), and so results were not included here.

Legend for Figure 2: Degree of immune infiltration correlation between RRN3P2 and A) CD8+ T lymphocytes, B) Neutrophils, C) CD4+ T lymphocytes, D) Macrophages (framed in green), E) B lymphocytes (memory), and F) Activated Dendritic Cells.

Discussion: This is the first known study to perform a comprehensive bioinformatics analysis of the RRN3P2, RPL7AP64, ACTR3C & RPL7AP30 genes as potential novel prognostic biomarkers for Glioblastoma Multiforme (GBM), the most aggressive type of glioma. In exploring the potential prognostic significance of the selected genes in GBM, various survival analyses showed the extent of the aforementioned risk signature's survival correlation in cohorts of GBM patients.

The treatment of GBM poses a substantial clinical challenge due to heterogeneity, chemoresistance, recurrence, and lack of effective treatments. Furthermore, the current TNM classification system remains ineffective for accurately predicting survival outcomes in GBM patients (Hanif et. al, 2017). If the biological behavior of the tumor could be reliably predicted at the initial time of diagnosis, the prognosis of patients with GBM will be improved considerably. This makes it essential to explore new molecular markers for the prognosis of GBM. Current research on GBM-related prognostic biomarkers is insufficient to meet the diverse genomic profiles seen in GBM patients and to offer an accurate prognosis. Therefore, newly-identified markers that relate to patient survival outcomes in GBM must be discovered and shared with the scientific and medical community.

Survival Analysis for Prognostic Signature

The survival curves in Figure 1 showed that 1) high expression of RRN3P2 gene correlates with worse survival outcomes and a poor prognosis in patients, 2) high expression of RPL7AP64 gene correlates with worse survival outcomes and a poor prognosis in patients, and 3) high expression of ACTR3C gene correlates with worse survival outcomes and a poor prognosis in patients. However, 4) high expression of the RPL7AP30 gene correlated with relatively better survival outcomes among patients, and therefore, a better prognosis, relative to patients with low expression of the same gene. Survival analysis indicates high expression of the aforementioned genes as potential molecular markers for the prognosis of GBM patients.

Immune Infiltration Analysis

One interesting observation was the degree of Macrophage immune cell infiltration with regards to the RRN3P2 gene. This has particular value because tumor-associated macrophages (TAMs) have been linked to facilitating the metastatic cascade often observed in tumor evolution (Lin et. al, 2019) and TIICs have been observed to have considerable prognostic value in various cancers (Chen et. Al, 2020). The strong positive correlation between expression of the RRN3P2 gene and macrophage infiltration, as cited in the results, is indicative of prognostic value.

Only immune infiltration for the RRN3P2 gene was shown (with six TIIC subsets), due to the RPL7AP30, RPL7AP64, and ACTR3C genes have no statistically significant immune infiltration correlation any of the six TIIC subsets, and therefore the results were not included in this article.

Further Discussion

This study presents high expression of RRN3P2, RPL7AP64, ACTR3C, and RPL7AP30 as having significant prognostic value in GBM. Validation of the prognostic signature was performed using Kaplan-Meier survival analysis and through analyzing various gene expression datasets. Patient RNA-sequencing data was acquired through TCGA, and DEG analysis and survival analysis were performed using the GEPIA database. Immune infiltration analyses were performed using the TIMER database.

KEGG/GO Analysis clarified high-level functions of the selected genes and the associated molecular/biological pathways. The RRN2P2 gene was shown to have a critical role in ribosomal RNA (rRNA) synthesis, RNA polymerase I binding, and transcription initiation. RPL7A64 and RPL7AP30 were shown to be involved in rRNA processing, peptide chain elongation, cytoplasmic translation, cadherin/RNA binding, and maturation of LSU-RNA. ACTR3C was shown to have involvement with actin filament binding, cytoskeleton assembly, and regulation of actin cytoskeleton (Kanehisa et. al, 2000). Both the RRN3P2 and ACTR3C were shown to have regular ubiquitous expression in brain tissues, among other tissues such as lymph nodes, bone marrow, thyroid, and adrenal tissues (Ashburner et. al, 2000).

For further validation of results, all aforementioned molecular markers should be tested and confirmed through in-vitro experimentation in molecular biology and cytology experiments. Limitations of this study include all data was confined to the TCGA-GBM dataset (the largest GBM patient sequencing dataset at the time of this study).

KEGG pathway analysis and GO enrichment analysis present various molecular pathways as potential therapeutic targets, including rRNA (ribosomal RNA) synthesis, rRNA processing/peptide chain elongation, and cytoskeletal-assembly pathways. Targeting the aforementioned pathways with known inhibitors or suppressors, such as Homoharringtonine (omacetaxine mepesuccinate/HHT), Cytochalasin, or additional alkaloids, offers potential therapeutic value.

This is the first study to explore the prognostic value of the RRN3P2, RPL7AP64, ACTR3C, and RPL7AP30 genes in GBM. A risk signature was developed that could potentially predict the clinical progression and evolution of GBM in patients, and provides new insight into the therapeutic value of the correlated molecular pathways of the aforementioned genes.

Conclusion: Overexpression of RRN3P2, RPL7AP64, and ACTR3C gene was significantly associated with poor survival outcomes in patients, while overexpression of RPL7AP30 gene was significantly associated with better survival in patients. Therefore, RRN3P2, RPL7AP64, ACTR3C & RPL7AP30 can potentially be used as auxiliary prognostic biomarkers for GBM, and upon further in-vitro experimentation, their respective molecular pathways can serve as potential therapeutic targets.

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Data Availability: All patient data included in this study were obtained from The Cancer Genome Atlas (TCGA) (portal.gdc.cancer.gov). All other datasets used include Krypto Encyclopedia for Genes & Genomes (KEGG), Gene Ontology (GO), and Tumor Immune Estimation Resource (TIMER).

Competing Interests Statement: The authors of this study declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Additional Statement: This study was conducted in accordance with guidelines and regulations relevant to bioinformatics-based biomarker studies.

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Figures

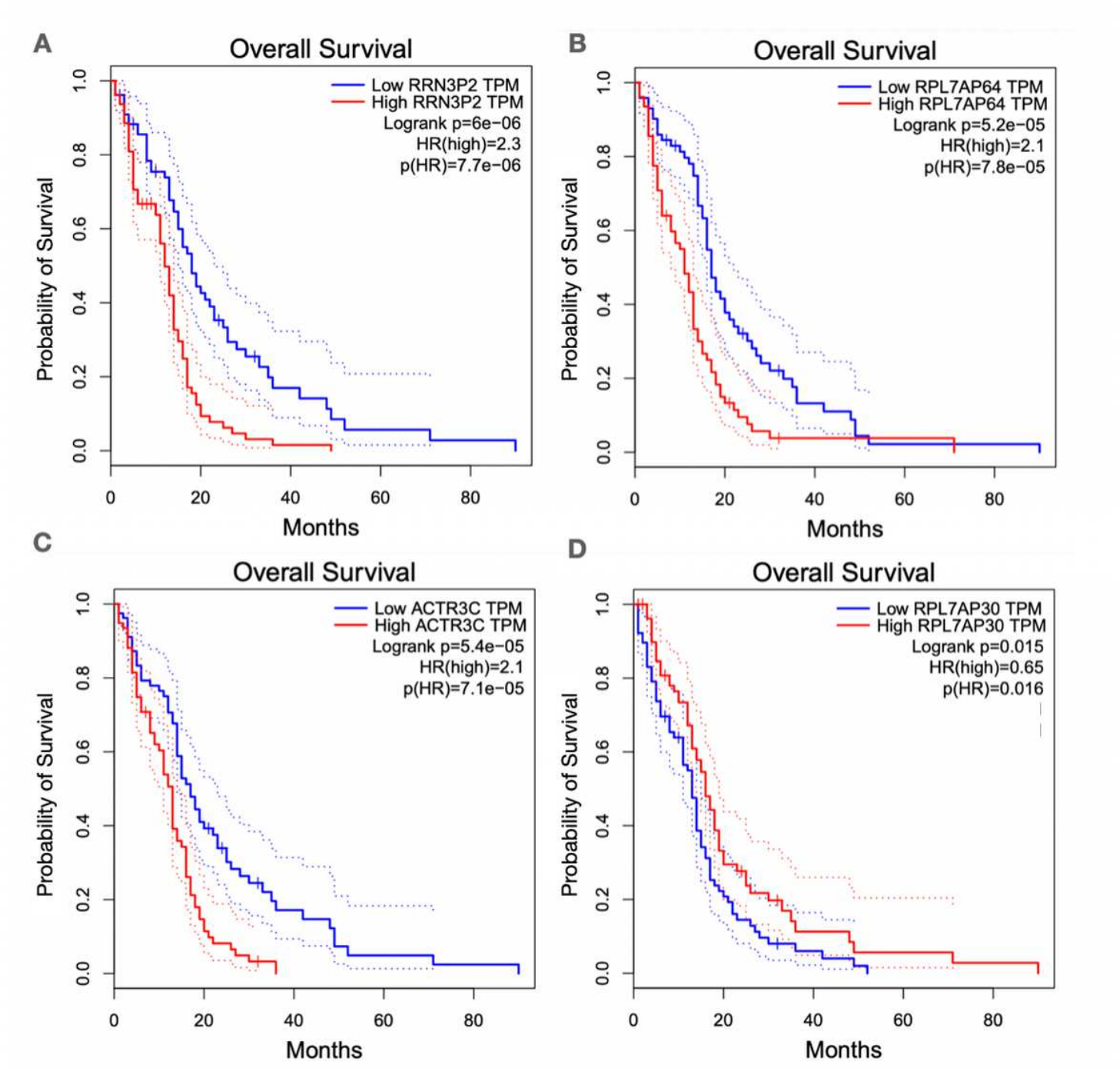

Figure 1

A) Survival outcomes in patients with high/low expression of RRN3P2. B) Survival outcomes in patients with high/low expression of RPL7AP64. C) Survival outcomes in patients with high/low expression of ACTR3C.

D) Survival outcomes in patients with high/low expression of RPL7AP30.

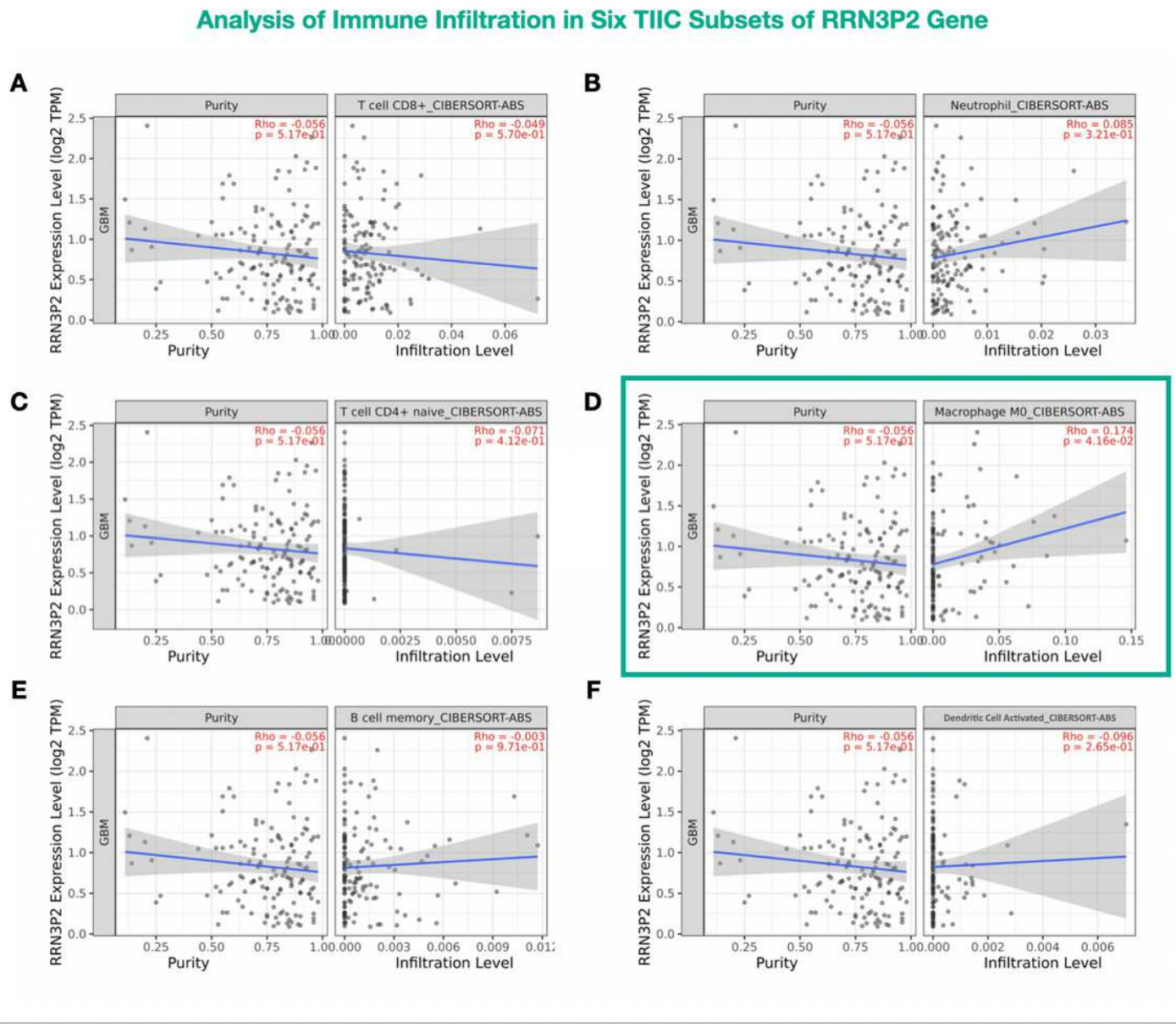

Figure 2

Degree of immune infiltration correlation between RRN3P2 and A) CD8+ T lymphocytes, B)

Neutrophils, C) CD4+ T lymphocytes, D) Macrophages (framed in green), E) B lymphocytes (memory), and F) Activated

Dendritic Cells.