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DIFFERENTIAL EXPRESSION OF RHD AND RHCE IN IMMUNE CELL POPULATIONS OF ACUTE MYELOID LEUKAEMIA: INSIGHTS FROM TCGA AND GTEX TRANSCRIPTOMIC ANALYSIS USING GEPIA PLATFORMS

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ABSTRACT – *Objective:* Acute Myeloid Leukaemia (AML) is an aggressive haematological malignancy with poor prognosis, mainly due to its complex interactions with the immune microenvironment. The *RHD* and *RHCE* genes, best known for their role in red blood cell biology, are emerging as potential regulators of immune processes. This study aimed to investigate the differential expression of *RHD* and *RHCE* in various immune cell types within AML tumour samples and healthy blood samples, utilising large-scale transcriptomic data (TCGA and GTEx).

Materials and Methods: Gene expression analyses were conducted using GEPIA, GEPIA2, and GEPIA2021, which integrate data from TCGA and GTEx. Normalised expression levels of *RHD* and *RHCE* were compared between AML tumour and normal blood samples across various immune cell types, including monocytes, macrophages, T cells, and NK cells, using log2(TPM + 1) values.

Results: Both *RHD* and *RHCE* were significantly overexpressed in AML tumour samples compared to healthy blood samples across several immune cell populations, including monocytes, macrophages, T cells, and B cells. Notably, *RHD* was highly expressed in T cells and monocytes, whilst *RHCE* showed elevated expression in macrophages and dendritic cells. In contrast, activated NK cells displayed higher *RHCE* expression in normal blood than in AML tumour samples.

Conclusions: The differential expression of *RHD* and *RHCE* in immune cells suggests their potential roles in modulating the immune microenvironment in AML. These findings provide a foundation for future research into the functional implications of *RHD* and *RHCE* in leukaemia progression and immune regulation. The study also highlights the utility of GEPIA platforms for comprehensive gene expression analysis in haematological malignancies.

KEYWORDS: Acute Myeloid Leukaemia, RHD, RHCE, Gene expression, TCGA, GTEx, Immune microenvironment, GEPIA, Immune cell deconvolution, Hematological malignancies.

INTRODUCTION

Acute Myeloid leukaemia (AML) is an aggressive haematological malignancy characterised by the rapid proliferation of abnormal myeloid progenitor cells, which leads to bone marrow failure and disruption of normal haematopoiesis[1]. Despite advances in treatment, the prognosis for AML remains poor, particularly in older adults, with relapse being a common challenge. As such, there is a pressing need to better understand the molecular mechanisms underlying AML to develop novel therapeutic strategies[1-3].

The *RHD* and *RHCE* genes, located on chromosome 1, encode proteins that are part of the Rh blood group system[4]. These proteins are primarily known for their role in red blood cell physiology, particularly in the context of blood transfusion and haemolytic disease[4]. However, emerging evidence suggests that the expression of *RHD* and *RHCE* may extend beyond erythrocytes, implicating these genes in broader biological processes[5], including immune regulation. Recent studies have highlighted their involvement in immune cell differentiation, migration, and activation, making them potentially relevant in the context of haematological malignancies such as AML.

The immune microenvironment plays a pivotal role in the progression of AML, where interactions between leukaemia cells and various immune cell populations contribute to immune evasion and tumour cell survival[6]. Given the established role of immune cells in the pathogenesis of AML[7], it is essential to explore how genes like *RHD* and *RHCE*, which are involved in immune-related processes, may influence the tumour immune microenvironment[8]. Understanding the expression patterns of these genes in different immune cell populations could provide valuable insights into their potential roles in AML progression and immune modulation[7].

Previous studies have primarily focused on the role of *RHD* and *RHCE* in red blood cell biology[9], leaving a significant gap in knowledge regarding their function in immune cells, particularly in the context of cancer[10]. This study aims to address this gap by profiling the expression of *RHD* and *RHCE* across various immune cell types in AML and normal blood samples. Investigating how these genes are differentially expressed in the tumour microenvironment *vs.* healthy tissues may uncover novel mechanisms contributing to AML pathogenesis.

Large-scale transcriptomic data from public databases such as The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) project offer a powerful resource for investigating gene expression patterns in cancer[11,12]. TCGA provides comprehensive RNA sequencing data from various tumour types, including AML, while GTEx references normal tissue expression. By comparing gene expression profiles from the TCGA-AML datasets with healthy blood samples from GTEx, researchers can identify dysregulated genes in AML and gain insights into their potential functional relevance in tumour biology[3,13].

The GEPIA (Gene Expression Profiling Interactive Analysis) platforms are well-established tools that allow researchers to explore and visualise RNA-seq data derived from TCGA and GTEx projects[14]. These platforms provide user-friendly interfaces for differential expression analysis, correlation analysis, survival analysis, and more. The subsequent versions, GEPIA2 and GEPIA2021[15], build on the original platform by incorporating updated datasets and enhanced functionalities, such as immune cell-type deconvolution.

Given the complexity of the AML immune microenvironment, the use of GEPIA2 and GEPIA2021 for immune cell-type deconvolution provides a critical advantage in this study. By breaking down gene expression patterns within specific immune cell subpopulations[16], we can better understand how *RHD* and *RHCE* are expressed in different immune contexts. This approach allows us to investigate whether these genes are associated with immune cell activation, suppression, or evasion within the tumour microenvironment.

This study aims to profile the expression of *RHD* and *RHCE* in various immune cell populations within AML tumour samples and compare them to their expression in normal blood samples. Specifically, we aim to determine whether *RHD* and *RHCE* are differentially expressed in immune cells from AML tumour samples compared to normal blood and identify immune cell subpopulations where these genes are significantly dysregulated. Finally, we explore the potential roles of *RHD* and *RHCE* in modulating immune cell behaviour within the AML microenvironment.

MATERIALS AND METHODS

Data Source and Selection

The data used in this study were obtained from The Cancer Genome Atlas (TCGA, Funded and managed by the NCI and the NHGRI, (Bethesda, MD, USA) and the Genotype-Tissue Expression (GTEx, Supported by the NIH, Bethesda, MD, USA.) projects. These datasets provide large-scale RNA sequencing (RNA-seq)

data from tumour and normal samples, enabling comprehensive gene expression profiling. The study focused on AML tumour samples (n=173) and corresponding healthy normal blood samples (n=70). The genes of interest were *RHD* and *RHCE*, which were selected based on their known biological significance in haematological malignancies.

Gene Expression Analysis

Gene expression analysis was performed using three web-based platforms: Gene Expression Profiling Interactive Analysis 1, 2 and 2021 (GEPIA, GEPIA2, and GEPIA2021) (Zhang's Lab, at Peking University, Beijing, China). These platforms facilitate RNA-seq data analysis across multiple cancer types, including TCGA-LAML, by integrating data from the TCGA and GTEx databases. Each platform was employed to cross-validate the results, ensuring robustness and reproducibility in the observed gene expression patterns.

Normalisation and Expression Quantification

Gene expression levels were normalised and quantified using log2(TPM+1) (Transcripts Per Million) to allow for variation across different samples and experimental conditions. TPM normalisation corrects sequencing depth and gene length, while log transformation helps stabilise variance across the dataset.

Differential Expression and Survival Analysis

The gene expression levels of *RHD* and *RHCE* were compared between AML tumour (n=173) and normal blood (n=70) samples across various immune cell types, including Monocytes, Macrophages (M0, M1, M2), T cells (CD8, CD4 Naïve, CD4 Memory Resting, CD4 Memory Activated), B cells (Naïve, Memory), Natural Killer (NK) cells (Resting, Activated), Dendritic cells (Resting, Activated), Mast cells (Resting, Activated), Eosinophils and Neutrophils.

To evaluate the survival rates of patients with AML based on gene expression levels, we utilised the GEPIA platform. The analysis focused on the *RHD* and *RHCE* genes, hypothesised to play a role in AML progression and immune microenvironment modulation. Patients (n=106) were stratified into high-expression and low-expression groups based on median gene expression levels. Kaplan-Meier plots were automatically generated by GEPIA, showing the proportion of surviving patients over time for each expression group. Additionally, Log-rank tests, hazard ratios (HR), and confidence intervals (CI) for survival comparisons were performed to compare survival distributions between the two groups, and the resulting *p*-values were used to determine statistical significance.

Data Visualisation

The results of the differential expression analysis were visualised using boxplots generated by the GEPIA platforms. These boxplots display the median expression levels, interquartile ranges, and outliers for *RHD* and *RHCE* in each immune cell type, allowing for easy comparison between LAML tumour and normal blood samples (n=173 and 70, respectively).

Immune Cell-Type Deconvolution

To gain deeper insights into the immune microenvironment of AML, immune cell-type deconvolution was performed using the functionalities available in GEPIA2 and GEPIA2021. This approach helped to characterise the relative contributions of different immune cell types to the expression levels of *RHD* and *RHCE*. The deconvolution process provides a clearer picture of how these genes may play distinct roles in the context of the immune response in AML.

Validation of Results

To ensure the accuracy and reproducibility of the findings, the results were cross validated across the three platforms (GEPIA, GEPIA2, GEPIA2021). The datasets for each platform were updated, and algorithm improvements were made in order to ensure that the observed trends in gene expression remained consistent. Additionally, previous studies in the literature were referenced to confirm that the findings aligned with known patterns of gene regulation in haematological malignancies.

Ethical Considerations

The Biomedical Research Ethics Committee at Umm Al-Qura University approved the project concept and protocol (HAPO-02-K-012-2023-03-153). All data used in this study were obtained from publicly available sources (TCGA and GTEx), and no patient-identifying information was involved. As a result, this study did not require specific ethical approval or patient consent.

Statistical analysis

All statistical analyses were conducted using the GEPIA platforms (GEPIA, GEPIA2, and GEPIA2021). The platform's internal algorithms automatically generated the visualisation and statistical summaries, which provided F-values and *p*-values for each comparison.

The statistical analysis was conducted using one-way ANOVA to determine the significance of the differences in gene expression between tumour and normal samples. F-values and *p*-values were calculated for each immune cell type to assess the magnitude and significance of the observed differences. A *p*-value <0.05 was considered statistically significant.

RESULTS

Differential Expression of *RHCE* and *RHD* Genes in LAML Tumour and Normal Samples

The gene expression levels of *RHCE* and *RHD* were assessed in tumour and normal samples of patients with AML, measured in log2 (TPM+1) units (**Figure 1**). The data revealed a significant difference in the expression patterns of both genes between tumour and normal tissues. *RHCE* expression showed a marked downregulation in tumour samples compared to normal tissues. The mean expression in tumour samples was 1.26, whereas the levels in normal samples were significantly higher (>3.5-fold) (**Figure 1**). This suggests a potential role of *RHCE* in suppressing tumour activity or its involvement in tumorigenesis. In contrast, *RHD* expression displayed the opposite trend, where tumour tissues exhibited a considerably higher expression level (>2-fold) compared to normal samples.

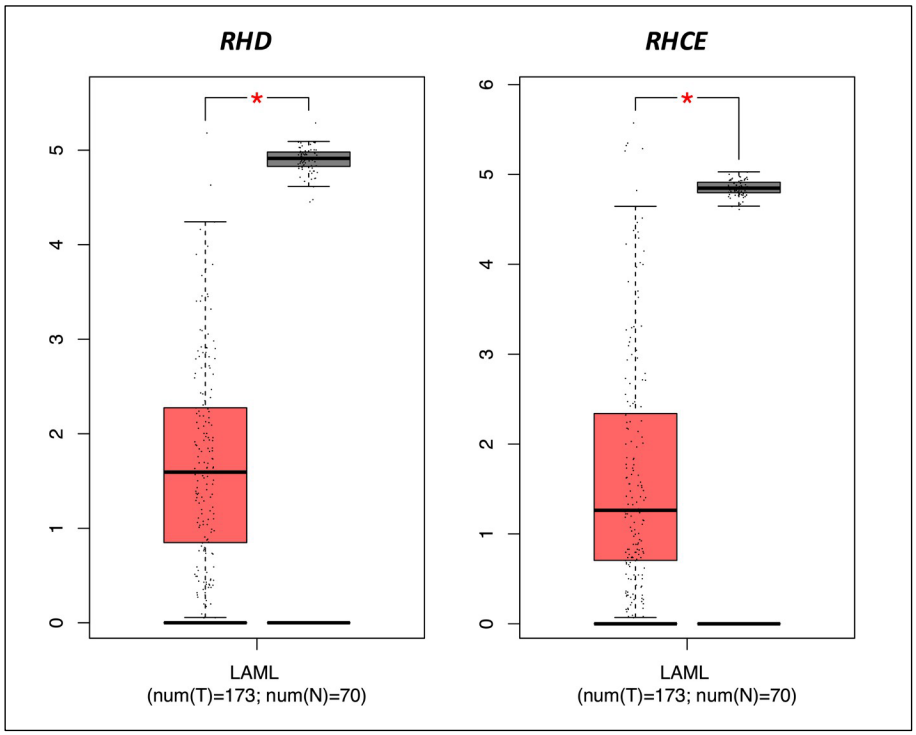

Figure 1. Boxplot of *RHD* and *RHCE* gene expression in tumour and normal AML samples. This figure shows the distribution of *RHD* and *RHCE* gene expression levels in tumour (T) and normal (N) samples from AML patients. The box represents the interquartile range, and the whiskers denote the range of non-outlier data points. Individual data points are shown as dots, indicating the variability within each group.

Survival Analysis of *RHCE* and *RHD* Expression in AML Patients

Survival analyses were performed to further explore the clinical relevance of *RHCE* and *RHD* expression in AML and evaluate the association between gene expression levels and patient overall survival (**Figure 2**). The survival analysis for *RHCE* expression divided patients into two groups based on high and low gene expression levels. The Kaplan-Meier survival curves demonstrated no significant difference between the two groups, as indicated by a log-rank *p*-value of 0.51. The hazard ratio (HR) for the high expression group was 0.83, with no statistically significant difference in overall survival between the high and low expression groups (p=0.51). The number of patients in both the high and low expression groups was 53 each. Similarly, survival analysis for *RHD* expression revealed no significant association between *RHD* expression levels and overall survival in AML patients.

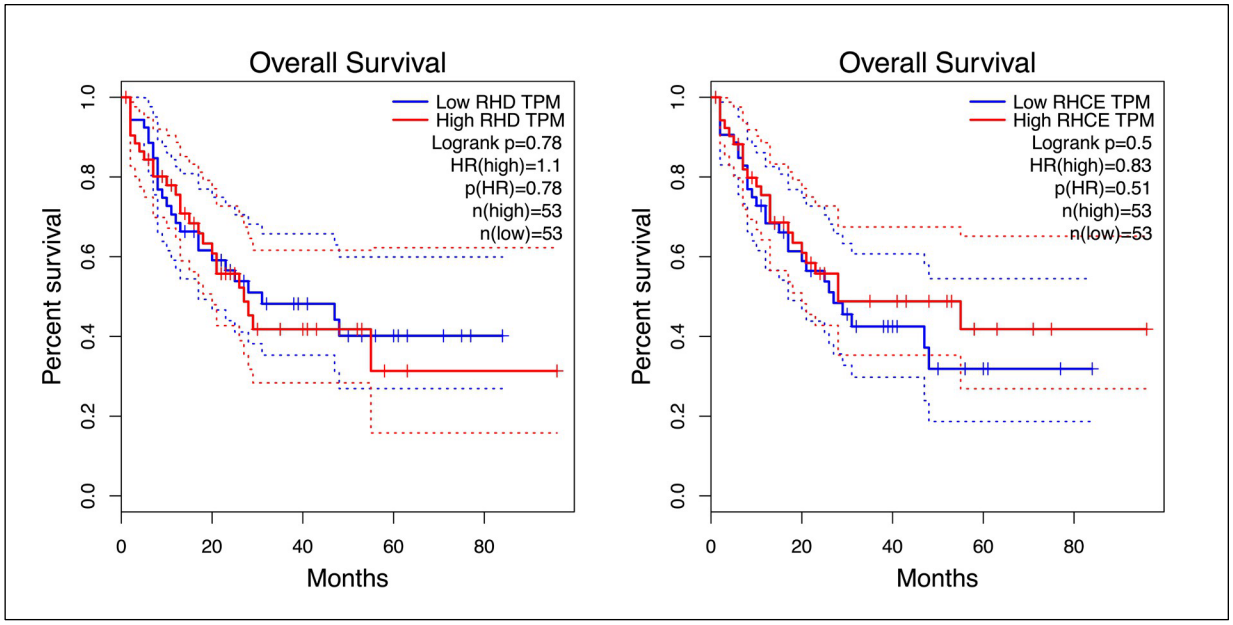

Figure 2. Kaplan-Meier survival curves for RHCE and RHD expression in AML patients. This figure illustrates the Kaplan-Meier survival analysis for AML patients grouped by high and low expression levels of *RHCE* and *RHD* genes. In both cases, there is no significant difference in overall survival between the high and low-expression groups. For *RHCE*, the log-rank *p*-value is 0.51 with a hazard ratio (HR) of 0.83, indicating a non-significant trend towards better survival in the high expression group. For *RHD*, the log-rank *p*-value is 0.78, with a hazard ratio of 1.1, showing no significant survival difference between the groups. Each group contained 53 patients.

Isoform Expression of *RHCE* and *RHD* Genes in AML Patients

The expression patterns of their isoforms were analysed to further investigate the complexity of *RHCE* and *RHD* gene expression in AML (**Figure 3**). Isoform expression profiles provide insights into the diversity of transcript usage and potential functional variation in cancer biology. Multiple isoforms of the *RHCE* gene were observed, each with varying expression levels across the AML samples. The most highly expressed isoforms included ENST00000527187.5 (*RHCE*-002) and ENST00000294413.11 (*RHCE*-001), showing relatively higher expression than the other isoforms. A total of 12 distinct isoforms were identified, with notable variation in their expression levels. This variation suggests a potential functional diversity of *RHCE* in AML tumour biology, although further functional studies are required to determine the specific roles of these isoforms. Similarly, the *RHD* gene displayed diverse isoform expression in AML patients. The most prominently expressed isoforms included ENST00000328664.8 (*RHD*-001) and ENST00000564398.5 (*RHD*-003). Ten distinct isoforms were identified, each contributing differently to the overall expression profile of the gene. The variability in isoform expression suggests that different *RHD* transcripts may play distinct roles in the pathogenesis of AML.

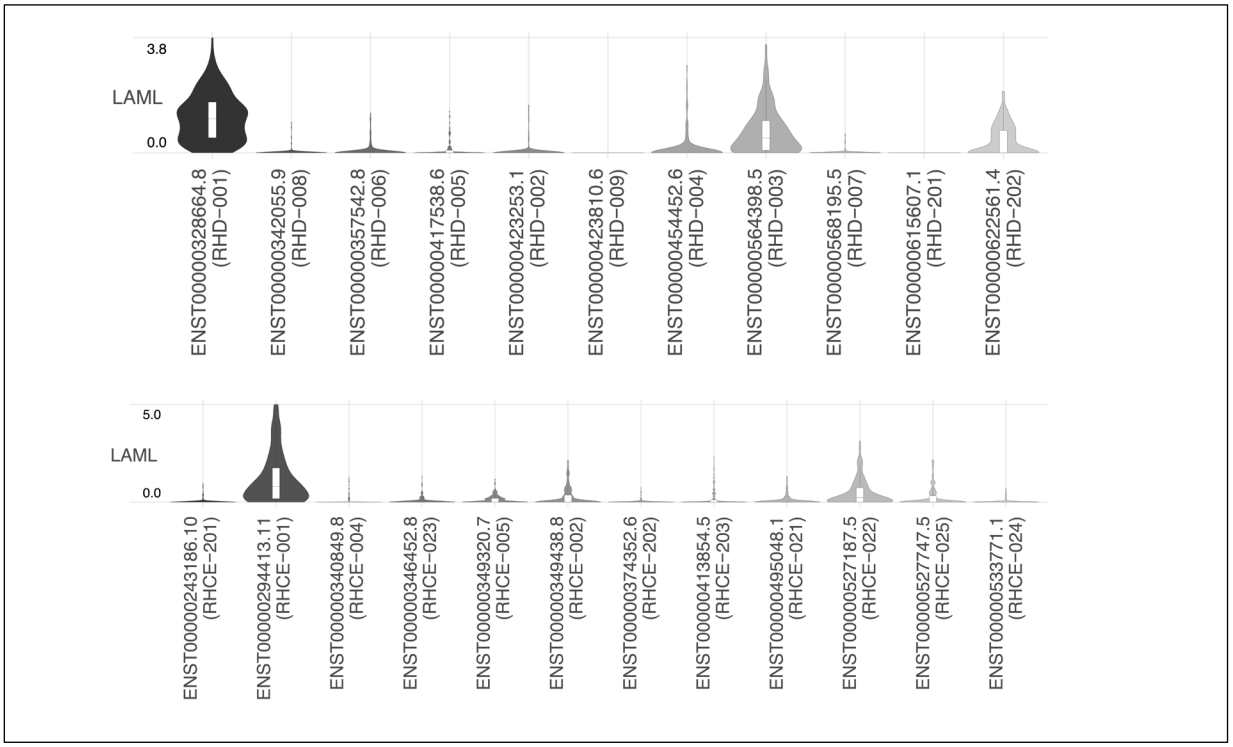

Figure 3. Isoform expression profiles of RHCE and RHD genes in AML patients. This figure displays the isoform expression patterns of *RHCE* and *RHD* genes in AML samples. For *RHCE*, a total of 12 isoforms were identified, with *RHCE*-001 and *RHCE*-022 being the most highly expressed. For *RHD*, 9 isoforms were detected, with *RHD*-001 and *RHD*-003 (showing the highest expression levels. The variation in isoform expression across both genes suggests potential functional diversity, which may influence AML pathogenesis. The x-axis represents the individual isoforms, and the y-axis indicates the expression level (TPM).

Correlation between *RHCE* and *RHD* Gene Expression in AML

Correlation analysis assessed the relationship between *RHCE* and *RHD* gene expression levels in AML samples (**Figure 4**). The gene expression values for both genes were log-transformed (log2 TPM), and their expression correlations were calculated (**Figure 4**). The analysis yielded a Pearson correlation coefficient (R) of 0.67, indicating a moderate positive correlation between the expression levels of *RHCE* and *RHD*. The *p*-value for this correlation was <0.05, demonstrating that the observed correlation is statistically significant. This suggests that higher expression of one gene is associated with higher expression of the other in AML samples. Furthermore, the scatter plot of log2-transformed TPM values for *RHCE* and *RHD* showed a clear upward trend, supporting the positive correlation. Moreover, the tight clustering of data points along this trend line further underscores the relationship between the expression profiles of both genes in the context of AML.

Differential Expression of *RHD* and *RHCE* in Various Immune Cell Types in AML and Blood Samples

The expression of *RHD* was analysed in various immune cell types in both LAML tumour samples and healthy blood samples (**Figure 5**). The expression levels are presented in log2(TPM+1) units, with the results summarised across the following immune cell populations. The expression of *RHD* was significantly higher (2-fold) in AML tumour samples compared to blood samples (F = 125.09, *p* = 1e-15). For M0 and M1 Macrophages, *RHD* expression was slightly elevated in tumour samples compared to blood, with a significant difference between them (F=65.73, *p*=5.55e-15; F=3.94, *p*=0.05; respectively). M2 Macrophages, however, had a notable increase in tumour samples (2-fold) compared to blood, with high statistical significance (F=401.56, *p*=1e-15). Naïve B and memory B cells exhibited elevated *RHD* expression in AML that was 3-fold (F=150.52, *p*=1e-15) and 2-fold (F=24.91, *p*=7.84e-7) significantly higher than

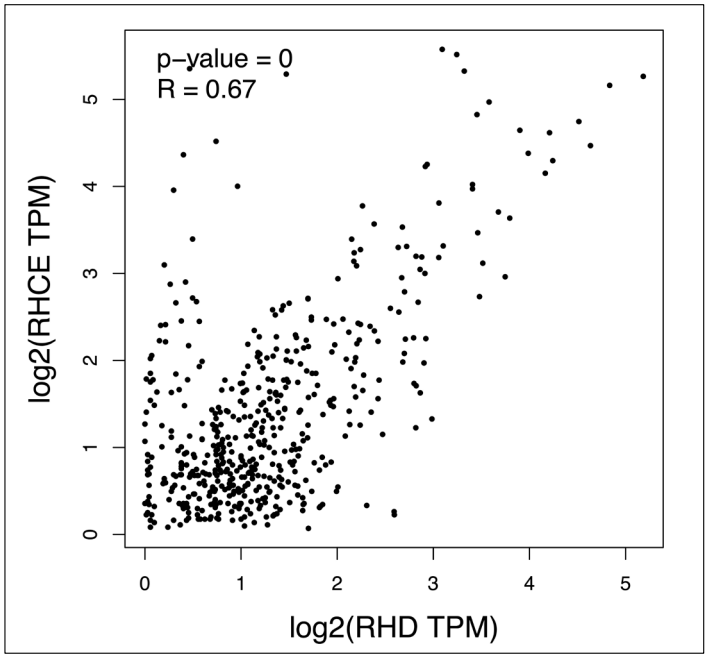

Figure 4. Correlation between *RHCE* and *RHD* gene expression in AML patients. This figure illustrates the scatter plot of log2-transformed TPM values for *RHCE* and *RHD* gene expression in AML samples. A moderate positive correlation is observed between the two genes, with a Pearson correlation coefficient (R) of 0.67 and a statistically significant *p*-value of <0.05. The data points show a clear upward trend, indicating that higher expression of *RHCE* is associated with higher expression of *RHD* in these samples.

blood. Plasma B cells also exhibited increased *RHD* expression in tumour samples (mean=1.0) compared to blood (mean=0.3), with statistical significance (F=139.42, *p*=1e-15). Whilst resting natural killer (NK) cells showed no significant difference in *RHD* expression between tumour and blood samples (F=0.80, *p*=0.37), activated NK cells displayed significantly increased expression in blood samples (2-fold) compared to tumour (F=13.90, *p*=2.11e-4). Furthermore, lymphoid derived CD8+ T cells did not significantly differ in *RHD* expression between tumour and blood samples (F=1.49, *p*=0.22). However, naïve CD4 T cells had 4-fold significantly higher expression in AML samples compared to blood (F= 28.08, *p*=1.62e-7). Both resting and activated CD4+memory T cells showed increased expression in tumour samples (mean = 0.6 and 0.8, respectively) compared to blood (mean=0.1 and 0.2, respectively), with high statistical significance (F=531.49, *p*=1e-15, and F=25.84, *p*=4.92e-7, respectively). Similarly, eosinophils and neutrophils displayed significantly higher expression of *RHD* in tumour samples (>3.5-fold and >3-fold, respectively) compared to blood.

The expression of *RHCE* was analysed in multiple immune cell types in AML tumours and healthy blood samples (**Figure 5**). The expression levels were reported in log2(TPM + 1), and the results highlight significant differences across immune cell populations. Although resting NK cells showed no significant difference in *RHCE* expression between tumour and blood samples (F=2.28, *p*=0.13), activated NK cells exhibited 2-fold significantly higher *RHCE* expression in blood samples than in tumour samples (F=12.08, *p*=5.47e-4). As with *RHD,* expression of *RHCE* was significantly higher in eosinophils and neutrophils in AML tumour samples (F=265.85, *p*=1e-15 and F=225.16, *p*=1e-15, respectively). Furthermore, monocytes displayed 2.5-fold significantly higher *RHCE* expression in AML tumour samples compared to blood samples (F=136.84, *p*=1e-15). Similarly, both M0 and M1 macrophages showed >2-fold pronounced and significant elevated *RHCE* expression in tumour *vs.* blood samples (F = 56.85, *p*=1.69e-13 and F=360.65, *p*=1e-15, respectively); however, M1 macrophages only marginally increased expression of the genes (F=3.80, *p*=0.05). A similar pattern to M1 macrophages was observed with resting dendritic cells, whereas *RHCE* expression was dramatically increased (5-fold) in activated dendritic cells in the tumour samples (F=46.39, *p*=2.31e-11). Resting mast cells had significantly higher *RHCE* expression in tumour samples (mean = 0.4) compared to blood (mean < 0.1), with a highly significant difference (F=442.42, *p*=1e-15). In contrast, activated mast cells showed a marginal difference between tumour and blood samples (F=3.85, *p*=0.05). Furthermore, naïve, memory and plasma B cells exhibited 2-fold significantly elevated *RHCE* expression in AML tumour samples compared to blood (F=145.16, *p*=1e-15; F= 27.36, *p*=2.31e-7; and F=132.31, *p*=1e-15; respectively). Although CD8+ T cells did not show any significant difference in *RHCE* expression between tumour and blood samples (F=0.03, *p*=0.86), in contrast, naïve, resting, and activated CD4+ T cells exhibited significantly higher *RHCE* expression in AML tumour samples compared to blood (F=27.54, *p*=2.12e-7; F=461.87, *p*=1e-15; F=17.19, *p*=3.85e-5; respectively).

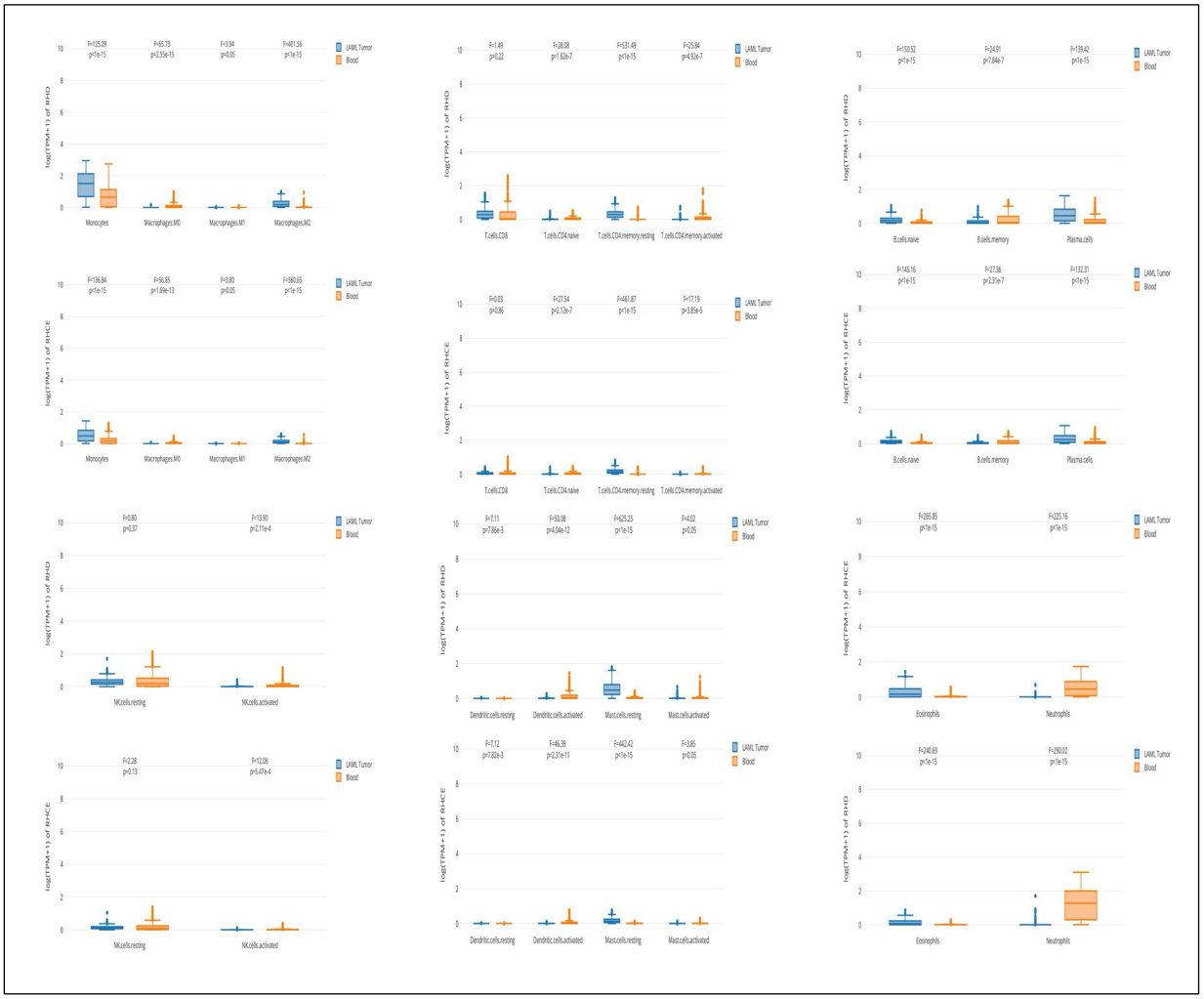

Figure 5. Differential expression of *RHD* and *RHCE* in various immune cell types in AML tumour and blood samples. This figure compares the expression levels of *RHD* and *RHCE* in multiple immune cell types between AML tumour samples (blue) and healthy blood samples (orange), measured in log2(TPM + 1). The plots highlight significant differences in expression across different immune cell populations, including Monocytes, Macrophages, T cells, B cells, and Plasma cells. In most cell types, both *RHD* and *RHCE* showed significantly higher expression in AML tumour samples, with notable exceptions such as Activated NK cells for *RHD* and *RHCE*, where expression was higher in blood. Statistical significance is indicated by F-values and *p*-values displayed above each plot.

DISCUSSION

Gene Expression Profiling of *RHD* and *RHCE* in AML

The current study utilised publicly available RNA-sequencing data from TCGA and GTEx databases[13,17] to investigate the differential expression of blood group genes[3], specifically *RHD* and *RHCE,* in AML and healthy blood samples. This analysis was performed using the GEPIA, GEPIA2, and GEPIA2021 platforms, providing robust and reproducible results that offer insights into the roles of these genes within the immune microenvironment of AML.

Differential Expression of *RHD* and *RHCE* in AML

The results revealed significant differences in the expression of *RHD* and *RHCE* between AML tumours and normal blood samples across various immune cell types. *RHD* was generally overexpressed in tumour samples, particularly in monocytes, macrophages, T cells, and B cells, indicating a potential role in AML tumourigenesis or immune evasion. Similarly, *RHCE* exhibited elevated expression in monocytes,

macrophages, and dendritic cells, highlighting its possible involvement in the modulation of the tumour immune microenvironment. The elevated expression of *RHD* in tumour samples could indicate its possible contribution to oncogenic processes in AML. These findings underscore the distinct expression profiles of *RHCE* and *RHD* in AML, suggesting a potential functional divergence between these genes in tumour progression or treatment and normal cellular processes[5,8,18]. Further functional studies are warranted to elucidate the biological implications of these expression differences.

These findings are consistent with previous reports that suggest *RHD* and *RHCE* play crucial roles in immune cell function, particularly in the context of haematological malignancies[5,9]. The elevated expression of these genes in tumour samples could indicate their involvement in the recruitment or activation of immune cells within the tumour microenvironment. Furthermore, the differential expression of *RHD* and *RHCE* in specific immune cell populations, such as T cells and macrophages, suggests that these genes may be differentially regulated depending on the immune cell subtype, which could have implications for targeted therapeutic approaches[19-21].

Immune Cell-Type Specific Patterns

The deconvolution of immune cell types in GEPIA2 and GEPIA2021 provided a deeper understanding of the immune landscape in AML. The results showed that activated NK cells displayed higher *RHCE* expression in healthy blood samples compared to AML samples, suggesting that *RHCE* expression may be suppressed in these cells during tumour progression[22,23]. Conversely, the significantly higher expression of *RHD* in AML tumour-associated immune cells, such as monocytes and T cells, points to a potential role in tumour-associated immune dysregulation[20,24].

Interestingly, plasma and naïve B cells exhibited distinct expression patterns of both genes in AML, potentially reflecting the involvement of *RHD* and *RHCE* in antibody production or B cell maturation within the tumour microenvironment[25,26]. The differential expression of these genes across various immune cell types underscores their complexity and highlights the need for further functional studies to clarify their roles in AML progression.

Biological Implications and Future Directions

The overexpression of *RHD* and *RHCE* in specific immune cell types in AML suggests that these genes may contribute to the immune escape mechanisms employed by the tumour[23]. By modulating immune cell activation, these genes could facilitate tumour growth and survival by creating an immunosuppressive environment[27]. Given the significant role of the immune system in controlling cancer progression[2,8,16,19,25,27], understanding how *RHD* and *RHCE* contribute to immune regulation in AML is critical for identifying new therapeutic targets.

Future studies should focus on further characterising the biological functions of these genes in AML. In particular, functional assays examining how *RHD* and *RHCE* influence immune cell activation, cytokine production, and tumour immune evasion would provide valuable insights into the underlying mechanisms that define their function in leukaemia. Moreover, developing targeted therapies to modulate *RHD* or RHCE expression in specific immune cell types could represent a novel strategy for improving immune response in AML patients.

Limitations

While this study provides important insights into the differential expression of *RHD* and *RHCE* in AML, several limitations should be acknowledged. First, the analysis relied solely on transcriptomic data from public databases, which may not capture the full complexity of gene regulation at the protein level. Further studies utilising proteomics data would be beneficial in validating the findings observed at the mRNA level. Additionally, while immune cell-type deconvolution estimates immune cell populations, single-cell RNA sequencing could offer more precise information regarding the specific cell types expressing these genes in AML.

Another limitation is the lack of longitudinal data, which prevents the understanding of how *RHD* and *RHCE* expression may change over the course of disease progression or in response to treatment. Future studies incorporating time-course data could help elucidate the dynamic nature of these genes in AML and their potential as biomarkers for disease monitoring.

CONCLUSIONS

This study provides valuable insights into the differential expression of *RHD* and *RHCE* in various immune cell types in AML using data from the TCGA and GTEx databases, analysed through the GEPIA, GEPIA2, and GEPIA2021 platforms. These established RNA-sequencing analysis tools allowed for robust comparisons between AML samples and normal blood samples, highlighting the complex roles of *RHD* and *RHCE* in the immune landscape of AML.

The findings revealed significant overexpression of *RHD* and *RHCE* in AML tumour-associated immune cells, particularly in monocytes, macrophages, T cells, and B cells, suggesting that these genes may be involved in modulating the immune microenvironment to support tumour survival and progression. The differential expression observed in NK cells, with higher expression of *RHCE* in normal blood samples, further suggests context-dependent regulation of these genes in different immune cell types, potentially influencing immune surveillance mechanisms.

The study results emphasise the importance of *RHD* and *RHCE* in shaping the tumour-immune interactions in AML. These genes could be potential biomarkers for disease progression or immune dysfunction within the tumour microenvironment. The observed expression patterns warrant further investigation into the functional roles of *RHD* and *RHCE* in immune cell activation and tumour immunology, to uncover novel therapeutic targets that could modulate these pathways to enhance anti-tumour immunity.

Moreover, this study underscores the utility of the GEPIA platforms in facilitating large-scale transcriptomic analysis and enabling the exploration of cancer-immune interactions. The ability to integrate data from multiple publicly available datasets and perform immune cell deconvolution strengthens the validity of the findings and provides a foundation for future research into gene expression profiles in haematological malignancies.

While this study has contributed to understanding the roles of *RHD* and *RHCE* in AML, further research is needed to validate these findings at the protein level and elucidate the functional consequences of gene expression changes. Single-cell RNA sequencing and proteomic analyses would offer greater granularity and confirm the specific immune cell populations driving the observed expression patterns. Additionally, longitudinal studies could provide insights into the dynamic expression of *RHD* and *RHCE* during disease progression and in response to therapeutic interventions.

In conclusion, this study has highlighted the potential significance of *RHD* and *RHCE* in the immune microenvironment of AML. By leveraging publicly available transcriptomic data and the analytical power of the GEPIA platforms, we have uncovered critical gene expression patterns that may influence tumour development and immune responses. Future work on these findings will help to advance our understanding of these genes in cancer biology and explore their potential as targets for therapeutic intervention.

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Author Contributions:

SK and AA are responsible for the conception, design, data collection, analysis, and interpretation of the study. They drafted the manuscript and approved the final version for submission. They also carried out all aspects of this work independently.

Conflict of Interest:

The authors listed in this manuscript certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria, educational grants, participation in speakers' bureaus, membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data Availability:

Details regarding the availability of data and its sources (TCGA and GTEx) are explicitly provided in the manuscript.

ETHICAL APPROVAL:

This study was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration; also, it was conducted in compliance with all the applicable institutional ethical guidelines for care and welfare. Additionally, the Biomedical Research Ethics Committee at Umm Al-Qura University approved the project idea and protocol (HAPO-02-K-012-2023-03-153).

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REFERENCES

1. Shimony S, Stahl M, Stone RM. Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. Am J Hematol 2023; 98: 502-526.
2. Khaldoyanidi S, Nagorsen D, Stein A, Ossenkoppele G, Subklewe M. Immune Biology of Acute Myeloid Leukemia: Implications for Immunotherapy. J Clin Oncol 2021; 39: 419-432.
3. Kabrah S, Alsaegh A, Almahroqi F, Flemban A, Mujalli A, Obaid AA, Halawani AJ, Bawazir W, Altayar MA, Almotiri A, Ar-baeen AF. Validation of ABO Gene Expression in Normal Tissues and Acute Myeloid Leukaemia Using In-Silico Approaches. J Umm Al-Qura Univ Med Sci 2024; 19: 1132-1142.
4. Rosenkrans D, Zubair M, Doyal A. Rh Blood Group System. Internet: StatPearls, 2024.
5. Flegel WA. Molecular genetics of RH and its clinical application. Transfus Clin Biol 2006; 13: 4-12.
6. Sendker S, Reinhardt D, Niktoreh N. Redirecting the Immune Microenvironment in Acute Myeloid Leukemia. Cancers (Basel) 2021; 13: 1423.
7. Yu S, Jiang J. Immune infiltration-related genes regulate the progression of AML by invading the bone marrow microenvironment. Front Immunol 2024; 15: 1409945.
8. Menter T, Tzankov A. Tumor Microenvironment in Acute Myeloid Leukemia: Adjusting Niches. Front Immunol 2022; 13: 811144.
9. Madgett TE, Tounsi WA, Halawani AJ, Avent ND. RHD molecular analysis—from discovery to next generation sequencing. Ann Blood 2023; 8: 36.
10. Floch A, Siegel DL, Westhoff CM. Rh and LW blood group antigens. In T. L. Simon & E. A. Gehrie (Eds.), Rossi's Principles of Transfusion Medicine - 6ª ed. Wiley-Blackwell 2022; pp.100-108
11. Marando L, Huntly BJP. Molecular Landscape of Acute Myeloid Leukemia: Prognostic and Therapeutic Implications. Curr Oncol Rep 2020; 22: 61.
12. Stanfill AG, Cao X. Enhancing research through the use of the genotype-tissue expression (GTEx) database. Biol Res Nurs 2021; 23: 533-540.
13. Albadrani HM, Hamed M, Zakariyah A, Binkheder S, Kabrah SM, Flemban AF. Differential expression of ABO in normal and tumor tissues: Implications for cancer biology and prognosis. J Taibah Univ Med Sci 2024; 19: 1132-1142.
14. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019; 47: W556-W560.
15. Li C, Tang Z, Zhang W, Ye Z, Liu F. GEPIA2021: integrating multiple deconvolution-based analysis into GEPIA. Nucleic Acids Res 2021; 49: W242-W246.
16. Taghiloo S, Asgarian-Omran H. Immune evasion mechanisms in acute myeloid leukemia: A focus on immune checkpoint pathways. Crit Rev Oncol Hematol 2021; 157: 103164.
17. Giordano TJ. The cancer genome atlas research network: a sight to behold. Endocr Pathol 2014; 25: 362-365.
18. Chen W, Liang W, He Y, Liu C, Chen H, Lv P, Yao Y, Zhou H. Immune microenvironment-related gene mapping predicts immunochemotherapy response and prognosis in diffuse large B-cell lymphoma. Med Oncol 2022; 39: 44.
19. Verneau J, Sautés-Fridman C, Sun CM. Dendritic cells in the tumor microenvironment: prognostic and theranostic impact. Semin Immunol 2020; 48: 101410.
20. Chandra DJ, Alber B, Saultz JN. The Immune Resistance Signature of Acute Myeloid Leukemia and Current Immunotherapy Strategies. Cancers (Basel) 2024; 16: 2615.
21. Ulu BU, Başcı S, Bakırtaş M, Yiğenoğlu TN, Batgi H, Yıldız J, Darçın T, Şahin D, Baysal NA, İskender D, Çakar MK, Dal MS, Altuntaş F. Could blood groups have prognostic significance on survival in patients with diffuse large B cell lymphoma? Leuk Res 2022; 115: 106810.
22. Guillerey C. NK Cells in the Tumor Microenvironment. Adv Exp Med Biol 2020; 1273: 69-90.
23. Lion E, Willemen Y, Berneman ZN, Van Tendeloo VF, Smits EL. Natural killer cell immune escape in acute myeloid leukemia. Leukemia 2012; 26: 2019-2026.
24. Miari KE, Guzman ML, Wheadon H, Williams MTS. Macrophages in Acute Myeloid Leukaemia: Significant Players in Therapy Resistance and Patient Outcomes. Front Cell Dev Biol 2021; 9: 692800.

25. Cencini E, Fabbri A, Sicuranza A, Gozzetti A, Bocchia M. The Role of Tumor-Associated Macrophages in Hematologic Malignancies. Cancers (Basel) 2021; 13: 3597.
26. Roex G, Feys T, Beguin Y, Kerre T, Poire X, Lewalle P, Vandenberghe P, Bron D, Anguille S. Chimeric Antigen Receptor-T-Cell Therapy for B-Cell Hematological Malignancies: An Update of the Pivotal Clinical Trial Data. Pharmaceutics 2020; 12: 194.
27. Hino C, Pham B, Park D, Yang C, Nguyen MHK, Kaur S, Reeves ME, Xu Y, Nishino K, Pu L, Kwon SM, Zhong JF, Zhang KK, Xie L, Chong EG, Chen CS, Nguyen V, Castillo DR, Cao H. Targeting the Tumor Microenvironment in Acute Myeloid Leukemia: The Future of Immunotherapy and Natural Products. Biomedicines 2022; 10: 1410.