Exploration of the Correlation Between GRHL1 Expression and Tumor Microenvironment in Endometrial Cancer and Immunotherapy

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Introduction: GRHL1 belongs to the family of Grainyhead-like (GRHL). Previous studies have shown that dysregulation of growth and survival pathways is associated with the GRHL family of gene cancers. Immunotherapy with checkpoint inhibitors has changed the treatment paradigm for many tumors, including endometrial cancer (EC). However, the effect of GRHL1 on immunotherapy in EC and its relationship with immune cell infiltration are poorly understood.

Methods: Differential expression of GRHL1 between EC and normal EC tissues was analyzed by searching the TCGA database, and the results were verified utilizing immunohistochemistry analyses. Next, the relationship between GRHL1, CD8+ T cells and tumor microenvironment (TME) was also investigated, and the effect of GRHL1 expression on immunotherapy in EC was evaluated.

Results: According to the findings, EC tissues had elevated expression levels of GRHL1 relative to normal tissues. Patients with EC who expressed GRHL1 at high levels experienced worse overall survival (OS) and Progression-free survival (PFS) than those whose expression was lower. In addition, GRHL1 expression was negatively correlated with CD8+ T cells, and patients with high GRHL1 expression were less effective in receiving immunotherapy.

Conclusion: The expression of GRHL1 was high in EC patients, and high expression of GRHL1 inhibits the proliferation of CD8+ T cells in the tumor microenvironment of EC and affect the efficacy of immunotherapy.

Keywords: GRHL1, endometrial cancer, tumor microenvironment, CD8+ T cells, immunotherapy

Introduction

Endometrial cancer (EC) is the sixth most common neoplasm in women worldwide. In the US, the mortality rate for people of the white race was 4.3–4.5 per 100,000 individuals, while this value for people of the black race was 8.2–8.9 per 100,000 population. In China, the mortality rate for people of the yellow race was 2.5 per 100,000 individuals. Even though high-grade EC often has a positive prognosis, it tends to recur. Because recurrent EC has such a dismal prognosis, preventing it is crucial. Currently, traditional surgery and minimally invasive surgery are the two most crucial treatment options for EC.

The GRHL family of genes has been implicated in the development of many cancers, and is involved in the regulation of embryogenesis, and growth and survival pathways in cancer, where it leads to dysfunction through dedifferentiation or loss of functional integrity. As a member of the GRHL family, GRHL1 is mainly associated with neuroblastoma and esophageal cancer.

In this study, the possible involvement of GRHL1 in EC and its expression in TME were validated. Using the TCGA database and in vitro tests, we discovered that GRHL1 expression was considerably upregulated in EC tissues. We also
explored the level of GRHL1 expression and its relationship with prognosis. In addition, the correlation of GRHL1 with the tumor microenvironment and CD8+ T cells was analyzed, and the effect of GRHL1 expression on EC immunotherapy was evaluated.

**Materials and Methods**

**Dataset Source and Pre-Processing**

The TCGA database was searched to retrieve clinical information on patients with uterine corpus endometrial carcinoma (UCEC), covering information on common gene expression, total mortality, and prognosis. TCGA pan-cancer tumor mutation burden (TMB) and microsatellite instability (MSI) data downloaded from the UCLA Xena Data Portal (https://xenabrowser.net/) and then scrutinized utilizing the R package TCGAbiolink. The FPKM values were converted to transcript per kilobase million values. To account for the non-biological technical bias-related batch effect, the “ComBat” method from the sva package was implemented. R (v 4.1.2) and the R Bioconductor package were applied to analyze all of the data.

**Clinical Sample Collection**

Patients undergoing EC surgery at the First Affiliated Hospital of Bengbu Medical College, Department of Oncology Gynecology, from January 2021 to December 2021, were recruited for this research, and samples were obtained from those patients. Immunohistochemistry (IHC) labeling was performed on 66 EC tissue specimens and 10 surrounding normal tissues. Before or after surgery, no patient had chemotherapy, radiation, or biological treatment, and there was no history of EC in any of the patients. Tissue samples collected after surgery were preserved in a refrigerator at −80 degrees Celsius until protein extraction.

**Experimental Materials**

CUSABIO (CSB-PA868368LA01HU) provided rabbit anti-human antibody GRHL1 (50μL, WUHAN, CH). Primary antibodies against actin and alpha rabbit monoclonal antibodies against CD8+ T cells were purchased from Cell Signaling Technology Inc. (Danvers, MA, United States). Jackson ImmunoResearch Inc. provided an anti-rabbit antibody coupled to horseradish peroxidase (HRP, West Grove, PA, US). Sigma-Aldrich provided bovine serum albumin (BSA, St. Louis, MO, United States). Skim milk and Tween-20 were supplied by Sangon Biotech Co., Ltd (Shanghai, China).

**Immunohistochemistry (IHC)**

Paraformaldehyde (4%) (PFA) was employed to fix all tissue samples, followed by embedding them in paraffin, cutting them into sections, and attaching them on slides. Extraction of antigens was performed by soaking the slides in citrate buffer (pH 7.8, 0.1M) for 24 mins at about 82°C after they had been deparaffinized, rehydrated, and exposed to xylene density gradients. To inhibit peroxidase activity, slides were coated uniformly with an endogenous blocking solution for 15 min at room temperature (RT). Overnight, slides were treated with anti-GRHL1 primary antibody, then rinsed gently in PBS. Following a 10-minute incubation at RT with a biotin-conjugated secondary antibody, the samples were treated with streptavidin peroxidase for 5 mins. Next, the slides were rinsed with hematoxylin dye to eliminate the remaining debris. After the slides had been dried and washed, an IHC examination was performed. Sections were scored by two pathologists with extensive experience in double-blind reading. To determine the extent of staining, a quantity score (0–4) denoted 0, 0%; 1, 1–10%; 2, 11–50%; 3, 51–80% and 4, 81–100% of positive cells, was used. The staining intensity was divided into three grades: weak, moderate, and strong staining. It should be noted that the corresponding intensity scores ranged from 1 to 3. The final IHC score was calculated by multiplying the quantity and intensity scores.

**Assessment of the Immune Characteristics of the EC Tumor Microenvironment**

Immunological features of TME in EC encompass the immunomodulator expression, the cancer immune cycle activity, TIIC infiltration levels, and suppressive immune checkpoint expression. Information on 48 immunomodulators was collected, including chemokines and their receptors. To examine the link between GRHL1 expression and the
infiltration levels of immune cells, we employed the Tumour Immune Estimation Resource (TIMER) and CIBERSORT algorithm to investigate how GRHL1 expression relates to immune cell activity. When the p-value was <0.05, it was considered to be significant. To avoid errors, correlations between GRHL1 and CD8+ T cells were calculated based on 7 different algorithms: XCELL, QUANTISEQ, CIBERSORT–ABS, CIBERSORT, MCP–COUNTER, TIMER and EPIC algorithms.

Immune Response Analysis
The Cancer Genome Atlas (TCGA) and other sources of next-generation sequencing (NGS) data for 20 solid tumors are provided with an extensive immunogenomic analysis by the Cancer Immunome Database (TCIA) (https://www.tcia.at/home). Using the ggpubr R program, the immunological phenomenon scores (IPS) of 560 EC patients from this database were used to assess immunotherapy.

Statistical Analysis
R software (version 4.1.2) and Perl (version 5.32.1.1) were utilized for data analysis. P <0.05 indicated the significance level.

Results
Expression of GRHL1 in Endometrial Cancer and Adjacent Normal Tissues and Its Prognostic Assessment in Endometrial Cancer
In the TCGA cohort, the GRHL1 mRNA expression level was found to be elevated in EC tissues compared to nearby normal tissues (p < 0.001, Figure 1A). The comparison of the mRNA expression of GRHL1 in 35 pairs of EC and adjacent normal tissues in the TCGA database showed that GRHL1 expression was higher in EC tissues than in adjacent normal tissues (p < 0.05, Figure 1B). IHC staining tests confirmed this finding at the tissue level (Figure 1C). KM analysis of survival of patients exhibiting varying GRHL1 expression levels based on TCGA-derived data was performed to evaluate the prognostic significance of GRHL1 in EC. According to the results, the 10-year OS was substantially higher in patients whose GRHL1 expression was lower as compared to those whose GRHL1 expression was higher (p =0.035, Figure 1D). We also discovered that GRHL1 overexpression was a poor predictor of EC PFS (p = 0.042, Figure 1E). Overall, remarkably higher expression levels of GRHL1 were found in EC, in comparison to nearby normal tissues. GRHL1 upregulation was associated with a shorter OS, and PFS for patients.

Clinicopathological and Predictive Value of GRHL1 Expression in EC Patients
Using box plots and heatmaps, we revealed that GRHL1 expression levels were correlated with clinicopathological parameters of EC patients (Figure 2A–E). The results showed that the expression level of GRHL1 did not correlate well with the clinicopathologic stage. Patients’ clinical stage, grade, and age were shown to have a substantial association with their OS, and GRHL1 was found to have statistically significant predictive value for EC patients, as shown in a univariate analysis (p = 0.044, Figure 2F). In addition, multivariate analysis demonstrated that clinical stage, grade, and age were substantially linked to EC patients’ OS, but the prognostic value of GRHL1 for EC patients was found to be insignificant (p = 0.165, Figure 2G).

GRHL1 Inhibits CD8+ T Cell Proliferation and is Associated with Immune Escape in EC
Herein, we assessed the correlation of chemokines and their receptors with GRHL1 expression (Figure 3A and B). We found that CCL2, CCL4, CCL5, CXCL9, CXCL10, CXCR3, and CCR5 were negatively correlated with the expression of GRHL1. Chemokines that bind to CXCR3 (such as CXCL9 and CXCL10) are essential and necessary for the trafficking of activated CD8+ T cells to tumor sites. Subsequently, we used the TCGA-derived data to calculate the TME scores using the EC expressions and the “estimate” package included R. Furthermore, we plotted TME differential analysis. The ImmuneScore, StromalScore, and ESTIMATEScore of TME were downregulated in the high GRHL1 expression group (Figure 3C).
The CIBERSORT algorithm was used to verify the correlation between \textit{GRHL1} and immune cells (Figure 4). The abundance of 22 different types of immune cells was measured and compared between the two groups (Figure 4A). \textit{GRHL1} was negatively linked to CD8+ T cells, T cells regulatory (Tregs), NK cells activated, and Mast cells resting, and positively correlated with Neutrophils, T cells CD4 memory resting, Dendritic cells activated, B cells naive, and Mast cells activated (Figure 4B–K). In previous studies, TME has been categorized into two subtypes as follows: an inflammatory TME dominated by T-cell infiltration and a non-inflammatory TME dominated by T-cell suppression.
Tumors with T-cell inflammation contain abundant CD8+ T cells. Tumors without T-cell inflammation lack these cells but contain blood vessels, fibroblasts, and macrophages, which support tumor growth.  

Next, we showed GRHL1 correlation with CD8+ T cells based on ssGSEA, MCPcounter, TIMER, ESTIMATE, CIBERSORT, XCELL, and EPIC algorithms (Figure 5A–H). Among the six algorithms, GRHL1 correlated negatively with CD8+ T cells (Figure 5B–G). TME cell infiltration is characterized by three types: immunoinflammatory phenotype (immunoinflammatory), immune exclusion phenotype (immune), and immune desert. The immune exclusion and the immune desert phenotypes are also known as non-immune inflammatory phenotypes.  

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Figure 2 Association of clinicopathological characteristics with GRHL1 expression levels. (A–D) The association of GRHL1 expression level with the clinicopathological features of individuals with EC for age (A), weight (B), Grade (C), and stage (D). (E) Heatmap illustrating the link between the clinicopathological characteristics of EC patients and the GRHL1 expression. (F and G) Forest plots show the results of univariate (F) and multivariate (G) Cox regression analyses for the OS of patients with EC.
immune microenvironment shaped by GRHL1 as an immunodeficient phenotype (immune exclusion phenotype), that is, a non-immune inflammatory phenotype.

Analyses of Immune Cell Infiltration in High- and Low-GRHL1-Expression Groups
Since GRHL1 was negatively correlated with CD8+ T cells, we investigated its potential role in the tumor immune microenvironment (TIME) of EC. IHC staining was performed to compare the tissue samples with low (+) and high (+++) GRHL1 expression.
expression levels (Figure 1C). To determine whether there was a link between high GRHL1 expression and CD8+ T cells in TIME, we used IHC labeling to measure the infiltration of CD8+ T cells into EC and the surrounding tissues. CD8+ T cells in EC tissues with high (+++) GRHL1 levels were particularly clustered in the peripheral tumor stroma, with just a small proportion of immune cells infiltrating the stroma to reach the tumor’s parenchyma (Figure 6A). Conversely, a greater number of immune cells infiltrated the stroma of the tumor parenchyma, and fewer CD8+ T cells were clustered throughout the tumor parenchyma in EC tissues with low (+) GRHL1 levels (Figure 6B). In summary, high expression of GRHL1 inhibited the proliferation of CD8+ T cells in the TIME of EC, as shown by the analysis.

Correlation Analysis of GRHL1 Expression with TMB and MSI and Evaluation of Immunotherapy

After exploring immune phenomenon scores (IPS) through TCIA,28 it was observed that patients with high GRHL1 expression were less effective in receiving immunotherapy (Figure 7A–D, p < 0.05). To exclude the influence of confounding factors on immunotherapy efficacy, we also assessed the correlation between TMB/MSI and GRHL1 expression. (Figure 7E and F, p > 0.05).
Endometrial cancer (EC) is among the three most prevalent gynecological malignancies. It has now become the most common gynecologic cancer in developed countries. Although the majority of patients are diagnosed at an early stage and have a favorable prognosis, there are still some patients with advanced stage at the time of initial diagnosis or...
recurrence and metastasis after treatment, and the 5-year survival rate is only 20–26%. The success of tumor immunotherapy has shown that immunotherapy plays a landmark role in cancer treatment. In recent years, treatment guidelines for EC have been updated to include targeted treatments such as immune checkpoint inhibitors.

Homologous sequencing has led to the identification of 3 GRHL genes in the human genome to date. Recent years have seen a surge in the publication of multiple studies on GRHL and cancer, and these findings provide compelling evidence that GRHL is strongly associated with cancer. GRHL3 is closely associated with epithelial cancers, including skin, breast, and head and neck cancers. GRHL2 acts as an oncogene to suppress epithelial-to-mesenchymal transition (EMT). In recent years GRHL1 has also been found to be closely associated with skin cancer, esophageal cancer and neuroblastoma.

We discovered that GRHL1 expression was upregulated in EC tissues in comparison to non-EC tissues. GRHL1 upregulation was associated with a shorter OS, and PFS for patients. Based on these results, GRHL1 could be a potential target gene for EC. It has been shown that immune infiltration and immune escape of tumors correlate with both the prognosis of cancer and the patient’s response to treatment. The majority of tumor cells express antigens that may facilitate the identification of the tumor by the host CD8+ T cells. The TME allows for the categorization of immune escape into two distinct subtypes. One of the primary subsets has a phenotype that is indicative of T cell inflammation. These tumors can resist immune

![Figure 6 Immune cell infiltration in the groups with high and low GRHL1 expression. (A) CD8+ T cell (a and b) infiltration in the group with high GRHL1 expression (c and d). (B) CD8+ T cell (e and f) infiltration in the group with low GRHL1 expression (g, h).](https://doi.org/10.2147/PGPM.S453061)
attack through the dominant inhibitory effects of immune system-suppressive pathways. The other group does not exhibit this T cell inflammation profile and hence is resistant to immunological assault due to being excluded or ignored by the immune system.

Standardized cancer immunotherapy requires that a target molecule exhibit TME-specific upregulation and immunosuppression action. To determine the potential role of \textit{GRHL1} in EC and its expression in TME, we explored the

**Figure 7** Correlation analysis of \textit{GRHL1} expression with TMB and MSI and evaluation of immunotherapy. (A–D) Association of IPS with the \textit{GRHL1} expression in individuals with EC based on the TCIA database: CTLA4–PD1– (A), CTLA4–PD1+ (B), CTLA4+PD1– (C), CTLA4+PD1+ (D). (E and F) Correlation analysis of \textit{GRHL1} expression with TMB (E) and MSI (F). (*p < 0.05; **p < 0.01; ***p < 0.001).
EC cohort from the transcriptome of specimens from the TCGA database dataset, and the results showed that EC expression was significantly overexpressed in tumor tissues. Additional research using IHC validated these findings. Our data also demonstrated a negative association between GRHL1 and the immunological states of TME in EC. Using seven different algorithms, we found that in the high GRHL1 group, T cell recruitment activity was remarkably downregulated, GRHL1 was negatively linked to CD8+ T cell activation, and the amount of TIIC infiltration was substantially decreased. These results suggest that high expression of GRHL1 in EC may inhibit the proliferation of CD8+ T cells, thereby affecting the efficacy of immunotherapy. Subsequent IHC experiments also verified this result. Therefore, targeting GRHL1 in EC may improve the success of immunotherapy. Previous studies have shown that about 30% of endometrial cancers have mismatch repair defects, which increase the adverse effects of drug therapy, while the defects increase the likelihood of a positive response to immune checkpoint inhibitors. And there is growing evidence that tumor mutational load is a promising predictor of immune checkpoint inhibitor therapy. To rule out the influence of the confounding factors TMB and MSI on the efficacy of immunotherapy, we assessed the correlation between TMB/MSI and GRHL1 expression. We found that there was no significant correlation between GRHL1 expression level and TMB/MSI in endometrial cancer. This result also verified our conclusion from other aspects.

Some limitations remain in this study. The TCGA data served as the foundation for our investigation of GRHL1’s involvement in EC. Despite our success in using IHC assays to verify the link between GRHL1 and immune cells infiltrating TME, the absence of equivalent confirmation in cellular and animal trials suggests where we should focus our future efforts. Second, the effect of GRHL1 on patient immunotherapy was derived from an analysis of the TCGA database and lacks direct evidence. Third, although we analyzed a direct link between GRHL1 expression and MSI, we did not stratify the mismatch repair status of the tumor on consecutive samples and TCGA data, and therefore could not obtain GRHL1 results for the mismatch repair good and mismatch repair bad subgroups, which is a direction for our future work.

Conclusion
Our results show that GRHL1 is highly expressed in EC tissues compared to normal samples and can be characterized as a specific biomarker to distinguish EC tissues from normal endometrial tissue. Unfavourable OS, and PFS were all highly correlated with GRHL1 upregulation in EC patients. Immune cell infiltration analysis showed that high expression of GRHL1 inhibited the proliferation of CD8+ T cells and affected the efficacy of immunotherapy. Our thorough analysis of GRHL1’s potential as an immune target for EC may offer a useful assessment system for clinical use.

Abbreviations
EC, endometrial cancer; GRHL1, Grainyhead like transcription factor 1; TCGA, The Cancer Genome Atlas; OS, overall survival; GSEA, Gene set enrichment analysis; IHC, Immunohistochemistry; RT, room temperature; DCs, resting dendritic cells; KM, Kaplan-Meier; FDR, false discovery rate; TME, tumor microenvironment; TIME, Tumor immune microenvironment; TMB, tumor mutation burden; ICI, Immune checkpoint inhibitor; ICB, immune checkpoint blockade; MSigDB, Molecular Signatures Database; FDRs, false discovery rates; TIMER, Tumour Immune Estimation Resource; TCIA, The Cancer Immunome Database; IPS, immune phenomenon scores.

Data Sharing Statement
The following online resources were screened based on this study’s analysis of used clinical data: TCGA (https://www.cancer.gov/), TCIA (https://tcia.at/).

Ethics Approval and Informed Consent
The study involving human participants has been reviewed and approved by the First Affiliated Hospital of Bengbu Medical College’s [2021] 143 ethics committee. The Helsinki Declaration, which outlines moral guidelines for medical research with human participants, was followed in conducting the study. Patients/participants signed a consent form to indicate their agreement to be included in the study with each patient providing their informed consent.
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Author Contributions
All authors contributed significantly to the research that was published, whether it was in the conceptualization, research design, implementation, data gathering, analysis, and interpretation, or each of these areas separately; participated in the report’s drafting, revision, or detailed evaluation; approved the final version for publishing; decided upon the journal to which the manuscript has been submitted; and accept responsibility for all aspects of the project.

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Disclosure
The authors report no conflicts of interest in this work.

References