Prognostic Value of MUC1 and its Correlation with Tumor-Infiltrating Immune Cells in Breast Cancer

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Abstract

Background: MUC1 is a transmembrane glycoprotein, aberrantly glycosylated and overexpressed in a variety of epithelial cancers, and plays a crucial role in cancer progression, especially in breast cancer. It is also an essential regulator for immune functionality, but the mechanisms whereby it effects immune infiltration in breast cancer remain uncertain.

Methods: In this research, MUC1 expression was analyzed by the Oncomine and TIMER database. The association between MUC1 and prognosis was evaluated by Kaplan-Meier plotter database. The correlations of MUC1 with immune infiltration and immunological markers were assessed by TIMER database.

Results: We found that MUC1 expression was significantly correlated with outcomes in multiple cancers, with the effect being particularly pronounced in breast cancer. Pathologically, elevated MUC1 expression was related with worse prognosis depending on intrinsic subtypes, ER status, patient stage, lymph node and TP53 mutation status in breast cancer. Specifically, low level of MUC1 seemed to be more favorable to luminal B patients with systemic treatment. MUC1 expression had significant negative correlations with infiltrating levels of CD8+ T cells, CD4+ T cells, B cells, macrophages, neutrophils, monocytes and dendritic cells (DCs) in breast cancer. Besides, MUC1 displayed strong regulations on macrophage polarization and diverse immunological gene markers. The patients with lower MUC1 and deeper immune infiltration predicted better prognosis.

Conclusions: MUC1 is significantly associated with clinical prognosis and potentially plays an essential role in modulating cytotoxic T lymphocytes (CTLs), tumor associated macrophages (TAMs), natural killer cells (NK cells) and DCs in breast tumors. Collectively, MUC1 could be served as a valuable biomarker of predicting prognosis and immune infiltration for breast cancer patients.

Background

Breast cancer is the most frequently diagnosed cancer and the second most common cause of cancer mortality in women worldwide, accounting for an estimated 28% of new cancers. It is a highly heterogeneous disease with different clinical manifestations and multiple signaling pathways can mediate its initiation and progression[1]. Based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), researchers have classified breast cancer into four types according to the hormone receptors: HER2 type (ER+, PR−, HER2+), luminal A (ER+, PR+, HER2−), luminal B (ER+, PR−, HER2+), and triple-negative breast cancer (ER−, PR−, HER2−,TNBC)[2]. Basically, the drugs applied in the treatment of breast cancer include chemotherapy and pharmacological agents for endocrine therapy, such as tamoxifen targeted at ER. Although there are numerous therapeutic options available for breast cancer patients, allowing for a number of approaches designed to inhibit ER or oncogene molecules, drug resistance and recurrence is the main problem for most treatments [3, 4].

Previous studies showed that patients with higher level of immune infiltration showed significant better outcomes than lower groups in many cancers, meanwhile, deeper immune infiltration displayed intensive drug effects for chemotherapy. Therefore, cancer immunotherapy, currently as the most promising treatment, has been developed and achieved great success in immune checkpoint blockade and chimeric antigen receptor T cell (CART) therapies, especially in melanoma and non-small cell lung cancer (NSCLC). These successes also underscore the importance of understanding basic tumor immunology for successful clinical application in treating patients with cancer. Immune checkpoint inhibitors, targeting PD-1/PD-L1 or CTLA1-4, are gradually utilized in breast cancer[5]. However, unlike melanoma and NSCLC, breast cancer, as a kind of immunologically cold cancer, has lower response to this treatment, probably due to its high intra-tumoral heterogeneity, cancer stem cells, low immune infiltration and complex tumor microenvironment (TME)[6]. In some breast cancers without immunogenic features, single immune agent activity occurs in less than 10% of patients with metastatic breast cancer. This is likely due to the dominant repression of tumor immunity, particularly through down-regulating the immune infiltration like CTLs. Therefore, finding methods to activating tumor immune environment is necessary to improve prognosis in breast cancer.

MUC1 is a transmembrane glycoprotein, aberrantly glycosylated and overexpressed in a variety of epithelial cancers, and plays a crucial role in cancer progression, especially in breast cancer[7]. For the past 30 years, MUC1 has been considered as a possible therapeutic target. Recently, the mechanism of MUC1 in TME and immunotherapy has been increasingly explored[8]. In NSCLC, targeting MUC1 was associated with the downregulation of PD-L1, induction of IFN-γ and enhanced effector function of CD8 + tumor-infiltrating lymphocytes (TILs) in MUC1 transgenic mouse model[9]. In addition, MUC1 drived PD-L1 transcription by NF-κB p65 promoter occupancy and activates NF-κB to repress the toll-like receptors[9], which was associated with decreased overall survival[10]. Furthermore, targeting MUC1 in transgenic mice was correlated with suppressed PD-L1, increased TILs and better survival[7]. These studies demonstrated that MUC1 was closely related with tumor TME and possibly be an essential regulator for breast tumor immunity, so it is urgent to elucidate the correlations between MUC1 and multiple immune infiltration in breast cancer.

In this study, we conducted a comprehensive assessment of the relationship between MUC1 and patient prognosis using databases including Oncomine, PrognoScan and Kaplan-Meier plotter. We further investigated the link between MUC1 and immune cell infiltration of tumors by the TIMER database. Our results offer novel insights into the immunological role of MUC1 in breast cancer, thereby highlighting a potential relationship and an underlying mechanism between MUC1 and immune cell interactions.

Methods

Oncomine Database Analysis

The expression level of the MUC1 gene in multiple types of cancers was identified in the Oncomine database (https://www. oncomine.org/resource/login.html)[11]. The threshold was determined according

to the following values: P-value of 0.001, fold change of 1.5, and gene ranking of all.

PrognoScan Database Analysis

The correlation between MUC1 expression and survival in various types of cancers was analyzed by the PrognoScan database (http://www.abren.net/PrognoScan/)[12]. PrognoScan analyzed for relationships between gene expression and patient prognosis as overall survival (OS) and disease-free survival (DFS), across a large collection of publicly available cancer microarray datasets. The threshold was adjusted to a Cox P-value < 0.05.

Kaplan-Meier Plotter Database Analysis

The Kaplan-Meier plotter offers a means of readily exploring the impact of a wide array of genes on patient survival in 21 different types of cancer, with large sample sizes for the breast (n = 6,234), ovarian (n = 2,190), lung (n = 3,452) and gastric (n = 1,440) cancer cohorts. We therefore used this database to explore the association between MUC1 expression and outcomes in patients with breast cancer, analyzing the impact of both clinicopathological factors and MUC1 expression on patient prognosis (http://kmplot.com/analysis/)[13]. The hazard ratio (HR) with 95% confidence intervals and log-rank P-value were also computed.

TIMER Database Analysis

TIMER (https://cistrome.shinyapps.io/timer/) is a database designed for analysing immune cell infiltrates in multiple cancers[14]. This database employs pathological examination-validated statistical methodology in order to estimate tumour immune infiltration by CD4/CD8 T cells, B cells, neutrophils, macrophages and dendritic cells. Gene expression levels against tumor purity is displayed on the left-most panel[15]. We used database to explore the association between this MUC1 expression and the degree of infiltration by particular immune cells. Then we investigated the correlations of MUC1 mutation status and sCNA with immune infiltration. We further conducted Kaplan-Meier curve analyses to explore differences in patient survival based on MUC1 expression and certain immune cell infiltration. Lastly, we assessed how MUC1 expression correlated with the expression of particular immune infiltrating cell subset markers. These gene markers are referenced in prior studies[16,17].

Gene Correlation Analysis in GEPIA

GEPIA is an online database which facilitates the standardized analysis of RNA-seq data from 9,736 tumor samples and 8,587 normal control samples in the TCGA and GTEx data sets (http://gepia.cancer-pku.cn/index.html)[18]. We used this database to assess the connection between MUC1 expression and

patient prognosis in multiple tumor types, and we further evaluated the association between MUC1 expression and the expression of particular markers associated with immune cell infiltration.

Statistical Analysis

Survival curves were generated by the PrognoScan and KaplanMeier plots. The results generated in Oncomine are displayed with P-values, fold changes, and ranks. The results of KaplanMeier plots, PrognoScan, and GEPIA are displayed with HR and *P* or Cox *P*-values from a log-rank test. The correlation of gene expression was evaluated by Spearman's correlation and statistical significance, and the strength of the correlation was determined using the following guide for the absolute value: 0.00–0.19 "very weak," 0.20–0.39 "weak," 0.40–0.59 "moderate," 0.60–0.79 "strong," 0.80–1.0 "very strong." P-values <0.05 were considered statistically significant.

Results

The mRNA expression levels of MUC1 in different types of human cancers

To determine differences of MUC1 expression in tumor and normal tissues, we examined RNA sequencing data in TCGA using TIMER. This analysis revealed that the MUC1 expression was higher in breast, ovarian and cervical cancers compared with the normal tissues (Fig. 1A). Lower expression of MUC1 was observed in colorectal, gastric, kidney cancers. In lung, esophageal, brain and CNS, head and neck cancer, lymphoma, pancreatic and leukemia cancers, MUC1 was either upregulated or downregulated depending on different cancer subtypes. The detailed results were summarized in Additional file 6. To further evaluate MUC1 expression in pan cancers, we examined MUC1 expression using the RNA-seq data of multiple malignancies in TCGA. Figure 1B showed that MUC1 was significantly higher in BRCA (breast invasive carcinoma), CHOL (cholangiocarcinoma), COAD (colon adenocarcinoma), GBM (Glioblastoma multiforme), PAAD (pancreatic adenocarcinoma), SKCM(skin cutaneous melanoma), STAD(stomach adenocarcinoma), THCA(thyroid carcinoma) and UCEC(uterine corpus endometrial carcinoma) compared with adjacent normal tissues. However, MUC1 expression was significantly lower in HNSC (head and neck cancer), KIRC(kidney renal clear cell carcinoma), KIRP(kidney renal clear cell carcinoma) and LUSC(lung squamous cell carcinoma) compared with corresponding adjacent normal tissues. GEPIA was also used to evaluate MUC1 levels (Additional file 1).

Prognostic value of MUC1 in human cancers

Then we investigated whether MUC1 expression was correlated with prognosis in cancer patients. The impact of MUC1 expression to survival was evaluated by PrognoScan (Fig. 2). The details were shown in Additional file 7. Notably, MUC1 expression significantly impacts prognosis in diverse cancer types, including breast, brain, bladder, soft tissue, ovarian, colorectal and lung cancers. Three cohorts (GSE9893, E-TABM-158, GSE1379) included 155,117 and 60 samples at different stages of breast cancer showed

that high MUC1 expression was significantly associated with poorer prognosis OS HR = 1.21, 95% CI = 1.09–1.34, Cox P = 0.000249; DSS HR = 1.65, 95% CI = 1.16–2.35, Cox P = 0.005401; RFS HR = 1.10, 95% CI = 0.81–1.50, Cox P = 0.032756). In brain and soft tissue cancer, the cohorts (GSE4271-GPL96, GSE30929) containing 77 samples and 140 samples presented that high MUC1 was significantly related with poor prognosis (OS HR = 1.39, 95% CI = 1.10–1.76, Cox P = 0.005886; DRFS (Distant Recurrence Free Survival) HR = 2.45, 95% CI = 1.66–3.62, Cox P = 0.000007). However, in bladder, ovarian, colorectal and lung cancer, the datasets (GSE13507, GSE9891, GSE17537, HARVARD-LC) demonstrated that high MUC1 predicted better prognosis in patients (OS HR = 1.26, 95% CI = 1.00–1.58, Cox P = 0.048454; OS HR = 0.86, 95% CI = 0.74–1.01, Cox P = 0.040599; DFS HR = 0.22, 95% CI = 0.08–0.58, Cox P = 0.002182; OS HR = 0.62, 95% CI = 0.43–0.90, Cox P = 0.012020). The results showed that MUC1 had different prognosis depending on cancer types and high level of MUC1 predicted worse prognosis in breast cancer. Besides, RNA sequence data in TCGA were also used to analyze the prognostic potential of MUC1 via GEPIA (Additional file 2). These results confirmed the prognostic value of MUC1 in specific types of cancers and increased or decreased MUC1 expression have different prognostic value depending on the type of cancers. TIMER database was also used to verify the results (Additional file 3).

High MUC1 expression impacts the prognosis of breast cancer in patients with different intrinsic subtypes, clinical factors and systemic treatment

To better understand the relevance and underlying mechanisms of MUC1 expression in breast cancer, we investigated the relationship between MUC1 expression and clinical characteristics of breast cancer patients in the Kaplan-Meier plotter database (Fig. 3). The multivariate Cox regression analysis was performed to further evaluate the association between MUC1 and the prognosis of intrinsic subtypes. Generally, MUC1 had no significant correlations with total breast cancer prognosis. However, in ER negative breast cancer, low MUC1 expression was related with better RFS ($P < 0.05$). In basal like, HER2 positive and luminal B breast cancer, overexpression of MUC1 was significantly associated with worse OS and RFS ($P < 0.05$). Conversely, high MUC1 expression was correlated with better OS in luminal A breast cancer. Specifically, high MUC1 expression was correlated with worse OS and RFS in stage 3 breast cancer patients ($P < 0.01$), but there was no significant difference observed in stage 1 or 2. On TP53 mutation status, overexpression of MUC1 was related with worse RFS in TP53 mutation patients compared with wild type ($P < 0.05$). We further found that elevated MUC1 was linked to better RFS in lymph node positive status instead of lymph node negative group ($P < 0.05$). Table 1 demonstrated the details about the clinical parameters and prognosis. Interestingly, we also noticed low level of MUC1 predicted better prognosis in breast cancer patients on the condition of systemic treatment. Although no significant difference was found, there was a trend among them. Patients with low MUC1 and received systemic treatment (adjavant, neoadjavant or both) had better prognosis (Additional file 4). The prognosis of luminal B patients (with low level of MUC1) who received systemic treatment was significantly better than those without chemotherapy ($P < 0.05$). On the whole, these results concluded that MUC1 impacted the prognosis in breast cancer patients depending on the intrinsic subtypes, stages, TP53 mutation status and lymph node status. Furthermore, low level of MUC1 seemed more beneficial to the luminal B patients with systemic therapy.

Table 1
Correlation of MUC1 expression and patient prognosis in breast cancer with different clinicopathological factors by Kaplan-Meier plotter.

Clinicopathological characteristics	Overall survival (OS) (N = 1402)			Relapse Free survival (RFS) (N = 3955)		
	N	Hazard ratio	P-value	N	Hazard ratio	P-value
ER status						
ER positive	548	0.91 (0.64 − 1.3)	0.61	2061	0.98 (0.83 − 1.15)	0.79
ER negative	251	1.52 (0.96 − 2.41)	0.075	801	1.29 (1.03 − 1.62)	**0.027**
PR status						
PR positive	83	1.23 (0.33 − 4.61)	0.75	589	1.08 (0.76 − 1.53)	0.67
PR negative	89	1.72 (0.67 − 4.45)	0.26	589	0.96 (0.72 − 1.29)	0.8
HER2 status						
HER2 positive	129	1.3 (0.64 − 2.64)	0.46	252	0.95 (0.61 − 1.46)	0.8
HER2 negative	130	1.1 (0.47 − 2.61)	0.82	800	0.72 (0.56 − 0.94)	**0.016**
Intrinsic subtype						
Basal	241	1.11 (0.68 − 1.82)	0.67	618	1.47 (1.14 − 1.9)	**0.0026**
Luminal A	611	0.8 (0.56 − 1.15)	0.23	1933	0.84 (0.71 − 0.99)	**0.042**
Luminal B	433	1.34 (0.92 − 1.94)	0.13	1149	1.24 (1.02 − 1.51)	**0.027**
HER2+	117	2.78 (1.37 − 5.63)	**0.0031**	251	1.22 (0.83 − 1.79)	0.3
Pietenpol subtype						
Basal-like 1	58	2.26 (0.69 − 7.34)	0.16	171	1.72 (1.06 − 2.79)	**0.027**

Bold values indicate P < 0.05.

Clinicopathological characteristics		Overall survival (OS) (N = 1402)			Relapse Free survival (RFS) (N = 3955)	
Basal-like 2	38	4.9 (1.04 – 23.21)	**0.027**	76	2.22 (1.06 – 4.63)	0.03
Immunomodulatory	100	1.59 (0.62 – 4.11)	0.33	203	1.17 (0.65 – 2.09)	0.61
Mesenchymal	73	1.12 (0.51 – 2.45)	0.79	177	0.95 (0.62 – 1.46)	0.82
Luminal androgen receptor	83	1.56 (0.78 – 3.1)	0.2	203	1.27 (0.85 – 1.91)	0.24
Lymph node status						
Lymph node positive	313	1.11 (0.75 – 1.65)	0.59	1133	0.8 (0.66 – 0.98)	**0.027**
Lymph node negative	594	0.87 (0.6 – 1.26)	0.47	2020	0.92 (0.78 – 1.09)	0.33
GRADE						
1	161	0.53 (0.2 – 1.43)	0.2	345	0.77 (0.46 – 1.31)	0.34
2	387	1.16 (0.76 – 1.78)	0.49	901	0.98 (0.77 – 1.24)	0.86
3	503	1.78 (1.27 – 2.5)	**0.00075**	903	1.35 (1.09 – 1.68)	**0.0069**
TP53 status						
Mutated	111	2.08 (0.94 – 4.64)	0.066	188	1.67 (1.03 – 2.71)	**0.035**
Wild type	187	0.81 (0.42 – 1.55)	0.53	273	1.3 (0.85 – 1.99)	0.22
Systemically untreated patients	382	0.96 (0.61 – 1.5)	0.85	1010	0.99 (0.8 – 1.22)	0.93
Chemotherapy						
Adjuvant only	129	1.38 (0.73 – 2.62)	0.32	421	1 (0.71 – 1.41)	0.99
Neoadjuvant only	135	1.02 (0.44 – 2.36)	0.96	197	1.27 (0.68 – 2.35)	0.45

Bold values indicate P < 0.05.

Clinicopathological characteristics		Overall survival (OS) (N = 1402)			Relapse Free survival (RFS) (N = 3955)	
Include all	248	1.55 (0.9 − 2.66)	0.11	602	1.15 (0.85 − 1.55)	0.38
Luminal B type	86	1.04 − 5.57	**0.035**	220	1.1 − 2.95	**0.018**
Endocrine therapy						
Tamoxifen only	109	0.94 (0.45 − 1.94)	0.86	733	0.93 (0.7 − 1.25)	0.65
Include all	172	1.07 (0.61 − 1.89)	0.81	867	1.01 (0.77 − 1.32)	0.95
Luminal B type	121	0.62 − 2.41	0.56	364	1.04 − 2.21	**0.028**
Bold values indicate P < 0.05.						

MUC1 expression is correlated with immune infiltration level in breast cancer

Tumor-infiltrating lymphocytes(TILs) are an independent predictor of sentinel lymph node status and survival in many cancers. As an independent predictor of response to neoadjuvant chemotherapy, the increased number of TILs in primary tumor tissue relating to good prognosis has been reported in severa cancers, including breast cancer[19]. Therefore, we investigated the correlations of MUC1 expression with immune infiltration levels in 40 cancer types from TIMER. The results showed that MUC1 expression widely had significant correlations with immune cells in many cancers (Additional file 8).

The association between MUC1 expression and the degree of immune infiltration in breast cancer was explored by TIMER database (Fig. 4). Tumor purity is an important factor that influences the analysis of immune infiltration in clinical tumor samples by genomic approaches[20], and TIMER and GEPIA have most of the homologous data from TCGA[14, 18]. We found that MUC1 had significant correlations with tumor purity in breast cancer (P = 1.69e-02) and basal breast cancer (P = 2.08e-02), however, no significant correlations were observed in HER2, luminal A and luminal B subtypes. Figure 4 (A-C) showed that MUC1 had significant negative correlations with infiltrating levels of CD8 + T cells (Rho = -0.197, P = 3.78e-10), naive CD8 + T cells(Rho = -0.259, P = 1.07e-16), central memory T cells(Rho = -0.148, P = 2.96e-06), DCs(Rho = -0.228, P = 3.56e-13), NK cells(Rho = -0.168, P = 9.57e-08), naive CD4 + T cell(Rho = -0.114, P = 3.15e-04), memory CD4 + T cells(Rho = -0.208, P = 3.86e-11), B cells(Rho = -0.234, P = 8.37e-14), naive B cells(Rho = -0.173, P = 4.31e-08), memory B cells(Rho = -0.2, P = 1.93e-10), M1 macrophages(Rho = -0.204, P = 9.27e-11), neutrophils(Rho = -0.186, P = 3.74e-09) and monocytes(Rho = -0.168, P = 9.70e-08). However, MUC1 had significant positive correlations with infiltrating levels of M2 macrophages (Rho = 0.176, P = 2.27e-08). Overall, these results showed that MUC1 plays an essential role in modulating immune cell infiltration and memory T cells recruitment in breast cancer, with a particularly strong

regulation on macrophage polarization. In basal breast cancer, Fig. 4 (D-E) presented that MUC1 expression level had significant negative correlations with infiltrating levels of CD8 + T cells (Rho = -0.179, P = 1.84e-02), naive CD4 + T cells (Rho = -0.171, P = 2.37e-02), CD4 + Th2 cells (Rho = -0.228, P = 2.46e-03), B cells (Rho = -0.234, P = 8.37e-14) and M2 macrophages (Rho = -0.311, P = 3.00e-05). Unexpectedly, elevated MUC1 was associated with improved immune infiltration of DCs (Rho = 0.203, P = 7.26e-03), neutrophils (Rho = 0.232, P = 2.03e-03) and M1 macrophages (Rho = 0.275, P = 2.39e-04). There was no significance on NK cells (Rho = 0.069, P = 3.63e-01) and monocytes (Rho = 0.001, P = 9.86e-01). Considering basal like breast cancer is more aggressive and easier to metastasis on clinical and MUC1 plays distinct roles in basal subtype from total breast cancer, implying that MUC1 could play a part in tumor metastasis or recurrence in basal type due to reshaping tumor immune environment. Overall, MUC1 was significantly correlated with immune infiltration in breast cancer.

We next analyzed the correlations between MUC1 copy number with immune cells infiltrating (Fig. 5). Compared with normal level, high amplification of MUC1 was significantly related with less immune infiltrating of CD8 + T cells (P = 0.025), CD4 + T cells (P = 0.00055), DCs (P = 0.029), macrophages (P = 0.00063) and monocytes (P = 0.015), but more infiltrating of neutrophils (P = 3e-05). Overall, high amplification of MUC1 predicted worse immune infiltration in tumor tissue, which was consistent with above results (Fig. 4). In addition, the relationship between MUC1 mutation status and immune infiltration was also investigated and the results from diverse datasets showed that mutated MUC1 predicted higher infiltrating of CD8 + T cells (P = 0.049), CD4 + T cells (P = 0.023, P = 0.011) and DCs (P = 0.0043, P = 0.0017 and P = 0.026) immune infiltration compared with wild type (Fig. 6).

To further investigate the role of MUC1 expression together with immune infiltration on clinical prognosis, we compared the cumulative survival in breast cancer patients carried with different levels of MUC1 and different levels of immune infiltration. Figure 7 exhibited that patients with low level of MUC1 and high level of immune infiltration of CD8 + T cells (HR = 0.546, P = 0.00148), CD4 + T cells (HR = 0.512, P = 0.0011), B cells (HR = 0.535, P = 0.0013), M1 macrophages (HR = 0.558, P = 0.00273) and DC cells (HR = 0.666, P = 0.034) had better prognosis. Opposite to M1 macrophages, patients with lower level of M2 macrophages (HR = 2.21, P = 4.03e-05) predicted better outcome, which was consistent with macrophage polarization (Fig. 4C). Obviously, in patients with high level of MUC1, no significance was observed. In summary, MUC1 regulated immune infiltration to effect prognosis possibly meditated by regulating CTLs, DCs, B cells and macrophage polarization.

Correlation analysis between MUC1 expression and immune marker sets

We then explored the links between MUC1 and related immunological markers in breast cancer by TIMER and GEPIA. Correlations between MUC1 and the immune markers of CD8 + T cells, T cells (general), B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells, DCs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, Tregs and exhausted T cells were assessed (Table 2). After the correlation adjusted by tumor purity, results revealed significant correlations between MUC1 and CD8 + T markers (CD8A, CD8B),

T cell markers (CD3D, CD3E, CD2), B cell markers (CD19, CD79A), NK cell markers (KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DS4), Th1 markers (T-bet, STAT4, IFN-γ), Th2 markers(GATA3, STAT6, IL13), Tfh markers(BCL6, IL21), Th17 markers(STAT3, IL17A), Treg markers(FOXP3, CCR8, STAT5B, TGFβ) and T cell exhaustion markers(PD-1, CTLA4, LAG3, GZMB) in breast cancer ($P < 0.001$). MUC1 had close relationships with several immune gene markers, particularly NK cells, CD8 + T cells and B cells (Additional file 5). However, the correlation between MUC1 and specific subtype was not significant (Additional file 9). We further testified the above immunological markers in GEPIA database, which was basically the same (Table 3).

Table 2
Correlation analysis between MUC1 and related genes and markers of immune cells in TIMER.

Description	Gene markers	Breast Cancer			
		None		Purity	
		Cor	P	Cor	P
CD8 + T cell	CD8A	-0.100	**	-0.144	***
	CD8B	-0.122	***	-0.166	***
T cell (general)	CD3D	-0.116	**	-0.165	***
	CD3E	-0.101	**	-0.148	***
	CD2	-0.108	**	-0.155	***
B cell	CD19	-0.115	**	-0.161	***
	CD79A	-0.115	**	-0.165	***
Monocyte	CD86	-0.076	0.012	-0.101	*
	CD115 (CSF1R)	0.052	0.086	0.027	0.400
TAM	CCL2	-0.129	***	-0.158	***
	CD68	-0.020	0.505	-0.039	0.220
	IL10	-0.117	**	-0.138	***
M1 Macrophage	INOS (NOS2)	-0.021	0.485	-0.027	0.400
	IRF5	-0.156	***	-0.187	***
	COX2(PTGS2)	-0.071	0.019	-0.104	*
M2 Macrophage	CD163	-0.105	**	-0.135	***
	VSIG4	-0.004	0.887	-0.032	0.317
	MS4A4A	-0.101	**	-0.129	***
Neutrophil	CD66b (CEACAM8)	-0.042	0.166	-0.058	0.069
	CD11b (ITGAM)	0.178	***	0.162	***
	CCR7	-0.086	*	-0.122	**
Natural killer cell	KIR2DL1	-0.130	***	-0.156	***

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

** P < 0.01; ** P < 0.001; *** P < 0.0001.*

Description	Gene markers	Breast Cancer			
	KIR2DL3	-0.115	**	-0.128	***
	KIR2DL4	-0.224	***	-0.246	***
	KIR3DL1	-0.109	**	-0.135	***
	KIR3DL2	-0.116	**	-0.142	***
	KIR3DL3	-0.098	*	-0.107	**
	KIR2DS4	-0.126	***	-0.148	***
Dendritic cell	HLA-DPA1	0.044	0.147	0.020	0.523
	HLA-DPB1	0.037	0.216	0.005	0.885
	HLA-DQB1	0.006	0.840	-0.011	0.722
	HLA-DRA	0.015	0.626	-0.017	0.583
	BDCA-1(CD1C)	0.041	0.175	0.016	0.621
	BDCA-4(NRP1)	0.193	***	0.182	***
	CD11c (ITGAX)	0.012	0.695	-0.010	0.762
Th1	T-bet (TBX21)	-0.136	***	-0.192	***
	STAT4	-0.086	*	-0.129	***
	STAT1	-0.075	0.013	-0.095	*
	IFN-γ (IFNG)	-0.170	***	-0.206	***
	TNF-α (TNF)	-0.082	*	-0.095	*
Th2	GATA3	0.314	***	0.353	***
	STAT6	0.262	***	0.245	***
	STAT5A	0.111	**	0.090	*
	IL13	-0.104	**	-0.110	**
Tfh	BCL6	0.198	***	0.183	***
	IL21	-0.145	***	-0.168	***
Th17	STAT3	0.308	***	0.293	***

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

** P < 0.01; ** P < 0.001; *** P < 0.0001.*

Description	Gene markers	Breast Cancer			
	IL17A	-0.099	**	-0.124	***
Treg	FOXP3	-0.176	***	-0.209	***
	CCR8	-0.159	***	-0.174	***
	STAT5B	0.116	**	0.109	**
	TGFβ (TGFB1)	0.130	***	0.114	**
T cell exhaustion	PD-1 (PDCD1)	-0.181	***	-0.236	***
	CTLA4	-0.225	***	-0.273	***
	LAG3	-0.247	***	-0.276	***
	TIM-3 (HAVCR2)	0.006	0.851	-0.007	0.827
	GZMB	-0.231	***	-0.282	***

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

** P < 0.01; ** P < 0.001; *** P < 0.0001.*

Table 3
Correlation analysis between MUC1 and related genes and markers of immune cells in GEPIA.

Description	Gene markers	Breast Cancer			
		Normal		Tumor	
		R	P	R	P
CD8 + T cell	CD8A	0.47	***	−0.11	***
	CD8B	0.45	***	−0.11	**
T cell (general)	CD3D	0.51	***	−0.096	*
	CD3E	0.46	***	−0.098	*
	CD2	0.43	***	−0.12	**
B cell	CD19	0.32	***	−0.099	*
	CD79A	0.48	***	−0.11	**
Monocyte	CD86	−0.035	0.71	−0.11	**
	CD115 (CSF1R)	−0.16	0.091	0.012	0.7
TAM	CCL2	−0.14	0.15	−0.12	***
	CD68	−0.25	*	−0.066	0.029
	IL10	−0.36	**	−0.15	***
M1 Macrophage	INOS (NOS2)	0.11	0.25	−0.04	0.18
	IRF5	0.2	0.034	−0.14	***
	COX2(PTGS2)	0.04	0.67	−0.087	*
M2 Macrophage	CD163	−0.32	***	−0.16	***
	VSIG4	−0.26	*	−0.032	0.29
	MS4A4A	−0.36	***	−0.13	***
Neutrophil	CD66b (CEACAM8)	−0.22	0.018	−0.035	0.25
	CD11b (ITGAM)	−0.15	0.11	0.15	***
	CCR7	0.37	***	−0.097	*
Natural killer cell	KIR2DL1	−0.13	0.18	−0.13	***

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

** P < 0.01; ** P < 0.001; *** P < 0.0001.*

Description	Gene markers	Breast Cancer			
	KIR2DL3	-0.14	0.14	−0.15	***
	KIR2DL4	−0.075	0.43	−0.22	***
	KIR3DL1	−0.25	*	−0.12	***
	KIR3DL2	−0.039	0.68	−0.15	***
	KIR3DL3	−0.053	0.58	−0.12	***
	KIR2DS4	−0.25	*	−0.13	***
Dendritic cell	HLA-DPA1	0.16	0.1	0.015	0.62
	HLA-DPB1	0.35	**	0.043	0.16
	HLA-DQB1	0.23	0.016	0.025	0.41
	HLA-DRA	0.13	0.18	−0.013	0.66
	BDCA-1(CD1C)	0.18	0.06	0.031	0.3
	BDCA-4(NRP1)	−0.57	***	0.12	***
	CD11c (ITGAX)	0.24	0.011	−0.0086	0.78
Th1	T-bet (TBX21)	0.089	0.35	−0.12	***
	STAT4	−0.096	0.31	−0.096	*
	STAT1	0.039	0.68	−0.14	***
	IFN-γ (IFNG)	−0.052	0.59	−0.18	***
	TNF-α (TNF)	0.12	0.21	−0.1	**
Th2	GATA3	0.68	0	0.29	***
	STAT6	0.19	0.046	0.2	***
	STAT5A	−0.58	***	0.089	*
	IL13	−0.012	0.9	−0.086	*
Tfh	BCL6	−0.23	0.014	0.17	***
	IL21	0.056	0.56	−0.21	***
Th17	STAT3	0.44	***	0.19	***

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

** P < 0.01; ** P < 0.001; *** P < 0.0001.*

Description	Gene markers	Breast Cancer			
	IL17A	0.13	0.19	−0.12	**
Treg	FOXP3	0.73	***	−0.18	***
	CCR8	0.077	0.42	−0.23	***
	STAT5B	−0.41	***	0.057	0.058
	TGFβ (TGFB1)	0.14	0.15	0.17	***
T cell exhaustion	PD-1 (PDCD1)	0.49	***	−0.16	***
	CTLA4	0.26	*	−0.22	***
	LAG3	0.3	*	−0.19	***
	TIM-3 (HAVCR2)	−0.16	0.1	−0.036	0.24
	GZMB	−0.13	0.16	−0.22	***

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

** P < 0.01; ** P < 0.001; *** P < 0.0001.*

Discussion

In this study, MUC1 was validated as an independent prognostic indicator in breast cancer, mainly depending on intrinsic subtypes and clinical parameters. Furthermore, MUC1 was significantly correlated with immune infiltration and diverse immune marker sets and patients with low level of MUC1 and deeper immune infiltration predicted better prognosis. These findings provide insights in understanding the potential role of MUC1 in tumor immunology and its use as a breast cancer biomarker.

We first confirmed that MUC1 was overexpressed in breast cancer by Oncomine and TIMER databases and high level of MUC1 was correlated with poor prognosis in breast cancer. The discrepancies in levels of MUC1 expression in different cancer types from different databases may be a reflection in data collection approaches and underlying mechanisms pertinent to different biological properties. Nevertheless, we found consistent correlations between MUC1 expression and breast cancer in these databases. MUC1 had significant relationships with prognosis in basal, HER2+, luminal A and luminal B subtypes, implying that MUC1 was closely related with intrinsic subtypes. Although, there was no significance in ER positive subtype, it has been reported that MUC1 induced tamoxifen(an estrogen analogue) resistance in ER positive breast cancer through regulating growth cascades and gene transcription[21]. It has been studied that breast cancer has higher heterogeneity than other cancers, and the classification of breast cancer is also more complicated[2], thus analyzing MUC1 according to subtypes is more accurate and feasible. High level of MUC1 was related with worse prognosis when

lymph node was positive, indicating that MUC1 is associated with cancer lymph metastasis and could be a reliable predictor for patients with lymph node positive. In addition, high level of MUC1 was correlated with poor prognosis in stage 3 patients, showing evidence that MUC1 could be used as a biomarker to predict death risk particularly in advanced breast cancer patients. In general, these findings strongly suggested that MUC1 is a credible prognostic biomarker in breast cancer patients.

Another key finding in this study is that the expression of MUC1 correlated with the degree of immune infiltration in breast cancer. Historically, breast cancer was not thought to be immunologically active, particularly when compared with tumors such as melanoma. However, recent evidence has emerged that TILs infiltrated in breast tumor tissue prior to treatment could predict better response to therapy and improved prognosis[19]. We found that MUC1 was negatively correlated with the degree of CD8 + T cells, CD4 + T cells, B cells, DCs, NK cells, neutrophils and monocytes infiltration in breast cancer ($P < 0.001$, Fig. 4). Increased CD8 + T cells have been shown to predict improved clinical outcome (HR 0.55 95 % CI, 0.39 to 0.78 P = 0.001) in one large study of 1334 breast cancer patients[22]. Another study of 256 TNBC tumors demonstrated every 10 % increase in TILs correlated with a 17 % decrease in the risk of recurrence ($P = 0.023$, HR 0.83; 95 % CI 0.71–0.98) and a 27 % decreased risk of death ($P = 0.035$, HR = 0.73; 95 % CI 0.54–0.98)[23]. In HER2 + and TNBC breast cancer, even incremental increases in TILs both in and surrounding the tumor have shown to predict better response to chemotherapy and improved survival in patients[24, 25]. However, our results revealed that high level of MUC1 significantly downregulated TILs infiltration, which could partly explain the weak immunological response in breast tumor. Therefore,MUC1 possibly hinders the adaptive immune activation in breast cancer. In addition, MUC1 had significant negative correlations with memory T cells, such as CD8 + central memory cells ($P < 0.001$, Fig. 4). Compared with naive T cells, memory T cells (central and activated), as a type of effective T cells, have more sensitive reactions to antigens, live longer and have stronger immune responses[26]. So MUC1 possibly impairs immune attack by inhibiting memory T cells stimulation.

DCs are considered as pivot cells of the immune system due to their exclusive ability to coordinate the innate and acquired immune responses, cross-present exogenous antigens, and prime naive T cells[27]. Normally, glycosylated MUC1 could be recognized as antigen to activate MHC class II presentation, however, the adaptive immune responses against mucin antigens is influenced by the expression of specific proteins or enzymes that regulate the processing of cancer-associated mucins, like heat-shock protein 70 (HSP70) and cathepsins, which destroyed the function of DCs[28, 29]. Tumor-associated mucins, especially MUC1, promoted an immunosuppressive microenvironment by masking Toll-like receptors (TLR) on antigen presenting cells (APCs) or inhibiting synapse formation between cytolytic NK cells and tumor cells[30]. O-linked glycosylation at either Thr3 or Ser4 sites renders MUC1 glycoforms unavailable for proteolytic processing by DCs, probably due to cleavage site masking[31]. Moreover, the formation of aberrantly glycosylated mucins conceals various receptors on the cell surface, hence obstructing ligand–receptor or cellular cascades. Membrane associated MUC1 has been reported to suppress TLR-mediated innate immune activation, inhibiting secretion of pro-inflammatory cytokines, such as IL-6, IL-8, and TNF-α[32]. Thus, high level of MUC1 is correlated with inefficient and reduced DCs infiltration in TME. NK cells usually exhibit cytolytic activity and kill cancer cells by the engagement of

receptors present on their surface upon cancer cell encounter. Mucins associated antigens, expressed in various adenocarcinomas, inhibit the cytotoxic activity of NK cells, and the effect is further enhanced in the presence of ammonium ions, which are known inhibitors of NK cell function[33]. MUC16, one of mucins family, has been identified as an ovarian tumor marker, could downregulate the expression of CD16 and CD94/NKG2A on NK cells, thus NK cells are unable to recognize and kill tumor cells[34]. Therefore, inhibition of immune synapse formation between NK cells and cancer cells represents another effective MUC1 mediated immunosuppressive mechanism[35].

We further found MUC1 plays an essential role in regulating TAMs polarization in breast cancer. Many researchers discovered that M1 macrophage is associated with tumor elimination and pro-inflammation by producing cytokines and NO, however, M2 macrophage involves in tissue repairment, remodeling, angiogenesis and retain homeostasis to promote tumor growth[36]. TAMs are a group of macrophages, functionally similar to M2 polarized, harboring the tumor tissue and closely associated with cancer cells, and have complicated effects on tumor behaviors. In most cancers, TAMs infiltration in solid tumors is associated with poor prognosis and leading to chemotherapy resistance[37]. In breast carcer, TAMs are the prominent components and high TAMs density correlates with worse prognosis [38]. Thus, high level of MUC1 activates M2 macrophages to promote tissue repairment like aggravating blood vessel growth or inducing EMT, while inhibits M1 macrophage to stimulate T cells. Li et al found that TP53-deficient and TP53-mutant mice showed increased expression of M2 polarized macrophages to induce M1-M2 transition[39]. It has been revealed that MUC1 cytoplasmic domain integrated directly to TP53 regulatory domain[40], so maybe there is a potential mechanism that MUC1 induces TAMs polarization to affect TME meditated by directly regulating TP53. We also found high level of MUC1 was associated with worse prognosis in TP53 mutation patients, supporting the above hypothesis. These researches demonstrated that overexpression and aberrant glycosylation of MUC1 modulates both innate and adaptive immune effectors to promote tumor surviving. High amplification of MUC1 was negatively related with most CTLs infiltration, but neutrophil was an exception (Fig. 5C). Neutrophils typically obliterate bacterial and fungal in our body, however, the role of neutrophils in tumor immunity remains controversial[41]. Previous research showed neutrophils escorted circulating tumor cells (CTCs) within the bloodstream and facilitated the metastatic potential [42]. Fridlender et al also discovered neutrophils had opposite roles in the TME [43]. Considering the double-face of neutrophils, interactions between MUC1 and neutrophils still remain vague and more research needs to be done. Finally, MUC1 was negatively correlated with most immunological markers in breast cancer (Table 2 and Table 3). Taken together, immune cells especially CTLs, were significantly downregulated by MUC1 in breast cancer.

To investigate the relationships between MUC1 expression, immune infiltration and prognosis, Kaplan Meier survival curves were made to confirm the exact role of MUC1 in immunology (Fig. 7). Overall, our results were consistent with clinical outcomes in breast cancer patients[3]. Interestingly, in low levels of MUC1 groups, the prognosis in patients with higher M1 macrophages or M2 macrophages was totally opposite, proving the different functions of TAMs in breast tumors, which was consistent with Fig. 4. However, none of such survival benefit was detected in high MUC1 groups, indicating that down regulating MUC1 could be a potential precondition for immunotherapy. Previous studies showed that

traditional chemotherapy prolongs survival in breast cancer patients meditated by improving CTLs infiltration, especially in TNBC, which is more sensitive to cancer agents[44]. Cytotoxic chemotherapy has been shown to increase type 1 T cell stimulation. Several chemotherapeutic agents have been found to trigger immune recognition in tumor by releasing stress proteins from dead cells[45]. Early data has further demonstrated that combining chemotherapy and immunotherapy may increase the number of clinical responses to checkpoint inhibitors in breast cancer. In this paper, we noticed that low level of MUC1 possibly improves clinical outcome and agent response by synergistically enhancing the CTLs immune infiltration in patients especially those who have received chemotherapy. In conclusion, high level of MUC1 could be partly an explanation for the immune suppression within breast tumors, so targeting MUC1 could be necessary to arouse immune infiltration for breast cancer patients, possibly meditated by recruiting effective immune cells, especially CTLs, DCs and NK cells. In the future, precisely targeting on MUC1 combined with chemotherapy or immunotherapy to enhance immune infiltration could be a promising strategy to improve prognosis in breast cancer patients.

Abbreviations

DCs: dendritic cells; CTLs: cytotoxic T lymphocytes; TAMs: tumor associated macrophages; NK cells: natural killer cells; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; TNBC: triple-negative breast cancer; CART: chimeric antigen receptor T cell; NSCLC: non-small cell lung cancer; TME: tumor microenvironment; TILs: tumor-infiltrating lymphocytes ; OS: overall survival; DFS: disease-free survival; HR: hazard ratio; BRCA: breast invasive carcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; GBM: Glioblastoma multiforme; PAAD: pancreatic adenocarcinoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; THCA: thyroid carcinoma; UCEC: uterine corpus endometrial carcinoma; HNSC: head and neck cancer; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal clear cell carcinoma; LUSC: lung squamous cell carcinoma.

Declarations

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None.

Authors' contributions

Xiaomin Xi: Methodology, Formal analysis, Project administration, Writing-review & editing.

Yilin You: Investigation, Formal analysis, Funding acquisition, Writing-review & editing.

Weidong Huang: Conceptualization, Writing-review & editing, Funding acquisition.

Jicheng Zhan: Conceptualization, Writing-review & editing, Supervision, Funding acquisition.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

The authors consent for publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

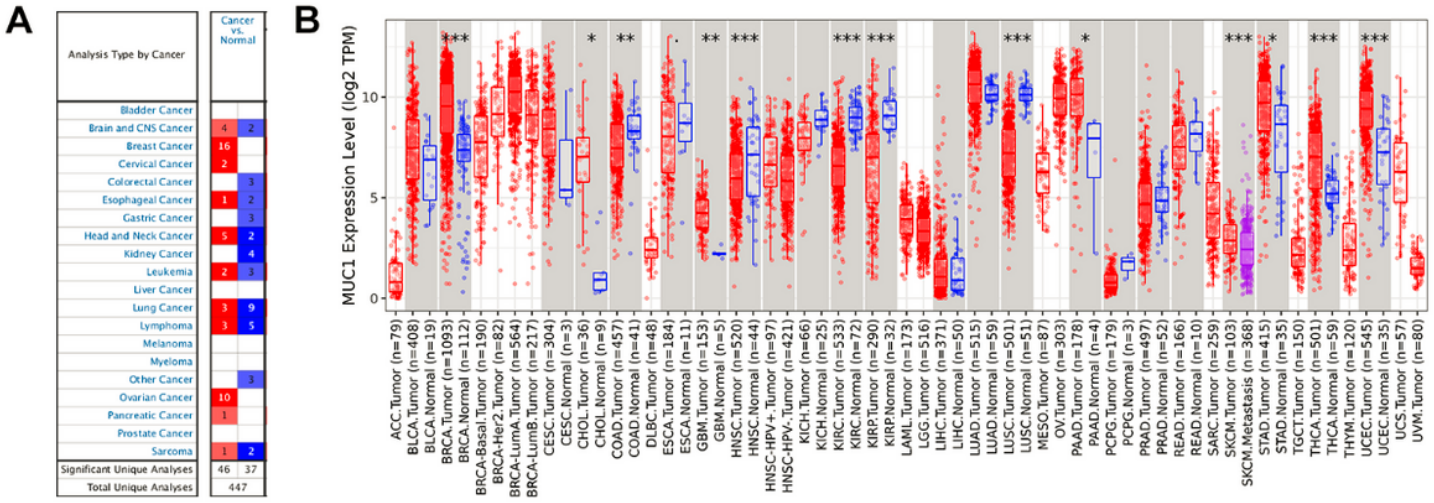

Figure 1

a Differentially expressed genes between LCCs and RCCs. b Differentially expressed DGIA lncRNAs between GU group and GS group. Red and blue circles indicate high and low genes expression, respectively. c Heat map depicts the differentially expressed DGIA lncRNAs in TCGA patients. d Unsupervised clustering of TCGA patients based on the expression pattern of 128 candidate DGIA lncRNAs. e In boxplot, cumulative number of somatic mutations in the GU-like group are significantly higher than those in the GS-like group. f Co-expression network of DGIA lncRNAs and intersection of mRNAs based on two methods. The red circles represent lncRNAs, and the blue circles represent mRNAs. LCCs and RCCs: Left and right-sided colon adenocarcinoma; DGIA lncRNAs: Genomic instability-associated long non-coding RNAs; GU: Genomic unstable; GS: Genomic stable.

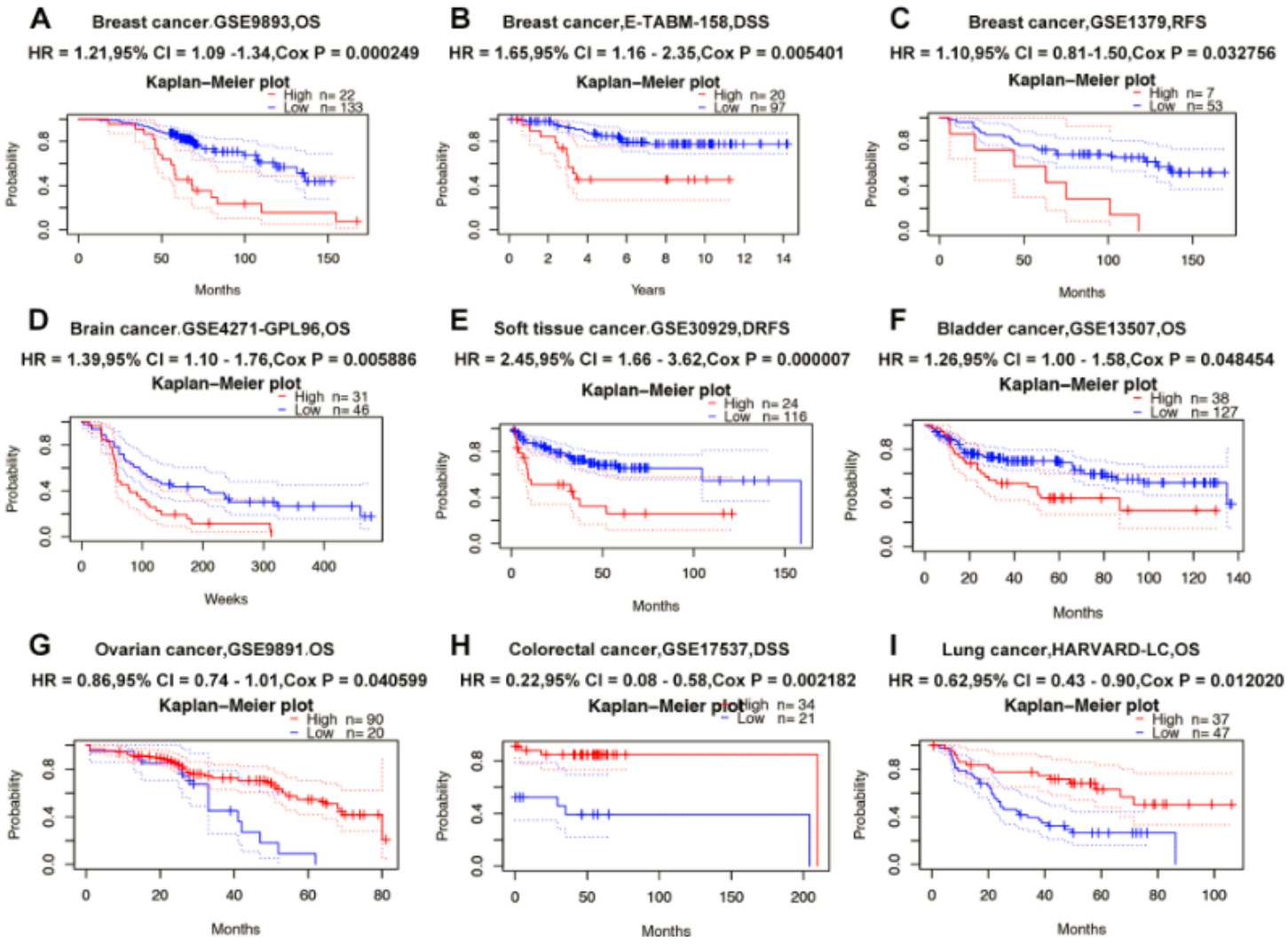

Figure 2

a Correlation between the gene modules and CNSMs. Each cell contains corresponding correlation coefficient and p-value. The correlation coefficient decreased in size from red to blue. b GO functional annotations for mRNAs co-expressed with lncRNAs. c KEGG enrichment analysis for mRNAs co-expressed with lncRNAs. CNSMs: Cumulative number of somatic mutations; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

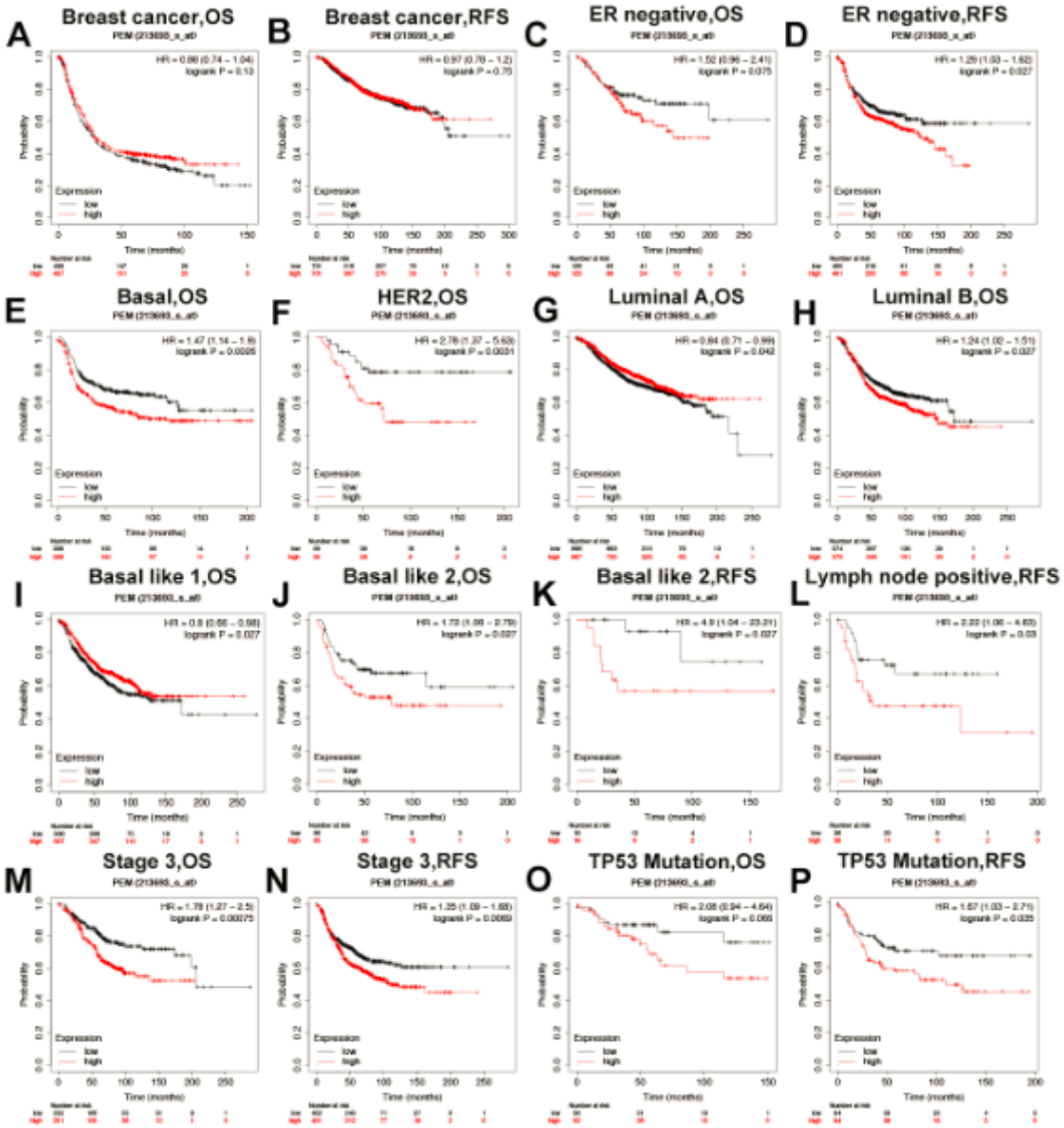

Figure 3

a Kaplan-Meier curves of overall survival in different groups. b ROC curves in different groups. c The heat map of 6 key lncRNA expression patterns with increasing risk score. ROC: Receiver operating characteristics

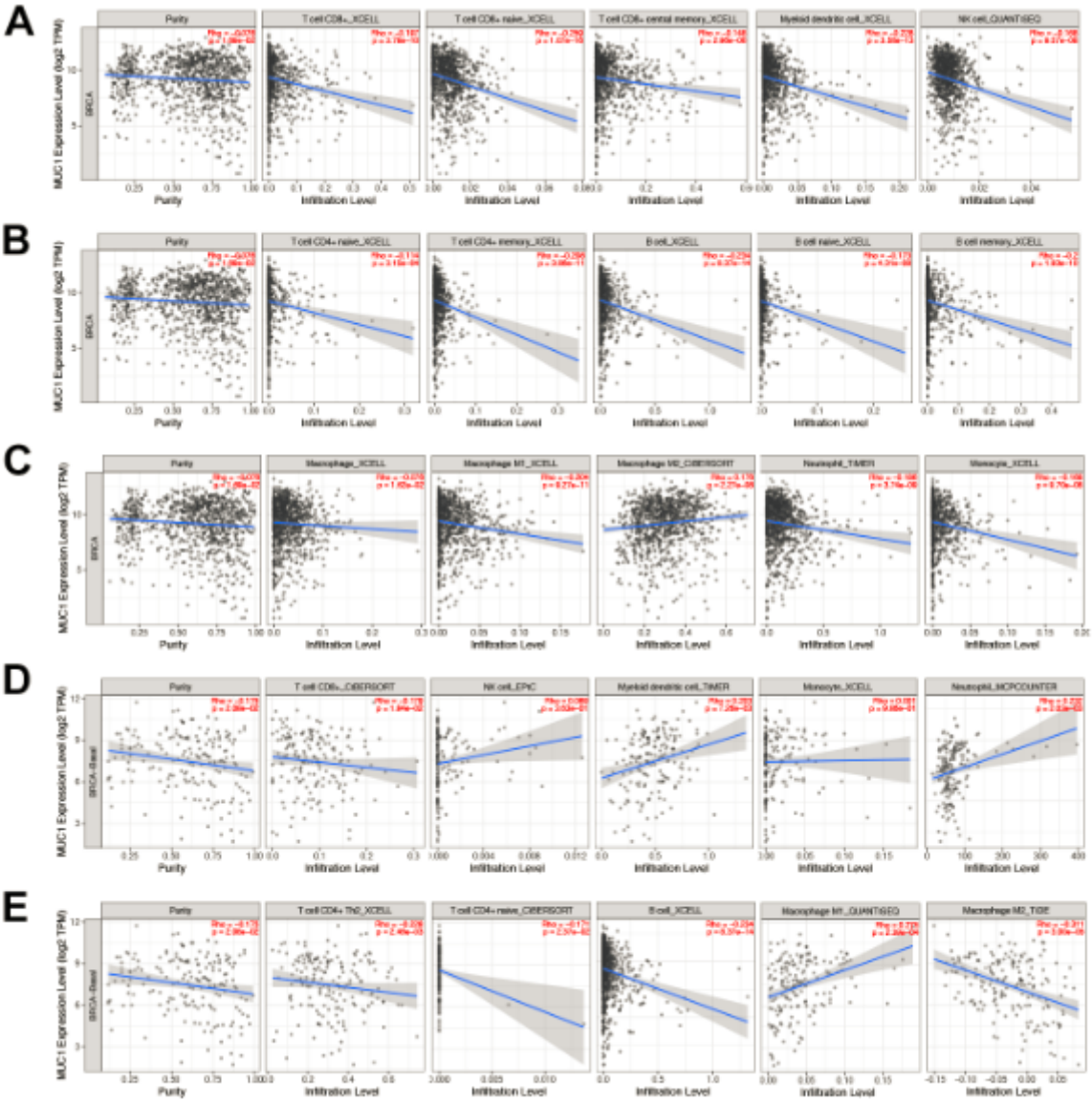

Figure 4

a Kaplan-Meier curves of overall survival in 6 key DGIA lncRNA. b The correlation between 6 key DGIA lncRNA and pathologic stages.

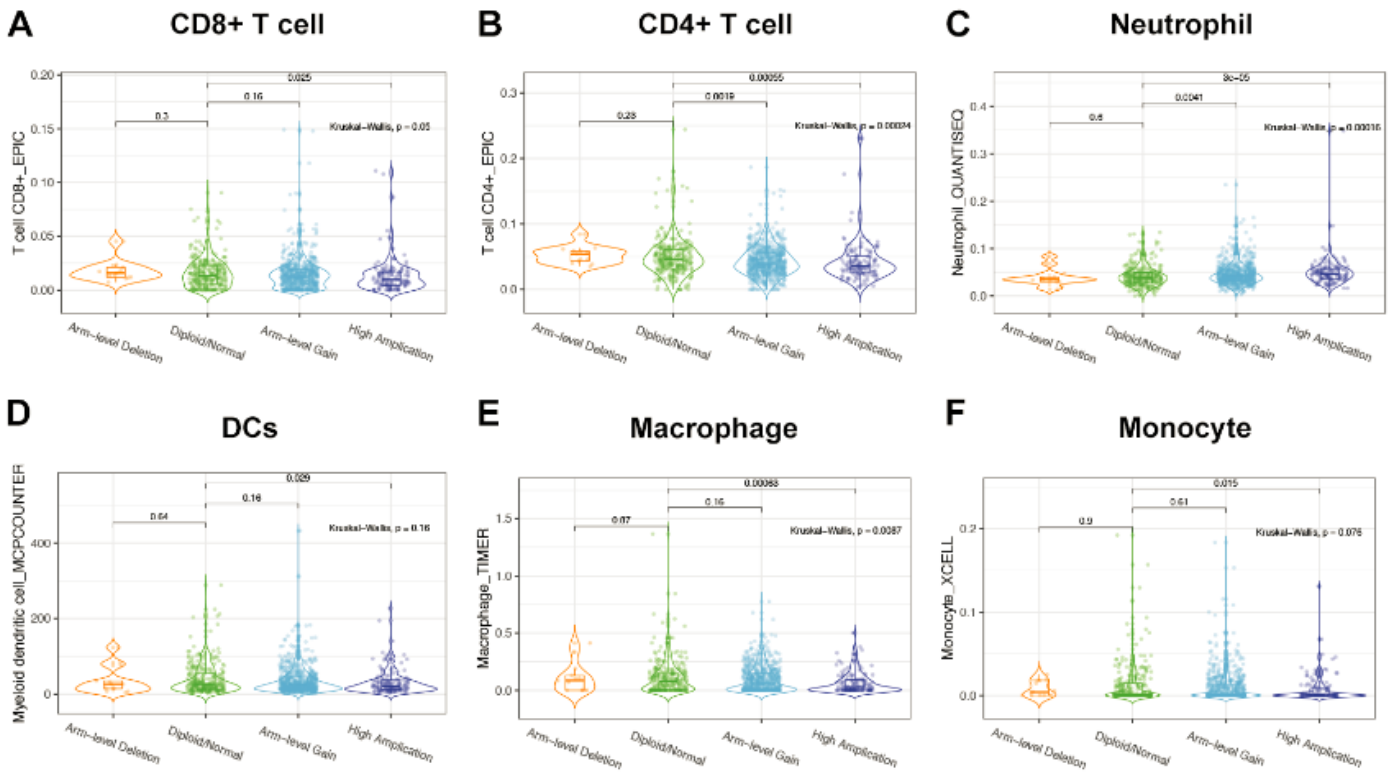

Figure 5

a Kaplan-Meier curve of overall survival in external validation group. b TimeROC curves for 1, 3, 5 years in external validation group. TimeROC: Time-dependent ROC.

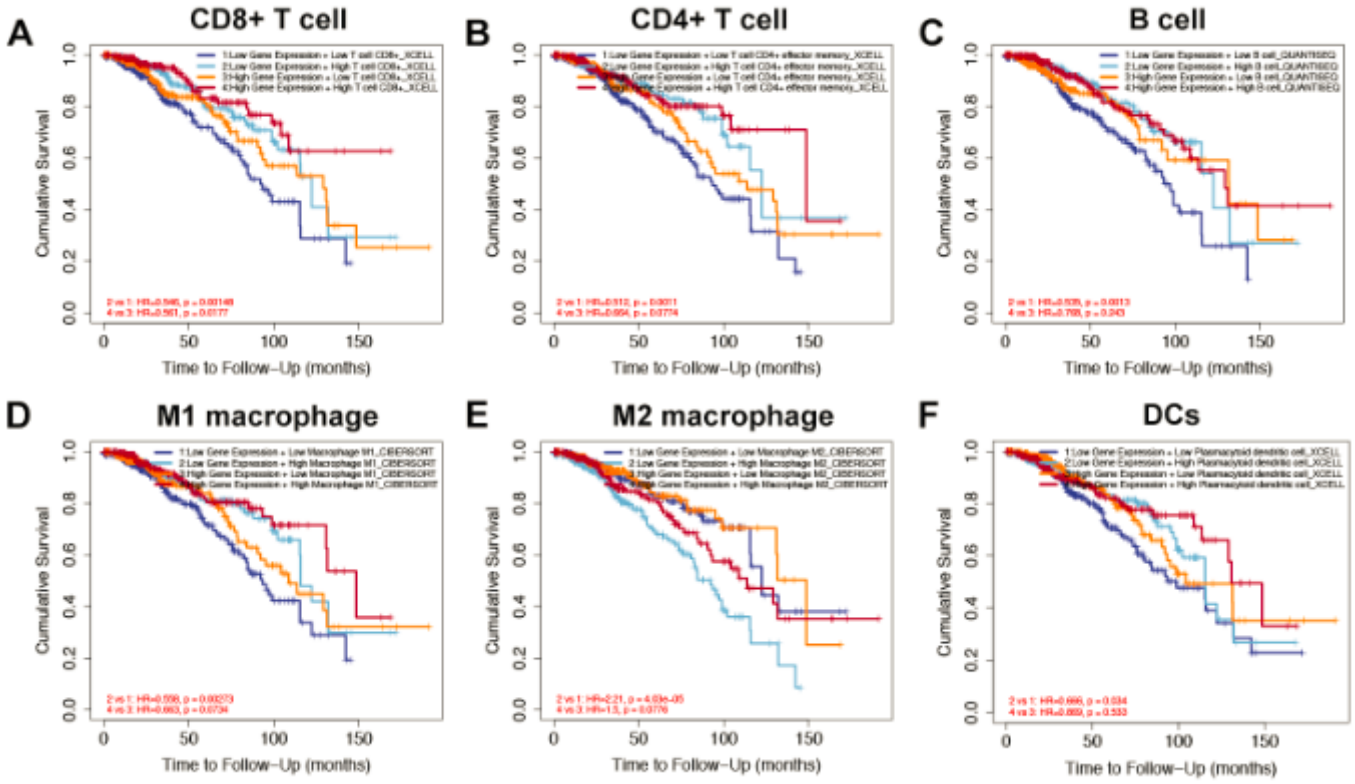

Figure 6

The differences of 22 immune cell types abundance within different risk groups.

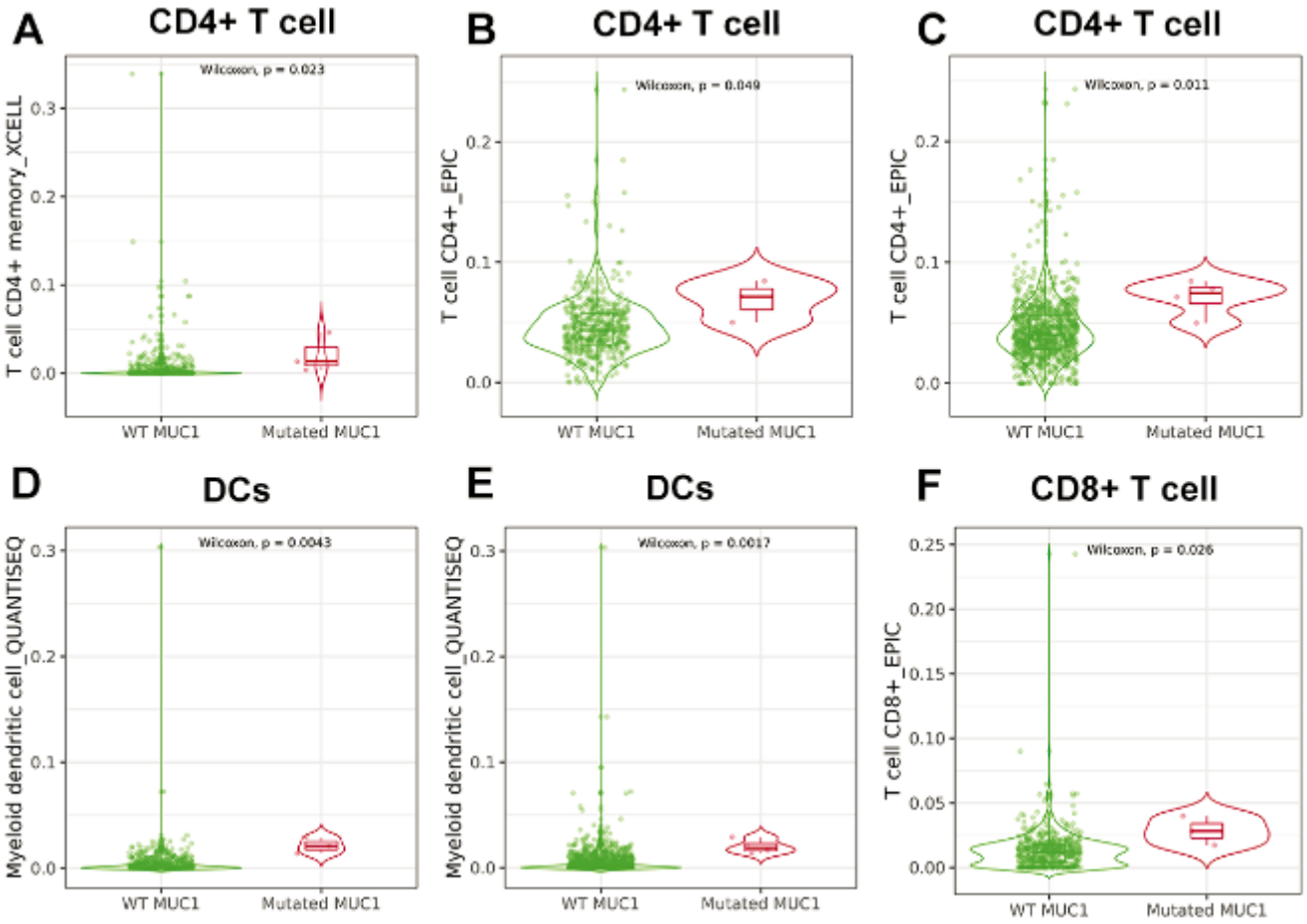

Figure 7

a BP of GO-related GSEA between different risk groups. b MF of GO-related GSEA between different risk groups. c KEGG-related GSEA between different risk groups. BP: Biological process; MF: Molecular function.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.tif
- Additionalfile2.tif
- Additionalfile3.tif
- Additionalfile4.tif
- Additionalfile5.tif
- Additionalfile6.xlsx

- Additionalfile7.xlsx
- Additionalfile8.csv
- Additionalfile9.xlsx