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MAL, a potential immunotherapy target, is associated with poor prognosis in cancer patients

Ruinian Zheng[1, 2, 3, #], Yamin Tian[4, #], NiYa Shang[3, #], Dan Yang[3], Yongmei Luo[3], Linxuan Huang[1], Caixiang Liu[1], Xuebing Pan[1], Yinjiao Yuan[1, 2], Xiarong Hu[5], Zhiming Wu[5], Ruijuan Luo[6], Yongkang Ye[7], Biao Yao[3, *], Jun Jia[1, *]

[1] Department of Oncology, Dongguan Institute of Clinical Cancer Research, Dongguan Key Laboratory of Precision Diagnosis and Treatment for Tumors, The Tenth Affiliated Hospital of Southern Medical University (Dongguan People's Hospital), Dongguan, Guangdong 523059, China
[2] The First School of Clinical Medicine, Southern Medical University, Guangzhou, Guangdong 510510, China
[3] Department of Oncology, Tongren People's Hospital, Tongren, Guizhou 554300, China
[4] Office of Administration, Tongren People's Hospital, Tongren, Guizhou 554300, China
[5] Department of General Surgery, The Tenth Affiliated Hospital of Southern Medical University (Dongguan People's Hospital), Dongguan, Guangdong 523059, China
[6] Department of Breast Surgery, The Tenth Affiliated Hospital of Southern Medical University (Dongguan People's Hospital), Dongguan, Guangdong 523059, China
[7] Department of Urology, The Tenth Affiliated Hospital of Southern Medical University (Dongguan People's Hospital), Dongguan, Guangdong 523059, China

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Abstract

The MAL gene encodes Myelin and Lymphocyte Protein, mainly expressed in T cells with immunomodulatory effects, showing the potential as a target for immunotherapy. However, the mechanism of MAL in the regulation of immune infiltration and its association with the prognosis in pan-cancer patients remain elusive. We used the TCGA, TIMER2.0, GTEx, UCSC, and TISCH databases and the R programming tool to explore the role of MAL in cancers. MAL was differently expressed in the majority of malignancies relative to the matched healthy controls. Patients with low MAL levels had adverse survival outcomes in the BRCA and LUAD cohorts. In all cancer types, MAL showed a significant correlation to specific immune-subpopulation abundance in particular T cells as well as B cells. MAL was also implicated in immunological pathways in BRCA and LUAD, suggesting the important role of MAL in cancer immune regulation. In conclusion, the pan-cancer study indicates that MAL with excellent prognostic value is a potential immunotherapy target in multiple cancers.

Keywords: Bioinformatics, Immunity, MAL, Pan-cancer analysis, Prognosis.

1. Introduction

Over the last few decades, cancer has become one of the major causes of human death across the world [1]. Modern immunotherapy is a new milestone for cancer treatment, and new alternative strategies in cancer immunotherapy are developed with a better understanding of the underlying mechanism of carcinogenesis [2]. Targeting co-inhibitory or immunological checkpoint receptors with immunosuppressive effects has achieved remarkable success in cancer treatment. However, there are still a large number of patients showing no positive response to the current immunotherapies [3]. Tumor immune cell interaction has attracted increasing attention in cancer immunotherapy. Analysis of these complicated connections may contribute towards predictive biomarker discovery as well as novel drug or therapy development [4].

The MAL gene encodes myelin and lymphocyte protein, an integral membrane protein of high hydrophobicity that forms part of the proteolipid MAL family. It has previously been found in the T cell endoplasmic reticulum where it was implicated as a potential linker protein in T cell signaling. The aberrant expression of MAL is related to various human cancers. For example, MAL is overexpressed in ovarian cancer and several lymphomas, and functions as an oncogene to promote cancer progression. Down-regulation of the MAL gene due to promoter hyper-

* Corresponding author.
E-mail address: 3157882462@qq.com (J. Jia), ybb223@163.com (B. Yao).
# These authors contributed equally
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methylation has been identified as a maker in many adenocarcinomas [5]. Moreover, MAL is located at the immunological synapse in T cells and is important for exosome secretion. Based on GSEA data from TCGA breast cancer samples, MAL is involved in vesicle transfer. In our preclinical study, we found that MAL may influence the prognosis of tumor patients by regulating the tumor immunological microenvironment through trafficking vesicles. However, no evidence has revealed the involvement of MAL in the control of the tumor immunological microenvironment, which still requires further investigation.

In terms of the role of MAL in immunity, MAL is indicated to be involved in exosome synthesis by T cells, suggesting it as a viable molecular target for artificially manipulating exosome production and the immune response for therapeutic purposes [6]. A new study used single-cell RNA sequencing to identify a population of T cells that co-express myelin and lymphocyte proteins in tumor-infiltrating T cells from breast cancer patients. In other tumor types, MAL is expressed by tumor-infiltrating CD8+ and CD4+ T cell subsets [7]. It is also reported that the MAL deficiency inhibits the release of exosome particles and markers. MAL is indicated as an important component of the exosome secretion apparatus [8]. Exosomes released by specific T-cell subsets can influence immune cell activity, including that of other T-cell subsets [6]. Thus, the MAL/exosomes pathway may be implicated in malignant development by mediating immune response. Currently, few studies have probed into the association between MAL with the prognosis and tumor immune microenvironment in different cancers. Therefore, in this work, we conducted a pan-cancer analysis to fully comprehend the role of MAL in tumor immunity in cancers. The expression profile and prognostic value of MAL were assessed using pan-cancer analysis. The findings of this study may provide novel target for immunotherapy in cancers.

2. Materials and methods

2.1. Data source

MAL expression profiles in various cancers in TCGA database were obtained utilizing UCSC Xena (https://xenabrowser.net/) [9]. Gene expression data for 54 normal tissue sites were also collected on the Genotype-Tissue Expression (GTEx) database [10]. For cancer-normal comparisons, normal samples collected in the TCGA and GTEx datasets were combined. The TISCH database is a tumor microenvironment-focused scRNA-seq database. The relation of MAL expression with tumor microenvironment was explored across cancer types using TISCH database in different cell types.

2.2. MAL expression profiles

We retrieved the consistent as well as normalized pan-cancer TCGA target GTEx (PanCAN) based on UCSC platform. Expression data for ENSG00000172005 (MAL) in a variety of samples was also retrieved. For each expression value, the samples were further log2 (x + 0.001) transformed. Finally, cancer with fewer than three samples was eliminated, resulting in expression profiles for thirty-four cancers.

2.3. Survival analysis

Survival analysis in different cancers was conducted using TCGA data. Hazard ratio (HR) was computed as well as log-rank P value with 95% confidence interval (CI). The "forestplot" package as well as "survival" package in R software were applied for result visualization with forest plots and survival curves.

2.4. Analysis of MAL expression and tumor immunity

The association of MAL expression with immunological checkpoints (such as PDCD1, CD28, and CTLA4) [11] and immunomodulatory genes (such as chemokine, receptor, MHC, immunoinhibitory, and immunostimulator) was analyzed with Spearman correlation analysis [12]. We also investigated the association between MAL expression and genomic features such tumor mutation burden (TMB) and microsatellite instability (MSI). MSI is a high mutator phenotype generated by frequent polymorphisms and single nucleotide changes in short repeat sequences, and successive MSI leads to TMB. These genetic markers are associated to carcinogenesis and are thought to be independent predictors of immunotherapy effectiveness [12]. The results were visualized as heatmaps by “reshape2” as well as “RColorBrewer” packages from R software.

2.5. Immune infiltration and enrichment analysis

"ESTIMATE" package in R was applied for the evaluation of tumor immune cell infiltration. The amounts of stromal and immunological components are shown in the stromal or immune fractions. ESTIMATE scores which depict tumor purity are calculated as the sum of stromal and immune values. Tumor Immune Estimation Resource 2.0 (TIMER 2.0) is a database that provides complete tools for determining immune infiltration across cancers [13]. The relationship between MAL and six different subsets of infiltrating immune cells or immune cell markers was explored using the TIMER methodology.

2.6. RNA sequencing of breast cancer samples (GSE103091)

An internal TNBC cohort of 238 patients was subjected to gene expression analysis using DNA chips. External data (n = 257) were used for validation, which were obtained using the same DNA chip. The groups were then functionally annotated after fuzzy clustering. The plasma cell and B lymphocyte infiltrations were tested with CD138 and CD20; MECA79 and CD31 were used to test for tertiary lymphoid structures; and UCHL1/PGP9.5 and S100 were used to test for neurogenesis.

2.7. Statistical analysis

The analysis of variance (ANOVA) tests and Student's t-test were used for comparisons of more than two groups and comparisons between two groups, respectively. Correlations were determined by applying the following R/rho values. 0-0.19 implied a markedly weak relation, 0.20-0.39 presented a weak relation, 0.40-0.59 represented a moderate relation, 0.60-0.79 implied a powerful relation as well as 0.80-1.00 implied an extremely high correlation. In most situations, a P value of less than 0.05 was set as the cut-off value.

3. Results

3.1. MAL expression and prognosis in human cancers

MAL mRNA was identified to be significantly up-regulated in the tumor samples of UCEC, PCPG, and CHOL compared to normal tissues, and was lowly expressed in

tumor samples of GBM, GBMLGG, LGG, LUAD, COAD, COADREAD, BRCA, ESCA, STES, KIRP, KIPAN, STAD, PRAD, HNSC, KIRC, LUSC, READ as well as KICH (Fig. 1A). Due to the small size of normal controls in some cancers in the TCGA database, we then combined the data of normal samples in both TCGA and GTEx databases, and found that MAL mRNA was also up-regulated in a few cancers not included in Fig. 1A, such as SKCM, THCA, OV, PAAD, TGCT and UCS, and was consistently down-regulated in the majority of cancers compared to normal controls (Fig. 1B). Tumor Immune Single-cell Hub (TISCH) was used to analyze MAL expression in TME across cancer types. As shown in the heatmap, MAL mRNA was lowly expressed in most malignant cells and was mainly secreted by T cells including CD4+ T cells, Tregs as well as CD8+ T cells (Fig. 1C).

The association between MAL expression and patient prognosis was analyzed using cox regression models based on clinical data and TCGA RNA-seq data of 44 TCGA tumor types. As shown in Fig. 1D, high MAL mRNA level exhibited significant association with the positive outcome in patients with KIPAN (N=855, p=6.6e-9, HR=0.88[0.85, 0.92]), KICH (N=64, p=7.2e-4, HR=0.57[0.37, 0.89]), KIRP (N=276, p=9.1e-4, HR=0.84[0.75, 0.93]), LUAD (N=490, p=1.7e-3, HR=0.85[0.78, 0.94]), GBMLGG (N=619, p=7.9e-3, HR=0.93[0.88, 0.98]), THYM (N=117, p=0.01, HR=0.81[0.68, 0.97]), KIRC (N=515, p=0.04, HR=0.94[0.88, 1.00]), BRCA (N= 1044, 0.05, HR= 0.93 [0.87, 1.00]). We also revealed that low MAL level was linked to adverse overall survival in GBMLUAD (p=2.7e-4, HR=0.62[0.48, 0.80]), LUAD (p=6.6e-4, HR=0.59[0.44, 0.80]), KIPAN (p=5.7e-9, HR=0.32[0.21, 0.48]), KIRC (p=0.01, HR=0.67[0.49, 0.92]), THYM (p=7.7e-4, HR=0.10[0.02, 0.50]), KICH (p=7.7e-4, HR=0.62[0.02, 0.50]) and BRCA(p=1.1e-3, HR=0.62[0.42, 0.81]) (Fig. 1E), suggesting the prognostic value of MAL across the cancers.

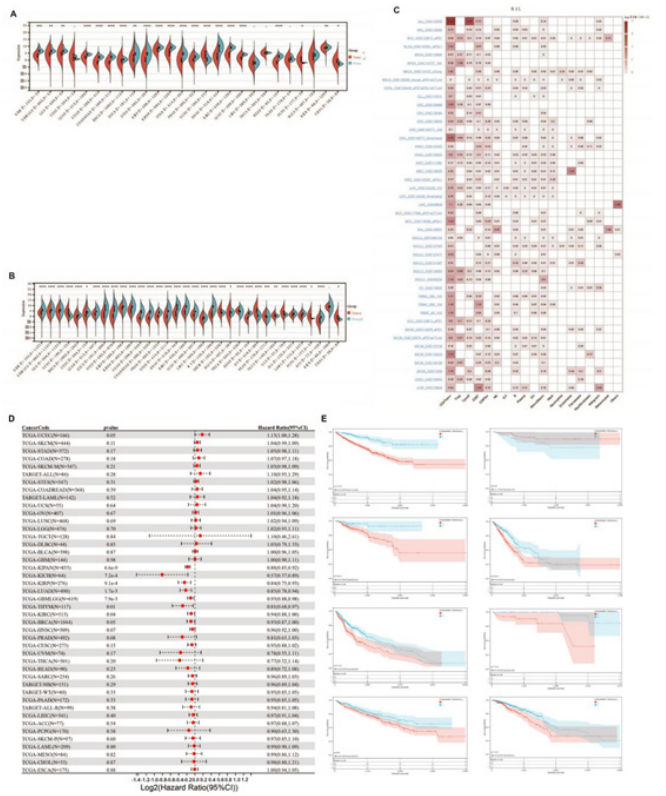

Fig. 1. MAL expression pattern and prognosis prediction across the malignancies. (A) Based on the TCGA database, MAL mRNA expression levels in tumor or normal samples in 26 types of cancer. (B) MAL mRNA levels in tumor as well as normal tissues in 33 types of malignancies of combined TCGA and GTEx datasets. (C) Heatmap of average gene expression across databases in a variety of cell types in different cancers. (D) A forest plot for the relation of high MAL mRNA levels with patient overall survival (OS) across the cancer. (E) The association of MAL mRNA with survival outcome in the GBMLGG, LUAD, KIRP, KIPAN, KIRC, THYM, KICH and BRCA cohorts.

3.2. Relation of MAL level and tumor immune infiltration

The association between MAL and tumor purity of GBMLGG, LUAD, KIRP, KIPAN, KIRC, THYM, and KICH was explored using the ESTIMATE method. MAL

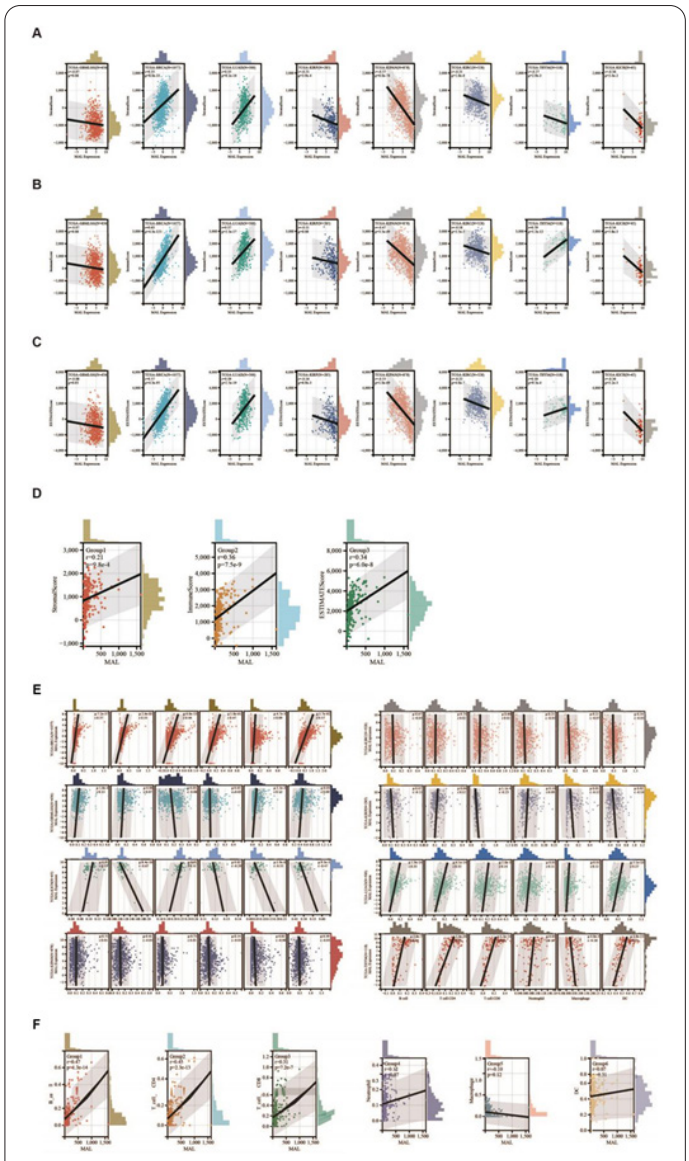

Fig. 2. Correlation between MAL expression and tumor purity or tumor immune infiltration in different cancers. (A-C) The connections of MAL levels and Stromal, Immune as well as Estimate Scores in GBMLGG, LUAD, KIRP, KIPAN, KIRC, THYM, KICH and BRCA. (D) The relationships between MAL levels and tumor purity were assessed by ESTIMATE method in 238 triple-negative breast cancer samples. (E) Scatter plots were used to show the correlation between ML expression and immune infiltration across the cancers. (F) Correlation between MAL level and immune cell infiltration in 238 triple-negative breast cancer samples.

level showed a positive correlation with stromal score in BRCA (r=0.35, p=8.03-33), LUAD (r=0.35, p=0.61e-16), and a negative correlation with stromal score in KIPAN (r=-0.57, p=6.0e-78) as well as KICH (r=-0.36, p=3.4e-3). We also found that MAL level was in positive association with the Immune Score in BRCA (r=0.63, p=0.43e-121), LUAD (r=0.37, p=2.5e-17) as well as THYM (r=0.59, p=1.2e-12) and negatively associated with the Immune Score in KIPAN (r=-0.47, p=3.5e-49) as well as KICH (r=-0.34, p=5.8e-3). Furthermore, MAL level was positively linked to prognostic score for BRCA (r=0.57, p=4.3e-95), LUAD (r=0.39, p=2.5e-19) as well as THYM (r=0.30, p=0.95e-4), whereas negatively correlated with prognostic score for KIPA (r=-0.55, p=1.0e-69) as well as KICH (r=-0.36, p=3.2e-3) (Fig. 2A-C). Then the association of MAL expression and tumor purity was explored in triple-negative breast cancer based on the GSE103091 dataset from the GEO database. MAL level was shown to correlate positively with immune scores in triple-negative breast cancer, which indicated the potential of MAL as a predictor of immune scores across the cancers (Fig. 2D).

Furthermore, the relation of MAL expression with tumor immune infiltration was explored. MAL levels were demonstrated to be in positive correlation with B cell infiltration level that occurred in BRCA (r=0.37, p=7.2e-37), LUAD (r=0.33, p=1.9e-14) as well as THYM (r=0.46, p=2.8e-7). MAL expression was found in positive correlation with CD4+ T cell infiltration in BRCA (r=0.55, p=2.6e-85), LUAD (r=0.33, p=4.1e-14) as well as THYM (r=0.73, p=1.4e-20), and negatively associated with the CD4+ T cell infiltration in KICH (r=-0.67, p=8.4e-10). For CD8+ T cells, the infiltration was found in positive association with the MAL level in BRCA (r=0.44, p=6.0e-53), KICH (r=0.31, p=0.01) as well as THYM (r=0.62, p=5.2e-14). Infiltration levels of neutrophils were in positive correlation with MAL expression in BRCA (r=0.47, p=1.8e-61). The macrophage infiltration was negatively related to MAL level in KICH (r=-0.55, p=1.9e-6) and THYM (r=-0.26, p=4.8e-3). MAL expression also showed a positive correlation with dendritic cell (DC) infiltration in BRCA (r=0.57, p=1.7e-92) and THYM (r=0.61, p=0.39e-13) but a negative correlation with DC infiltration in KICH (r=-0.47, p=0.93e-5) (Fig. 2E). In GSE103091 dataset, positive relationships were detected between MAL expression and B cells (r=0.47, p=1.3e-14), CD4+ T cells (r=0.45, p=2.3e-13) as well as CD8+ T cells (r=0.31, p=7.2e-7), suggesting that MAL level was related to immune infiltration in triple-negative breast cancer (Fig. 2F).

3.3. Relation of MAL expression with immune checkpoints and immunomodulatory genes across cancers

We further explored the association between MAL levels and immunological checkpoints as well as immunomodulatory genes across the cancers. MAL was significantly correlated with the expression of inhibitory or stimulatory immunological checkpoints such as ADORA2A, BTLA, CD27 and CCL5 in most malignancies, and the negative relation between MAL level with immune checkpoint expression was mainly found in THYM, KICH and KIPAN (Fig. 3A). Additionally, MAL level was mainly associated in a positive manner with chemokines, chemokine receptors, MHC, immunoinhibitory, and immunostimulatory across the cancers while showed negative correlation in the THYM and KIPAN (Fig. 3B), suggesting that MAL was co-expressed with the immunomodulatory genes.

3.4. Correlation of MAL levels with TMB and MSI across the cancers

Microsatellite instability and tumor mutation burden were independent predictors of ICB efficacy and essen-

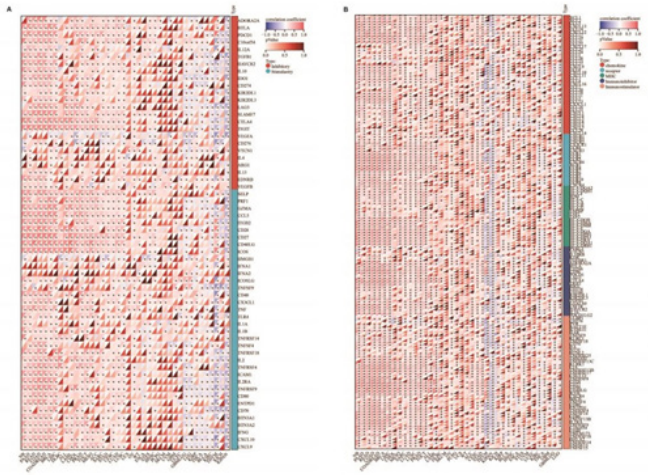

Fig. 3. Correlation of MAL expression with (A) immunological checkpoint and (B) immunomodulatory gene expression across forty cancer types. ***P < 0.001.

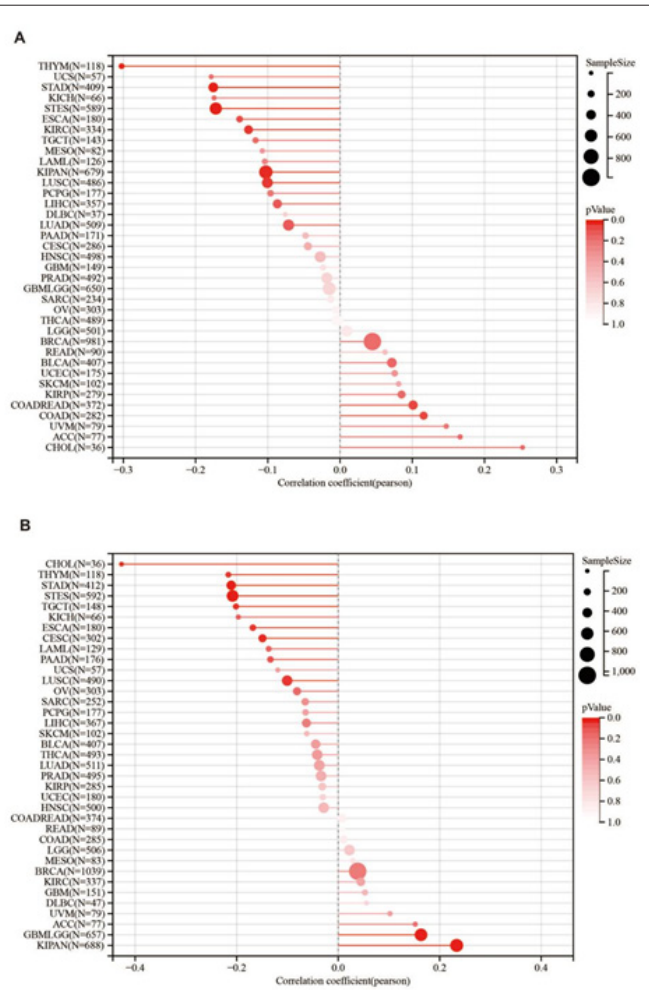

Fig. 4. Pearson correlation analysis between MAL level and (A) tumor mutation burden or (B) microsatellite instability across 37 cancer types

tially connected with the sensitivity of immune checkpoint inhibitors, and were critically involved in cancer initiation and development. Thus, we investigated the link between MAL expression and TMB or MSI across the cancers. We found that MAL expression was inversely associated with TMB in STES, KIPAN, STAD, KIRC, LUSC, and THYM ($P < 0.001$) (Fig. 4A). Additionally, MAL level was negatively connected with MSI in CESC, ESCA, STES, STAD, LUSC, THYM, TGCT, and CHOL, and positively related to MSI in GBMLGG and KIPAN (Fig. 4B).

3.5. Function analysis of MAL in different cancers

Enrichment of AML in KEGG pathways in different cancers was further analyzed using GSEA. MAL enrichment in LUAD as well as BRCA immune-related signaling pathways like B cell receptor signaling pathway, chemokine signaling pathway, cytokine receptor interaction and T cell receptor signaling pathway was observed (Fig. 5A-B). Additionally, HALLMARK enrichment analysis revealed that MAL was enriched in inflammation reactions as well as the IL6-JAK-STAT3 or IL2-STAT5 pathways (Fig. 5C-D). These results suggested that MAL was implicated in the regulation of immune response across human malignancies. Based on the TIMER2.0 database, we explored the association between MAL expression and immune infiltration levels in LUAD and BRCA with immunological markers. In both the LUAD and BRCA cohorts, the results were adjusted on the basis of tumor purity, and MAL was significantly correlated with the levels of markers of immune cells such as T cells, T helper cells, B cells as well as Natural killer T cells (Supplement Table 1).

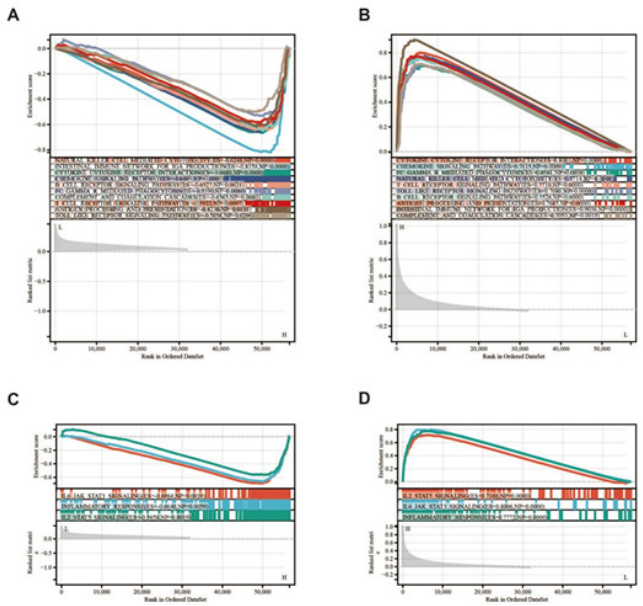

Fig. 5. GSEA for functional enrichment of KEGG and HALLMARK terms of MAL. (A-B) The top ten enriched KEGG pathways in. (C-D) The top three enhanced HALLMARK pathways in LUAD and BRCA.

4. Discussion

In the present study, we explored MAL expression patterns across cancers and identified MAL's differential expression pattern between tumor as well as normal tissues among human malignancies. MAL was lowly expressed among multiple malignancies observed in GBM, GBMLGG, LUAD, COAD, COADREAD, BRCA, ESCA, STES, KIRP, KIPAN, STAD, PRAD, HNSC, KIRC, LUSC, READ as well as KICH compared to GTEx normal controls based on TCGA and GTEx data. MAL, which is primarily expressed in T cells, was also revealed to be involved in immune infiltration of tumor microenvironment, suggesting a critical role for MAL in carcinogenesis.

Despite the effectiveness of new therapies such as immunotherapy, existing care of cancers such as locally advanced or metastatic breast cancer cannot achieve a positive prognosis [14]. The reason is that a large number of cancer patients do not respond to the current immunotherapy options. Some parameters can be examined at the gene level to assess the therapeutic potential of immunotherapy in cancers. Immune checkpoint genes such as CTLA-4 and IDO1 related to ICI efficiency are promising targets for immune checkpoint therapy [15]. Our findings showed that MAL with potential for risk classification was a promising biomarker for prognosis prediction in cancers. Similar to the MAL/exosomes system in T cells, the MAL/exosomes pathway is suggested to be involved in malignancies to mediate immune response in the oncologic milieu. Furthermore, in patients with LUAD or BRCA, the strong link between elevated MAL expression and a better prognosis was identical to that of CCL5 [16], CD27 [17], CD40LG [18], TNFRSF14 [19], IL2 [20], IFNG [21], CXCL9 [22], BTLA [23], PDCD1 [24] and IDO1 [15].

Another crucial conclusion of the current work was that MAL levels were strongly linked to immunological infiltration. In LUAD or BRCA, MAL expression is related to the immune cell infiltrates. These findings were validated with breast cancer samples from the GEO database. However, more research is needed to determine whether MAL performs these duties. Because MAL was engaged in multiple immunological pathways (KEGG and HALLMARK) in LUAD and BRCA, it was suggested to affect cancer patient prognosis via modulating immune process. Additionally, MAL was positively linked to immune cell markers including activated T cells and B cells, implying the immunity-dependent effect of MAL on patient survival. Moreover, MAL is also indicated to affect immune infiltration through the tumor microenvironment, altering their distribution and subsequent interactions with malignancies, and thus resulting in different survival outcomes in certain cancers.

There were certain limitations of the research. First, the projected outcomes have not been experimentally validated. Experimental validation should be carried out in future research using a variety of methodologies, such as immunocytochemistry, western blotting, and fluorescent quantitative polymerase chain reaction (qPCR). Furthermore, for diseases that have a long course of development, such as malignancies, Transcriptomic profiles do not always mirror proteomics, at least at some stages. The relation of protein aberrations with cancer malignancy requires further validation. Furthermore, because of the significant heterogeneity across different populations, the conclusion of our investigation is possibly not applicable to other research cohorts. As a result, more experiments and clinical researches of the expected outcomes are necessary in future research.

5. Conclusion

In conclusion, based on an integrated bioinformatics technique we found that MAL predicted favorable pro-

gnosis in cancer patients and mediated immune infiltration across the malignancies. It was suggested that MAL could be employed as a predictive biomarker and provide a novel option for targeted therapy in these tumors. Immunotherapy combining MAL targeted therapy with already available checkpoint inhibitors, is suggested as a highly successful and viable method against malignancies.

Conflict of interests
The authors declare no competing interests.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
Not applicable.

Informed consent
Not applicable.

Availability of data and material
If you have any additional questions about the study's original contributions, please contact the corresponding author.

Authors' contributions
ZR and JJ contributed to the study conceptualization. PX and HL contributed to the methodology. Formal analysis and investigation were performed by TY and SN. Writing original draft preparation was performed by LC, YY and YY. YD and YB commented on previous versions of the manuscript. Visualization was performed by HX, WZ and LR. Project administration was performed by LY and JJ. ZR and HL were responsible for the funding acquisition. JJ was in charge of supervision.

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