Integrated Pan-Cancer Analysis Reveals Distinct Clinical, Genomic and Immunological Features of the LILRB Immune Checkpoint Family in Acute Myeloid Leukemia

Zi-jun Xu  
Affiliated People's Hospital of Jiangsu University

Xin-long Zhang  
Affiliated Danyang Hospital of Nantong

Ye Jin  
Affiliated People's Hospital of Jiangsu University

Shi-sen Wang  
Affiliated People's Hospital of Jiangsu University

Yu Gu  
Affiliated People's Hospital of Jiangsu University

Ji-chun Ma  
Affiliated People's Hospital of Jiangsu University

Xiang-mei Wen  
Affiliated People's Hospital of Jiangsu University

Jia-yan Leng  
Affiliated People's Hospital of Jiangsu University

Zhen-wei Mao  
Affiliated People's Hospital of Jiangsu University

Jiang Lin  
Affiliated People's Hospital of Jiangsu University  
https://orcid.org/0000-0002-4704-9157

Jun Qian  
Affiliated People's Hospital of Jiangsu University

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Abstract

Background

Leukocyte immunoglobulin (Ig)-like receptor Bs (LILRBs), a family of type I transmembrane glycoproteins, are known to inhibit immune activation.

Methods

We comprehensively evaluated the transcriptional levels and prognostic significances of LILRB members in a broad spectrum of cancer types, focusing on its role in AML. In addition, we systematically characterized the genomic and immune landscape in AML patients with altered LILRBs expression.

Results

Here, we show that LILRBs were significantly dysregulated in a number of cancers, especially in acute myeloid leukemia (AML). Clinically, high expression of LILRB1-LILRB4 predicted poor survival in six independent AML cohorts. Genetically, LILRB1 was associated with more mutational events than other LILRB members, and multiple genes involving in immune activation were deleted in LILRB1-high patients. Epigenetically, LILRB4 was significantly hypomethylated and marked by MLL-associated histone modifications in AML. Immunologically, LILRBs were positively associated with monocytic cells including M2 macrophages, but were negatively associated with tumor-suppressive CD8 T cells.

Conclusions

Our findings reveal critical immunological and clinical implications of LILRBs in AML, and indicate that LILRBs may represent promising targets for immunotherapy of AML.

Background

Acute myeloid leukemia (AML) is a highly fatal hematopoietic malignancy marked by various cytogenetic and molecular abnormalities and variable responses to treatment [1–3]. Currently, the mainstay of treatment for AML is cytotoxic chemotherapy [4], yet chemoresistance and relapse are commonly seen in clinical practice. Some regimens under study, such as the combination of hypomethylating agents (HMAs) and Venetoclax, have shown promising results in certain subsets of AML patients [5, 6]. However, there remains an urgent need to develop novel effective therapies for various subsets of AML.

Noteworthy, immune checkpoint inhibitors (e.g., anti–PD-1 and anti–PD-L1 antibodies) have revolutionized cancer treatment during the past decade in treating cancers such as non–small-cell lung carcinoma and melanoma [7, 8]; however, the transfer of immunotherapy to AML has been less
successful than to other cancers [9]. Indeed, the AML microenvironment is predominantly immunosuppressive. For example, we have previously demonstrated that M2 macrophages, a classical immunosuppressive component, was preferentially enriched in AML than other hematological malignancies and normal controls [10]. Also, a recent single-cell RNA-seq study has reported proportionally fewer T cells and CTLs in AML than normal controls, and the function of these T cells are profoundly impaired, probably mediated by CD14+ monocyte-like cells [11, 12]. Moreover, Noviello et al. have reported that bone marrow T cells at AML relapse showed an exhausted phenotype, which was absent in patients maintaining long-term complete response [13]. These findings suggest encouraging therapeutic opportunities by modulating the immune environment in AML.

The leukocyte immunoglobulin (Ig)-like receptor subfamily B (LILRB) proteins are a group of type I transmembrane glycoproteins with extracellular Ig-like domains that bind ligands and intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [14]. This group of receptors contains 5 members (LILRB1-LILRB5) that are mainly expressed in hematopoietic lineage cells and also various types of tumors [14]. As these proteins negatively regulate immune activation [15–17], they are often considered as immunosuppressive component in the tumor microenvironment (TME). In AML, the TME-modulating role of LILRBs have recently came into focus, especially for LILRB4. As demonstrated by the Gui group that LILRB4 facilitates tissue infiltration of AML cells by substantially suppressing T cell activities, and blocking LILRB4 activity efficiently inhibited AML development in vitro and in vivo [18, 19]. In addition, LILRB1, which showed predominant expression in monocytic AML cells as LILRB4 did [20], was found to be up-regulated in dysfunctional CD8+ T cells from AML than T cells from healthy controls [21]. Interestingly, a non-immunological AML-promoting role was reported for LILRB2, which binds Angptl2 to maintain stemness of normal stem cells and support leukemia development by inhibiting differentiation of AML cells [22]. Despite the functional importance of LILRBs in cancers, there currently lacked a systematic study to explore the expression patterns and clinical implications of all LILRB members in pan-cancers, especially in AML. Therefore, in this study, drawing on rich multi-omics data in the public domain, we comprehensively evaluated the transcriptional levels and prognostic significances of LILRB members in a broad spectrum of cancer types, focusing on its role in AML. In addition, we systematically characterized the genomic and immune landscape in AML patients with altered LILRBs expression.

Materials And Methods

Analysis of gene expression data

Briefly, the mRNA expression data of LILRB family in normal tissues were obtained from the Genotype-Tissue Expression (GTEx) project (www.gtexportal.org/) [23]. To confirm the expression patterns of LILRBs in normal tissues, we then explored the HPA (Human protein atlas) and FANTOM5 dataset from the human protein atlas database (http://www.proteinatlas.org/) [24]. Expression data of LILRBs for over 1000 cancer cell lines from various organ sites were accessed through Cancer Cell Line Encyclopedia (CCLE) (https://www.broadinstitute.org/ccle) [25]. Furthermore, RNA-seq data of 64 cell lines from The
Human Protein Atlas (HPA) ([https://www.proteinatlas.org/](https://www.proteinatlas.org/)) [24] were used to validate expression patterns of **LILRBs** in cancer cell lines.

To determine the expression patterns of **LILRBs** between tumor and adjacent normal tissues across a broad range of cancer types, we systematically analyzed the gene expression data of 9465 tumor and 7831 normal samples based on RNA sequencing data from the TCGA and the GTEx projects. All these datasets were downloaded from the UCSC Xena project and were normalized between arrays using the limma package [26]. To validate the differential expression of **LILRBs** between AML and normal controls, we further retrieved two datasets containing both healthy and AML samples from Gene Expression Omnibus (GEO) ([https://www.ncbi.nlm.nih.gov/geo/](https://www.ncbi.nlm.nih.gov/geo/)) (accession number GSE63270 and GSE30029). We also used a gene-expression dataset of normal hematopoietic cells (GSE42519) to assess the expression patterns of **LILRB** family genes at various stages of normal hematopoiesis.

We used the Hemap dataset curated by Dufva et al. [27], which includes datasets of AML, pre-B-ALL, DLBCL, and MM, to analyze the association between **LILRBs** expression and common molecular subtypes. The molecular subtypes were defined as previously described [27]. Three datasets-BeatAML, TCGA, and GSE13159—which have detailed cytogenetic information, were used to determine whether **LILRB4** expression was associated with MLL-rearranged AML. The counts per million (CPM) table of BeatAML were downloaded from the Supplementary Materials from Tyner et al. [28]. **LILRB4** expression level were log2 (1 + CPM) transformed before visualization. We computed single-sample GSEA (ssGSEA) enrichment scores for four MLL-r-related gene signatures from MSigDB ([http://www.gsea-msigdb.org/gsea/msigdb/](http://www.gsea-msigdb.org/gsea/msigdb/)) using the R ‘GSVA’ package.

**Analysis of AML single-cell RNA-sequencing (scRNA-seq) data**

For single cell RNA-seq (scRNA) data analysis, previously published scRNA-seq data from 16 AML samples at diagnosis consisting of 30,712 bone marrow (BM) cells (Van Galen AML scRNA) were downloaded from GEO (GSE116256) [11], another scRNA-seq data for 8 patients consisting of 30,579 AML BM cells (FIMM AML scRNA) were retrieved via the Synapse Web Portal ([https://www.synapse.org](https://www.synapse.org) and doi: 10.7303/syn21991014). Data was processed and visualized using custom scripts provided by Dufva et al. [27].

**Analysis of genetic alteration data**

The genetic alterations of **LILRBs** from TCGA PanCancer Atlas studies (10967 patients), including somatic mutations, amplification, and deep deletion were assessed through the cbioportal for Cancer Genomics ([http://www.cbioportal.org](http://www.cbioportal.org)). To determine the association between **LILRBs** expression and common gene mutations, patients from the TCGA LAML cohort were first stratified into two groups by the median expression value of respective member genes, then mutation status for 24 most frequently mutated genes were identified using TCGA mutational data. The relationships between mutation status and **LILRBs** expression were analyzed by two-sided Fisher exact tests. The mutational profiles of patients
with high or low \textit{LILRB1}/\textit{LILRB5} expression were displayed as co-bar plots using the “Maftools” package \cite{29}. To detect copy number alterations (deletions and amplications) in high- and low-\textit{LILRB1} expressers, we analyzed filtered segmented copy number data in each group (Affymetrix SNP 6.0 platform) using the GISTIC 2.0 algorithm \cite{30}.

### Analysis of gene methylation data

For comparison of methylation status of \textit{LILRBs} between tumor and normal samples, beta values of Illumina 450k probes at the promoter region of five genes were retrieved by DiseaseMeth version 2.0 web portal (http://bio-bigdata.hrbmu.edu.cn/diseasemeth/analyze.html). Correlation between \textit{LILRBs} methylation with expression and survival data across cancers were analyzed through the GSCALite platform \cite{31}. For AML, the DiseaseMeth dataset contains methylation data of 271 AML (from TCGA, GSE62303, and GSE64934) and 10 normal samples (from GSE58477). Another methylation dataset-GSE63409-contains DNA methylation profiles of 20 leukemia stem cells, 24 blast cells and 30 normal hematopoietic stem and progenitor cells. Beta values of Illumina 450k probes nearest the transcription start site of the genes were selected to represent methylation level of the gene promoter area. Heatmaps displaying methylation levels of \textit{LILRBs} across samples were generated using ‘pheatmap’ package.

### Analysis of Chromatin immunoprecipitation-sequencing (ChIP-seq) data

ChIP-seq data for MLL-fusion proteins and three histone marks (H3K79me2, H3K27ac, and H3K4me3) from MV4-11 and THP-1 cells were obtained from GSE79899 \cite{32}. For validation purpose, the three epigenetic marks from five other ChIP-seq datasets (H3K79me2 from GSE82116 and GSE71779; H3K27ac from GSE89336 and GSE71776; H3K4me3 from GSE61785 and GSE82116) were also included in our analyses. The gene tracks were generated by uploading the wiggle files as custom tracks onto the UCSC Genome Browser, assembly hg19. Wiggle files that have failed to be loaded as custom tracks were first converted to bigwig using the UCSC wigToBigWig tool.

### Survival Analysis

We used the “Gene Outcome” module of TIMER2.0 (http://timer.cistrome.org/) \cite{33} to investigate the association between the expression of \textit{LILRB} members and clinical outcomes across 33 cancer types. The association between transcript levels of \textit{LILRB} members and overall survival (OS) across cancers were assessed by univariate Cox regression. To confirm the prognostic value of \textit{LILRBs} in AML, we further obtained five independent GEO datasets (GSE10358, n = 304; GSE37642 [U133A], n = 422; GSE37642 [U133plus2], n = 140; GSE106291, n = 250; GSE71014, n = 104) with available survival information. AML patients from these datasets and the TCGA dataset were divided into those with high and low gene expression, according to the optimal cut-off determined by the X-tile method \cite{34}. We then performed Kaplan-Meier analysis (log-rank test) to compare the survival differences of two groups regarding overall survival (six datasets) and event-free survival (EFS) (only in TCGA dataset).

### Immune response analysis
The relative abundances of 22 immune cell populations in AML patients were estimated using the CIBERSORT algorithm as previously described [10]. As CIBERSORT may not be suitable for the use of the RNA-seq data [35], this algorithm was exclusively applied to the TCGA LAML microarray dataset. For validation purpose, the relative fractions of immune cells were also estimated in two relatively large GEO datasets: GSE10358 and GSE6891. In addition, we used other deconvolution methods to quantify the proportions of monocytes (quanTIseq, MCP-counter, CIBERSORT abs, and xCell) and CD8 T cells (EPIC, TIMER, quanTIseq, MCP-counter, CIBERSORT abs, and xCell). These methods have been integrated as a unified interface by Sturm et al. [36] and are freely available through the TIMER 2.0 web portal (http://timer.comp-genomics.org/).

We evaluated the relationship between LILRBs and several notable immune checkpoint genes describe by De Simone et al. [37]. Spearman correlation analysis was used to test the association between LILRBs expression and these parameter estimates.

**Differential gene expression analysis and functional enrichment analysis**

Differential gene expression analysis for RNA sequencing data was performed using the raw read counts with the R/Bioconductor package “DESeq2”, controlled for the false discovery rate (FDR) by the Benjamini–Hochberg procedure. Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of LILRB1-coexpressed genes were performed using the STRING database (http://www.string-db.org/). GO and KEGG terms with false discovery rate (FDR)-corrected p values less than 0.05 were considered as significantly enriched. For displaying purposes, the top 10 GO terms of each three GO categories—biological process (BP), cellular component (CC), and molecular function (MF), and the top 10 KEGG pathway terms were visualized as bar plots.

**Protein-protein interaction (PPI) network analysis**

We applied STRING (http://string.embl.de/) to construct a protein-protein interaction (PPI) network of the differentially expressed genes (DEGs). We chose a confidence score > 0.9 as the judgment criterion. Cytoscape visualization software (version 3.6.1) was used to present the LILRB1-related sub-network.

**Gene Set Enrichment Analysis (GSEA)**

Gene set enrichment analysis (GSEA) was performed on the TCGA dataset using GSEA v4.1.0 software (http://www.broad.mit.edu/gsea). Statistical significance of GSEA results was determined by 1,000 gene set permutations, with signal-to-noise gene ranking. All the gene sets used in this study were obtained from GSEA MSigDB website (http://software.broadinstitute.org/gsea/msigdb/index.jsp), and the gene sets were considered to be significantly enriched at a false discovery rate < 0.25 and normalized P-value < 0.05. Three categories of gene sets were used in this study: C2, curated gene sets containing genes coregulated in response to specific perturbations; C7, immunologic signature gene sets that represent cell states and perturbations within the immune system; and H, hallmark gene sets which represent well-defined biological states or processes.
Statistical analysis and visualization

Wilcoxon rank sum tests were used to compare differences between two groups. Specifically, for Fig. 6a, we determined differential expression of LILRBs between each molecular subtype and the remaining samples for each disease subtype. The average fold changes (FCs) and Bonferroni-adjusted p-values (false discovery rate [FDR]) were computed using Wilcoxon rank sum tests. The FDR values were then combined for each LILRB members using Stouffer’s method and categorized into five groups based on significance cutoffs for visualization (0.05, 0.01, 0.001, 1e-5, 1e-16). All statistical analyses and visualizations were performed using either indicated web servers or R version 4.0.4. Specifically, the expression map of LILRBs was generated using the “gganatogram” package [38], the box, bar, scatter and bubble plots were produced with the R package “ggplot2”, “ggpubr” and “ggsci”, the volcano plots were generated using the “EnhancedVolcano” package, and survival curves were made using the “survival” package. The correlation matrix was calculated and visualized using the “corrplot” or “ggcor” library. Finally, “circlize” was used to create the chord diagrams. All statistical tests were two-sided with p-values less than 0.05 considered significant.

Results

Expression patterns of LILRBs in normal tissues and cancer cell lines

In our first attempt, the expression patterns of LILRBs in different human tissues were determined based on RPKM values using GTEx [39] (http://www.GTExportal.org/home/). We note that the highest expression of LILIBs were observed in spleen, followed by blood and the lung tissue, while in other tissues, they were only weakly expressed (Fig. 1a and Supplementary Figure S1). Importantly, the preferential enrichment of LILRBs in spleen was further validated in the FANTOM5 and HPA (Human protein atlas) dataset (Supplementary Figure S2 and S3). Next, we explored the expression profiles of LILRBs in cancer cell lines from Cancer Cell Line Encyclopedia (CCLE). As shown in Fig. 1b and Supplementary Figure S4, LILRBs showed relatively high expression in cell lines of malignant hematological cell lines, such as acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), lymphomas, and multiple myeloma (MM). We also analyzed the expression levels of LILRBs based on RNA-seq in 64 different cell lines, as part of the Cell Atlas in the HPA. This analysis confirmed that LILRBs was highly expressed in cell lines of the myeloid and lymphocytic origin (Supplementary Figure S5). Notably, LILRB1 and 2 showed higher expression in monocytes and the THP1 monocyte cell line. Together, these findings indicated a cellular-, tissue-, and disease- specificity of LILRBs expression.

Analysis of LILRB family gene expression levels in tumor and non-tumor tissues
Previous studies revealed that members of the *LILRB* family were upregulated in a number of cancers [40, 41]. Here, using pan-cancer datasets from the TCGA project, we found that the expression levels of *LILRB1*-*LILRB5* were positively correlated with each other (Fig. 1c), suggesting they may share some common features or functions; of them *LILRB5* showed relatively weak correlation with the other four genes. Combining the normal tissue of the GTEx dataset as controls, we then systematically compared *LILRBs* expression between tumor and adjacent normal tissue across 28 cancer types (9465 tumor and 7831 normal samples). Surprisingly, *LILRBs* were significantly dysregulated in almost all cancer types (Fig. 1d and Supplementary Figure S6). For *LILRB1*, *LILRB2*, and *LILRB4*, increased expression in tumors was more commonly seen; whereas *LILRB3* and *LILRB5* were significantly down-regulated in the majority of cancer types (Fig. 1d and e). For *LILRB1*-*LILRB4*, the most remarkable difference was observed between AML and normal its normal counterparts (Fig. 1d and Supplementary Figure S6). We also found that *LILRB1*-*LILRB4* were highly expressed in glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), pancreatic adenocarcinoma (PAAD), and skin cutaneous melanoma (SKCM), whereas they were markedly decreased in adrenocortical carcinoma (ACC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and thymoma (THYM), as compared with normal controls (Fig. 1d and Supplementary Figure S6).

**LILRBs were significantly up-regulated in AML and dynamically expressed during normal hematopoiesis**

Our observations, together with previous findings [18], reflect an AML-specific expression patterns of *LILRBs*. We thus further explored the differential expression of *LILRBs* in two independent AML datasets with normal controls available. For these two datasets, only *LILRB1* and *LILRB3* were consistently up-regulated in AML (Fig. 1f and g), whereas *LILRB5* was down-regulated (Supplementary Figure S7a and b). Interestingly, *LILRB4*, which was previous shown to be increased in AML [18], exhibited no difference in expression in the two datasets (Fig. 1f and g).

We next sought to determine the expression patterns of *LILRBs* during normal hematopoiesis using a published data (GSE42519) [42]. We found that the transcripts of *LILRBs* was relatively high in bone marrow (BM) hematopoietic stem cells (HSCs), with a gradual diminishment when HSC differentiated to committed myeloid progenitors. The expression remained low in megakaryocyte-erythroid progenitor (MEP) and then steadily increased during myeloid maturation, reaching the highest level of expression in the mature polymorphonuclear cell (PMN) (Figure S7c). This dynamic regulation of *LILRBs* transcripts suggests a potential role of *LILRBs* in HSC biology.

**Landscape of genetic alterations of LILRBs in tumors**

We also investigated genetic alterations (including mutations, amplifications, and deletions) frequencies of *LILRBs* across pan-cancers. The average alteration frequencies of five genes were summarized in Fig. 4a and the oncoprint was present in Supplementary Figure S8a. The highest mutation loads of *LILRBs* was observed in SKCM (31.53%), followed by uterine corpus endometrial carcinoma (UCEC).
(14.56%), LUAD (14.49%), and LUSC (13.14%) (Fig. 4a and c). Overall, \textit{LILRB1} was the most highly mutated and \textit{LILRB3} the least; the most frequent genomic variants were missense mutations for five genes (Fig. 4b and Supplementary Figure S8b). For diffuse large B-Cell lymphoma (DLBC), mesothelioma (MESO), and THYM, copy number alterations (CNAs) were the only genetic events (Fig. 4a). Amplifications were more commonly seen in cancers such as ACC, uterine carcinosarcoma (UCS), and bladder urothelial carcinoma (BLCA), while deletions were mostly found in cancers like brain lower grade glioma (LGG), ovarian serous cystadenocarcinoma (OV), and testicular germ cell tumor (TGCT) (Fig. 4a and d). In AML where \textit{LILRBs} expression were markedly changed, however, genetic alteration frequencies of these genes were extremely low, suggesting other mechanisms might contribute to the abnormal \textit{LILRBs} expression in AML (Fig. 4a, c, and d).

**LILRBs were hypomethylated in AML**

We next asked whether DNA methylation regulates the expression of \textit{LILRBs} in cancers. We first retrieved the methylome data of \textit{LILRBs} across 30 cancer types with matched controls through the human disease methylation database Diseasemeth version 2.0 (http://bio-bigdata.hrbmu.edu.cn/diseasemeth/). Surprisingly, we found that \textit{LILRB} members were significantly hypomethylated in almost all cancer types analyzed as compared to normal samples (Fig. 3a). We then investigated the correlation between methylation and expression levels of \textit{LILRBs} using GSCALite [31]. We found that methylation level of \textit{LILRB1} was negatively associated with its mRNA expression in most cancer types (Fig. 3b). For other \textit{LILRB} members, methylation and expression levels were also mainly negatively correlated, with only a few positive correlations (Fig. 3b). Importantly, consistent with the increased expression of \textit{LILRBs} in AML, significantly hypomethylated promoters of \textit{LILRBs} were observed in both the Diseasemeth (AML, n = 271; normal, n = 10) and GSE63409 dataset (AML, n = 44; normal, n = 30) (Fig. 3c and d). We then studied the correlation between promoter methylation and expression of \textit{LILRBs} in TCGA AML dataset. Interestingly, expression of \textit{LILRB2}, \textit{LILRB3}, and \textit{LILRB4} correlated negatively with promoter methylation and the most significant correlation was observed for \textit{LILRB4} (Fig. 3b and e). This observation is consistent with a previous report that decitabine (DAC, a demethylating agent) treatment with AML cells remarkably promoted expression of \textit{LILRB} family members, especially \textit{LILRB4} [43]. Collectively, these results suggest that DNA hypomethylation might contribute to the activation of \textit{LILRB} members in AML. Further, analyzing the relation between methylation and survival revealed that hypomethylation of \textit{LILRBs} predicted worse survival in most cancers (Fig. 3f). In AML, hypomethylation of \textit{LILRB4} was associated with adverse outcome and hypomethylation of \textit{LILRB5} with favorable outcome (Fig. 3f).

**Adverse prognostic impact of LILRBs in AML**

Next, we used Cox regression analyses to explore the association between \textit{LILRBs} expression and overall survival (OS) in TCGA pan-cancer datasets. Overall, we found the significance and direction of the prognostic significances varied, depending on the cancer types analyzed. For example, increased expression of \textit{LILRB} family members were generally associated with worse OS in KIRC, LAML, LGG, TGCT, THYM, and uveal melanoma (UVM). While in SKCM and metastatic SKCM, the reverse was observed
Interestingly, a previous study has also performed Cox analyses in TCGA data, showing that **LILRB1-LILRB4** negatively impacts the survival of AML patients. It is of particular interest to validate the prognostic value of **LILRBs** using Kaplan-Meier methods in larger patient cohorts of AML. To this end, we collected five independent datasets from GEO; X-tile was used to determine the optimal thresholds for each **LILRB** members in TCGA and GEO datasets. First, we were able to validate the adverse prognostic impact for **LILRB1-LILRB4** in the TCGA cohorts (Fig. 4b), whereas high **LILRB5** was associated with favorable outcome (Supplementary Figure S9). Importantly, the prognostic value of **LILRB1-LILRB4** also extended to the event-free survival (EFS) endpoint and cytogenetically normal (CN)-AML subsets (Supplementary Figure S10a-c). Furthermore, the adverse prognostic impact of **LILRBs** was validated in TCGA microarray data (n = 183) (Supplementary Figure S10d) and other five independent cohorts of AML patients (GSE10358, n = 304; GSE37642 [U133A], n = 422; GSE37642 [U133plus2], n = 140; GSE106291, n = 250; GSE71014, n = 104) (Fig. 5a-e), although in some cases only a trend for shorter OS was observed. For subsequent analyses, we will focus on the role of **LILRB1-LILRB5** in AML, due to frequent alterations and high prognostic values of **LILRBs** in this malignancy.

**LILRB4 is aberrantly overexpressed in MLL-rearranged AML and may be a target of MLL fusion proteins**

We next asked whether **LILRBs** expression could be associated with specific molecular subtypes in AML. To this end, we examined the expression differences of **LILRBs** across published transcriptomic subtypes in the Hemap dataset (including AML, pre-B-ALL, DLBCL, and MM) [27]. As expected, all five **LILRB** members were more highly expressed in monocyte-like AML, while their expressions were relatively weak in the other three malignancies (Fig. 6a). One unanticipated exception to this overall trend was the strong enrichment of **LILRB4** in MLL-rearranged AML (Monocyte-like - MLL) and ALL (KMT2A) (Fig. 6a). To confirm this observation, we subsequently analyzed the transcript levels of **LILRB4** in 15 leukemia cell lines with or without MLL-rearrangements from the CCLE database. Leukemia cell lines with presence of MLL-fusion genes exhibited markedly higher **LILRB4** expression than those lack MLL-fusion genes, whether **LILRB4** expression was detected by RNA-seq (Fig. 6b) or Affymetrix microarray (Supplementary Figure S11a). Accordingly, analysis of three large primary patient datasets (BeatAML, TCGA, and GSE13159) revealed consistently highest **LILRB4** expression in MLL-rearranged AML as compared to other cytogenetic/clinicopathologic leukemia entities (Fig. 6c, Supplementary Figure S11b and c). To further confirm the relevance of **LILRB4** expression in MLL-rearranged AML, we collected four MLL-rearrangement-related gene signatures from MSigDB and computed ssGSEA scores of these signatures for each sample in the TCGA dataset. Then, we compared the ssGSEA scores computed for high **LILRB4**-expressing samples with those in low **LILRB4**-expressing samples. We found gene-sets down-regulated in MLL-rearranged AML (MULLIGHAN_MLL_SIGNATURE_1_DN) showed significantly lower ssGSEA scores in **LILRB4**-high patients than in **LILRB4**-low patients; whereas for gene-sets up-regulated in MLL-rearranged AML (MULLIGHAN_MLL_SIGNATURE_1_UP), the opposite was seen (Fig. 6d). Also, the ssGSEA scores of two MLL-rearranged-governed signatures (ROSS_AML_WITH_MLL_FUSIONS and
VALK_AML_WITH_11Q23_REARRANGED) were significantly up-regulated in high LILRB4 expressers (Supplementary Figure S11d).

It has been shown that target genes of MLL-fusions were often hypomethylated [44, 45], which is consistent with our previous observations. Also, promoters of these genes were often enriched with transcription activation-associated histone marks (H3K79me2, H3K27ac, and H3K4me3) [32]. To determine whether LILRB4 expression could be directly regulated by MLL-fusion gene, we analyzed a published ChIP-seq dataset (GSE79899) of MLL-fusion proteins, H3K79me2, H3K27ac, and H3K4me3 for MV4-11 (MLL-AF4) and THP-1 (MLL-AF9) cell lines. We found a significant enrichment of MLL-N proteins in the promoter regions of LILRB4 gene for both cell lines, while punctuated binding peaks of H3K79me2, H3K27ac, and H3K4me3 were observed in both the promoter and gene body of LILRB4 (Fig. 6e). Importantly, a similar enrichment of the three epigenetic marks was seen in five other ChIP-seq datasets (H3K79me2 from GSE82116 and GSE71779; H3K27ac from GSE89336 and GSE71776; H3K4me3 from GSE61785 and GSE82116) (Fig. 6f). Overall, these results suggest that MLL fusion proteins may be a direct regulator of LILRB4 expression.

LILRB1 expression correlates with distinct genomic alterations in AML

We then examined the associations between LILRBs expression and the clinical and genetic characteristics in the TCGA AML cohort. We found an association between LILRBs expression and the French-American-British (FAB) classification of AML: a higher percentage of myelomonocytic or monocytic morphology (M4/M5 subtypes) and a lower percentage of FAB M2/M3 was observed in patients with high LILRBs expression (Fig. 7a). Moreover, high LILRBs expressers were more likely to be >60-year-old and less likely to present with favorable cytogenetics (Fig. 7a).

To determine whether LILRB1-LILRB5 correlated with distinct mutational profiles characterized for AML, we identified significantly mutated genes occurred in patients with high and low LILRB1-LILRB4 expression (as stratified by the median expression value of respective genes), using curated mutational data from TCGA. Overall, we found LILRB1 and LILRB5 expression was associated with more mutational events than the other three genes (Fig. 7b). As shown in Fig. 7c, patients with high LILRB1 expression had higher frequency of mutations in U2AF1 (7% vs 1%) and RUNX1 (14% vs 4%), while IDH1 (14% vs 4%) was more frequently mutated in those with low LILRB1 expression. High LILRB5 expression was positively correlated with TP53 mutations and negatively correlated with FLT3 and WT1 mutations. For other three genes, LILRB2 was associated with mutations in IDH1 and STAG2, LILRB3 with WT1, and LILRB4 with RUNX1 (Fig. 7b).

To further explore the association between LILRBs expression and copy number variation (CNV), we performed GISTIC2.0 analysis of TCGA copy number data and assessed copy number alterations between in two patient groups. We focused on LILRB1, as it was consistently dysregulated and showed the greatest mutational events in AML patients. Interestingly, LILRB1-low patients had no somatic copy number alterations (Supplementary Figure S12), whereas LILRB1-high patients had 14 significantly
deleted regions and four significantly amplified regions (FDR = 0.25) (Fig. 7d). Interestingly, the majority of genes deleted in LILRB1-high patients were involved in inflammatory responses (including cytokines and genes essential for microbial killing and antigen processing and presentation, see Supplementary data 1 for detail). Also, a number of genes belong to the cadherin (CDH) and protocadherin (PCDH) family, which often exert tumor-suppressive functions [46], were significantly deleted. In contrast, LILRB-high AML patients had recurrent amplification at loci essential in AML pathogenesis, including KMT2A and ERG [47, 48] (Fig. 7d).

Correlations Between LILRBs and Tumor Immune Infiltrating Cells (TIICs) in AML

Considering that LILRBs might play important roles in the TME, we further explored the correlations between LILRBs and the level of immune cell infiltration in TCGA AML cohort. It is noteworthy that, among the 22 cell types, monocytes had the highest positive correlations with LILRB1-LILRB4 (Fig. 8a), consistent with previous finding that LILRBs were preferentially expressed in monocytic AML [19, 49]. This monocytic preference was also confirmed in two recently published scRNA-seq datasets of AML (Van Galen AML scRNA, Fig. 8b and FIMM AML scRNA, Supplementary Figure S13a). Interestingly, LILRB4 was exclusively correlated with M2 macrophages (Fig. 8a), a high immunosuppressive component in the TME. By contrast, LILRB1-LILRB4 were negatively correlated with the infiltrating levels of tumor-suppressive immune cells, such as resting T cells CD4 memory cell, CD8 T cells, memory B cells, plasma cells, and resting NK cells (Fig. 8a). Similar results were found by analyzing the CIBERSORT estimates in the GSE10358 and GSE6891 dataset (Supplementary Figure S13b and c). Importantly, when other methods were used for calculating the relative fractions of TIICs, positive associations between LIRB1-LILRB4 and monocytes were consistently seen, while negative associations between LILRB4 and CD8 T cells were proved for most-if not all-methods in all three datasets (Supplementary Figure S13d-f). Further analysis of normal cell populations from the Hemap dataset revealed that LILRBs were highly expressed in myeloid lineage immune cells (monocytes, macrophages, dendritic cells, myeloid progenitors, and neutrophils), with consistent low expression in T cells (CD4+ T cells and T/NK cells) (Fig. 8c). Collectively, these findings further confirmed the immunosuppressive roles of LILRBs in cancer TME.

Correlation between LILRBs and immune checkpoints in AML

Given that immune checkpoints have been proved to be promising therapeutic target for cancer treatment, we therefore evaluated the relationship between LILRBs and a collection of checkpoint genes describe by De Simone et al. [37]. Results from Spearman correlation analyses are given in Supplementary Data 2. As shown in the Circos plots, LILRB1-LILRB3 all showed strong positive correlations with CD86, VISTA, and HAVCR2 (Fig. 8d-f), indicating a possible synergistic effect between these genes. In contrast, no significant correlations were observed between LILRB4/5 and these
checkpoints (Supplementary Figure S14a and b). These results further highlight LILRBs potentially as major signaling pathways involved in immunosuppression in the AML microenvironment.

The biological significance of LILRBs expression in AML

We then sought to investigate the biological features associated with LILRBs in AML. Since the expression of five LILRB members were highly correlated, a comparison of gene expression profiles of patients with high and low LILRB1 expression (as determined by the median expression value) was performed. Overall, 799 genes (490 up- and 309 downregulated; adjusted p < 0.05; log2FC ≤ −1.5 or log2FC ≥ 1.5) were differentially expressed in LILRB1<sup>high</sup> versus LILRB1<sup>low</sup> patients (Fig. 9a and Supplementary Data 3). Among the genes positively correlated with LILRB1 were, as expected, the other members of the LILRB family (Fig. 9a). Also, genes associated with presence of monocytes/macrophages (CD14, CD68) or M2 macrophage polarization (MSR1, MRC1, CD163) were significantly up-regulated in high LILRB1 expressers (Fig. 9a), in line with our previous findings. Next, we used STRING database to construct a protein-protein interaction (PPI) network of the differentially expressed genes (DEGs), with a confidence score > 0.90. Genes interacted with LILRB1 and their sub-networks were shown through Cytoscape software (Fig. 9b). We found 12 genes directly interacting with LILRB1: PILRA, TLR8, SIGLEC7, CD300C, FCGR2A, FCGR2B, FCGR3A, CD86, FGR, HCK, IL10, ITGAX.

Among them, CD300C, FCGR2A, FCGR2B, and FCGR3A also had connections with the other four LILRB members (Fig. 9b). GeneMANIA results also revealed that genes of the FCGR and CD300 family were closely correlated with LILRBs. These genes were mainly involved in negative regulation of leukocyte mediated immunity and negative regulation of immune system process (Supplementary Figure S15a).

We then performed GO analysis using these DEGs and the top 10 significant terms of BP, MF and CC enrichment analysis were shown (Fig. 9c). Notably, in terms of BP, immune response-related processes were significantly enriched, such as inflammatory response, immune system process, and immune response. KEGG and Reactome Pathway analyses also revealed immune response pathways, including cytokine – cytokine receptor interaction, cytokine signaling in immune system, innate immune system, antigen processing – cross presentation, and adaptive immune system were mainly enriched (Fig. 11d and Supplementary Figure S15b).

Finally, GSEA was conducted in the LILRB1<sup>high</sup> and LILRB1<sup>low</sup> cohorts. For the C2 collection of curated gene sets from the MSigDB, the VALK_AML_CLUSTER_5 gene set (96% of the samples are FAB M4 or M5 subtype) was predominantly enriched in LILRB1<sup>high</sup> group. Also enriched were gene sets of MLL-fusion and NPM1-mutation, two distinct entities often associated with monocytic features of AML (Fig. 10a). For the C7 immunologic collection, the LILRB1<sup>high</sup> group had principal enrichment in genes up-regulated in monocytes compared to other immune cells (Fig. 10b), and multiple immune activities were enriched in the LILRB1<sup>high</sup> group for HALLMARK gene sets (Fig. 10c).

Discussion
The LILRB family members-LILRB1-5-are a group of proteins containing the immune-inhibitory ITIM motifs which negatively regulate immune cell activation [14]. Here, using RNA-seq data of normal tissues from GTEx, FANTOM5, and HPA, we showed that LILRB members were predominantly enriched in the spleen, consistent with their immune modulatory functions. In cancer cell lines, LILRBs showed relatively high expression in cell lines of malignant hematological origin, in line with the selective expression of LILRBs in hematopoietic lineage cells. Indeed, abnormal expression of LILRBs has been documented in various cancers, such as lung cancer [50], hepatocellular carcinoma (HCC) [51], and certain types of subtypes of adenocarcinoma [41]. In this study, based on combined datasets from TCGA and GTEx, we comprehensively analyzed LILRBs expression between tumor and adjacent normal tissue across 28 cancer types (9465 tumor and 7831 normal samples). Our data showed that LILRBs were significantly dysregulated in the majority of tumor types. For LILRB1-4, the most striking difference was seen between AML and its normal counterparts. Although previous study has reported increased expression of LILRB4 in AML [19, 49], this was not seen in two independent validation sets of AML patients, probably due to technical/biological heterogeneity across studies. However, we do note a strong enrichment for LILRBs in the monocytic lineage; this observation was confirmed in HPA pan-cancer cell lines dataset, single-cell transcriptomics of immune cells, immune cell abundances estimated using bulk TCGA samples, and GSEA analysis of monocyte-related gene sets, in agreement with previous reports [19, 20, 22, 49].

Despite being positively correlated with monocytes, LILRB1-4 were negatively correlated with the density of CD8+ T and NK cells, which are considered essential for effective anti-tumor immunity [27]. It has been shown that activated LILRB4 on monocytic AML cells recruits SHP-2 and upregulates NFκB, leading to increased ARG1 and uPAR accompanied by a concomitant suppression of T cell activity [18, 19]. This might provide a potential mechanistic explanation to our observations. It should be noted that bone marrow (BM)-T cells in AML are often functionally impaired [11–13, 52], possibly mediated by malignant monocyte-like cells from AML [11, 19, 21, 53]. Further research aimed to unravel the underlying molecular mechanisms is clearly warranted, as this may provide opportunities for identification of new drugs targets and therapeutics that can circumvent the T cell suppression state in AML.

Immunosuppressive factors, such as indoleamine 2,3-dioxygenase 1 (IDO1), CD200, and TIM-3 were reported to be closely associated with a poor outcome in AML [54–56]. In a preliminary analysis, Deng et al. studied the prognostic relevance of several co-stimulating and co-inhibitory receptors in TCGA AML dataset, including LILRB1-LILRB4 [19], but there is limited data and require validation in large datasets. Here, we independently validated the prognostic significances of LILRB members in five independent datasets. Strikingly, we showed that LILRB1-4 adversely impacted survival in almost all analyzed datasets. Of interest, we also noticed that LILRB4 was significantly associated with M2 macrophage abundances. This observation raises the possibility that LILRB4 might contribute to leukemogenesis through M2 macrophages. Our group has recently reported that M2 macrophages fractions were selectively upregulated in AML than other four hematological malignancies and normal controls [10]. Importantly, we also demonstrated a superior predictive performance of the M2 marker CD206 (MRC1) than classical prognosticators in AML. Interestingly, in this study we found that CD206 was significantly up-regulated in high LILRB1 expressers. As CD206+ and/or LILRB4+ monocytes could suppress T-cell
proliferation and create an immunosuppressive microenvironment in AML [19, 53], it could be hypothesized that at least part of the prognostic value of LILRBs could be attributed to the immune-suppressive TME it contributed. Acute monocytic leukemia often harbors mixed-lineage leukemia (MLL) rearrangements, an aggressive phenotype with limited treatment options and poor survival rates, which might also explain the observed result. Indeed, we demonstrated that LILRB4 was aberrantly overexpressed in MLL-rearranged AML and might be a direct target of the MLL fusion proteins.

In a recent pan-hematological-malignancies study, the authors have found that LILRB2 could distinguish lymphoma and leukemia subtypes with high immune infiltration from those harboring lower cytolytic score [27]. We consistently found multiple genes involving in immune activation (including cytokines and genes essential for microbial killing and antigen processing and presentation) were deleted in LILRB1-high patients, indicating a delicate balance between immune activation and suppression in the TME.

Indeed, an integrated analysis of transcriptomic and proteomic data has uncovered and ranked LILRBs among the top potential chimeric antigen receptor (CAR) targets in AML [57]. Preliminary evidence from the Gui group also suggests that blocking LILRB4 activation effectively reversed T-cell suppression and inhibited AML cell infiltration [18]. Given that LILRBs are selectively dysregulated in AML, it is tempting to speculate that AML positive for these proteins might be good candidates for immunotherapy. Future cancer immunotherapy clinical trials will be critical to further validate these findings.

In this study, we provided a comprehensive analysis of the expression patterns and clinical significances of LILRBs across pan-cancers, focusing its role in AML. We also analyzed the association of LILRBs expression with genomic features and tumor immunity in AML. Our data revealed up-regulated expression of LILRBs in AML and that higher expression levels of these genes predicted worse outcome. In addition, LILRBs were associated with an immune-suppressive TME in AML. Overall, these findings suggest important immunological and clinical implications of LILRBs in AML, which warrants further clinical investigation with immunotherapy specifically targeting AML with LILRBs dysregulations.

Declarations

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Availability of data and materials
The datasets analyzed in this study are available in the following open access repositories:

GTEx, www.gtexportal.org/

HPA, https://www.proteinatlas.org/

CCLE, https://www.broadinstitute.org/ccle

TCGA, https://portal.gdc.cancer.gov/, http://www.cbioportal.org

GEO, https://www.ncbi.nlm.nih.gov/geo/ (GEO accession numbers: GSE63270, GSE30029, GSE42519, GSE13159, GSE116256, GSE63409, GSE79899, GSE82116, GSE71779, GSE89336, GSE71776, GSE61785, GSE10358, GSE37642, GSE106291, and GSE71014)

FIMM AML scRNA data, https://www.synapse.org (doi: 10.7303/syn21991014)

DiseaseMeth, http://bio-bigdata.hrbmu.edu.cn/diseasemeth/analyze.html

**TIMER 2.0**, http://timer.comp-genomics.org/

**Author contributions**

JQ, JL, and Z-WM conceived and designed the study; Z-JX, X-LZ, YJ, S-SW, and YG collected and assembled data; Z-JX, J-CM, X-MW, and J-YL performed data analysis; Z-JX drafted the manuscript; JQ, JL, and Z-WM participated in study supervision and commented on the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**Figures**
Figure 1

Expression patterns of LILRBs in normal tissues, cancer cell lines, and primary tumor samples. (a) Heatmap showing mRNA expression levels of LILRBs in normal tissues from the Genotype-Tissue Expression (GTEx) database. (b) Heatmap showing mRNA expression levels of LILRBs in various tumor cell lines from the Cancer Cell Line Encyclopedia (CCLE) database. (c) Correlation matrix of the expression of LILRB1-LILRB5 in the TCGA pan-cancer dataset. (d) Heatmap of differential expression profiles of LILRBs between tumor and normal samples, combining data from TCGA and GTEx databases.
The color depicts the log2-transformed fold change (Log2FC) between tumor and normal tissues. *P < 0.05; **P < 0.01; ***P < 0.001. (e) Bar plot showing genes significantly upregulated and downregulated (P < 0.05) across different cancer types. Red, up-regulated expression; blue, down-regulated expression. (f and g) Box plots showing expression levels of LILRB1-4 in normal controls and AML in the GSE63270 (f) and GSE300029 (g) datasets.

Figure 2

Landscape of genetic alterations of LILRBs in tumors. (a) Genetic alteration frequencies of LILRBs across different tumors from TCGA. (b) Bar plot showing the percentages of various mutation types for five LILRB genes. (c) Heat map showing mutation frequencies of LILRBs across different cancer types. Numbers on the cells represent mutation percentages. (d) Pie plots showing the percentages of various copy number alteration (CNA) types for five LILRB genes.
Figure 3

DNA methylation aberration of LILRBs in tumors. (a) Heatmap of differential methylation profiles of LILRBs between tumor and normal samples, using data from the Diseasemeth database. The color depicts methylation differences between tumor (T) and normal (N) tissues. *P < 0.05; **P < 0.01; ***P < 0.001. (b) Correlation between methylation and mRNA expression of LILRBs analyzed via the GSCALite platform. Blue dots indicate negative correlation and red indicate positive correlation. The size of the
point represents the statistical significance. (c and d) Heatmap showing methylation of LILRB genes in the Diseasemeth dataset (AML, n = 271; Normal, n = 10) (c) and the GSE63409 dataset (AML, n = 44; Normal, n = 30) (d). (e) Scatterplot showing the correlation between mRNA expression and DNA methylation levels of LILRBs in the TCGA AML dataset. Spearman correlation coefficients and p-values are indicated. The linear models describing the correlations are depicted as straight lines. The marginal rugs drawn on the axis of the scatter plots were used to show the distributions of two variables. (f) Correlation between LILRBs methylation and survival across cancers analyzed via the GSCALite platform. Red dots indicate a higher risk conveyed by hypomethylation and blue indicate lower risk. The size of the point represents the statistical significance.

Figure 4
The prognostic impacts of LILRBs in cancers. (a) Association between LILRBs expression and patient prognosis across 33 cancer types as determined by the TIMER2.0 database. (b) Kaplan-Meier curves representing OS of AML patients from the TCGA cohort based on the expression of indicated LILRB members (LILRB1-LILRB4).

Figure 5
Independent validation of the prognostic significance of LILRBs in five GEO datasets. (a-e) Kaplan-Meier curves representing OS of five AML cohorts from GEO (GSE10358, n = 304; GSE37642 [U133A], n = 422; GSE37642 [U133plus2], n = 140; GSE106291, n = 250; GSE71014, n = 104) based on the expression of indicated LILRB members (LILRB1-LILRB4).

(a) Hemap
(b) CCLE RNAseq
(c) BeatAML
(d) MULLIGHAN_MLL_SIGNATURE_1_DN
(e) MV4-11 cells (MLL-AF4)
(f) H3K79me2

Figure 6

LILRB4 is aberrantly overexpressed in MLL-rearranged AML and is likely a direct target of MLL-fusion proteins. (a) Expression differences of LILRB genes in molecular subtypes of AML, and pre-B-ALL, DLBCL, and MM. The expression fold change between each subtype and the remaining samples in the same
disease were compared using the Wilcoxon rank sum test. The color of the dots indicates fold changes (log2) and size indicates the FDR values. The FDR values were categorized into five groups based on significance cutoffs for visualization (0.05, 0.01, 0.001, 1e-5, 1e-16). (b) Bar plot showing LIRB4 expression (RNA-seq) in non-MLL-rearranged (HEL, MEG01, KASUMI1, KG1, NB4, K562, HL60, U937) and MLL-rearranged (SEM, MONOMAC6, NOMO1, RS411, MOLM13, THP1, MV411) cell lines from the CCLE database. The dotted line represents the mean expression of LIRB4 across all cell lines analyzed. (c) Comparison of LIRB4 expression among human primary AML cases with MLL rearrangements and those without MLL rearrangements in the BeatAML dataset. (d) Box plots showing ssGSEA scores of two MLL-related gene signatures (MULLIGHAN_MLL_SIGNATURE_1_DN and MULLIGHAN_MLL_SIGNATURE_1_UP) between patients (TCGA dataset) with high and low LIRB4 expression. (e) ChIP-seq tracks for MLL-fusion proteins, H3K79me2, H3K27ac, and H3K4me3 at LIRB4 gene loci in MV4-11 and THP-1 cell lines. ChIP-seq data were obtained from GSE79899. (f) ChIP-seq tracks for H3K79me2, H3K27ac, and H3K4me3 at LIRB4 gene loci in MV4-11 and MLL-AF9 transformed blast cells. ChIP-seq data were obtained from GSE82116, GSE71779, GSE89336, GSE71776, and GSE61785.

Figure 7

LILRBs expression correlate with distinct genomic alterations in AML. (a) Heatmap showing association between LILRBs expression and clinical characteristics in the TCGA AML cohort. (b) Bubble plot showing associations between the expression of LILRB1-LILRB5 and common mutational events in the TCGA dataset. Bubble size indicates −log10 (Fisher test p-value). Color signifies −log10 (odds ratio), positive
association is indicated with red circles, negative with blue circles, and non-association with gray circles. (c) Co-bar plots showing the comparison of mutational profiles between patients with high and low LILRB1/LILRB5 expression in the TCGA dataset. (d) GISTIC analyses identified recurrent copy number alterations in AML patients with high LILRB1 expression.
The relation between LILRBs expression with immune cell infiltration and immune checkpoints. (a) Correlation matrix plot showing correlations between LILRBs and tumor immune infiltrating cells (TIICs). The overall immune cell compositions were estimated by CIBERSORT in the TCGA dataset. (b) Dot plot showing expression patterns of LILRBs in annotated cell types from 16 AML scRNA-seq samples (Van Galen AML scRNA). The color of the dots indicates average expression and size indicates percentage of cells with detectable expression. (c) Heatmap showing LILRBs expression in normal cell populations from the Hemap dataset. (d-f) Circos plots showing correlation between the expression of LILRB1 (d), LILRB2 (e), and LILRB3 (f) and immune checkpoint genes in the TCGA dataset.

Figure 9

The biological significance of LILRBs expression in AML. (a) Volcano plot showing differentially expressed genes (DEGs) between high and low LILRB1 expressers. (b) Cytoscape analysis of LILRB1-related network using PPI information obtained from STRING database (http://stringdb.org/). Red nodes represent up-regulated genes and blue represent down-regulated genes. (c and d) GO (c) and KEGG (d) analysis of DEGs.
Figure 10

Gene set enrichment analysis (GSEA) of patients with high and low LILRB1 expression, with curated (a), immunologic (b), and hallmark (c) gene sets obtained from the Molecular Signatures Database (MSigDB).

Supplementary Files

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

- SupplementaryData1.xlsx
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