scientific reports

OPEN

A bioinformatics analysis and experimental validation of PDGFD as a promising diagnostic biomarker for acute myeloid leukemia

Lulu Yang[1,7], Shuangshuang Ren[3,7], Lijiang Lou[1], Jiasu He[5], Qianlei Huang[6], Xiaojin Wu[4✉] & Ranran Zhao[2✉]

Acute myeloid leukemia (AML) is a malignant blood cancer resulting from leukemia stem cells (LSCs) supplanting normal stem cells. Platelet-derived growth factors (PDGFs) are important for LSCs but have not been studied in the development of AML. In this study, transcriptome data of PDGFs were sourced from The Cancer Genome Atlas (TCGA) and GTEx databases, and relevant differential expression and prognosis analysis were performed using R software packages and online tools (UCSC-Xena Shiny tools, GEPIA2, Kaplan-Meier Plotter databases, etc.). Then, we focused on PDGFD expression in AML, along with its clinical and diagnostic importance, drug resistance studies, and association with immunotherapy. The real-time quantitative polymerase chain reaction (RT-qPCR) was performed to verify the expression and clinical characteristics of PDGFD. Analyses of public data and clinical samples revealed that PDGFD expression was upregulated compared with other PDGF genes, and only this upregulation was associated with poor prognosis in AML. High expression of PDGFD showed a significant positive correlation with intermediate-high cytogenetic risk, NPM1 mutation, FLT3-ITD mutation, and unfavorable prognosis. ROC curve analysis indicated that PDGFD holds substantial diagnostic potential for AML patients. Functional enrichment analysis revealed the role of PDGFD in calcium and Rap1 signaling pathways. Additionally, PDGFD expression exhibited a significant positive correlation with natural killer cells and dendritic cells. Furthermore, we propose that MiR-203-3p targeting PDGFD has potential anti-leukemic effects in AML. In conclusion, PDGFD serves as a possible diagnostic and prognostic biomarker, as well as a target for cellular immunotherapy in AML.

Keywords Acute myeloid leukemia, PDGF family, NPM1 mutation, Natural killer cell, Prognosis

Acute myeloid leukemia (AML) is a hematological malignancy noted for its high aggressiveness and heterogeneity. The pathogenesis of AML involves the accumulation of acquired genetic mutations and epigenetic modifications in hematopoietic progenitor cells, resulting in the emergence of leukemic stem cells (LSCs). These alterations disrupt normal cellular processes such as growth, proliferation, and differentiation[1]. Despite progress in prognostic risk assessment and targeted therapy, the mortality rate of AML continues to be elevated. The long-term survival rates for patients aged under 60 years are below 40%, while for those over 60 years, the rates are less than 15%, leading to significant social and economic challenges[2]. Hence, elucidating the mechanisms that underlie the progression of AML, and the improvement of prognostic outcomes is paramount.

[1]Department of Hematology, Ninghai First Hospital, Ningbo 315600, Zhejiang, China. [2]Department of Hematology, The Second Affiliated Hospital of Xuzhou Medical University, Xuzhou 221006, Jiangsu, China. [3]Department of Ultrasound, Dongyang People's Hospital, Jinhua 322100, Zhejiang, China. [4]Department of Hematology, National Clinical Research Center for Hematologic Diseases, Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China. [5]Department of Anesthesiology, Ninghai First Hospital, Ningbo 315600, Zhejiang, China. [6]Department of Hematology, The First Affiliated Hospital of Hainan Medical College, Haikou 570102, Hainan, China. [7]Lulu Yang and Shuangshuang Ren contributed equally to this work. ✉email: wuxiaojin@suda.edu.cn; zhaoranran_zrr@163.com

Leukemia stem cells (LSCs) serve as the origin of naive myeloid progenitor cells, which amass in the bone marrow (BM), displacing healthy hematopoietic stem and progenitor cells (HSPCs), impairing regular hematopoiesis, and eventually disseminating to other tissues[3]. Regardless of the genetic mutations that initiate the illness, LSCs rely on various growth factors to sustain their malignant biological equilibrium[4]. Previous studies have found that the growth factors associated with this leukemic hematopoiesis process typically align with those observed in normal hematopoiesis. However, in individuals with preleukemic and leukemic conditions, the precise control of cytokines is compromised, leading to abnormal secretion of cytokines[5–7]. It is worth noting that the enhanced proliferation of patient-derived leukemic cells in response to elevated hematopoietic growth factors (HGFs) in vitro is associated with unfavorable clinical consequences in AML patients. These repercussions include reduced rates of complete remission, heightened risk of relapse, and decreased overall survival[8]. Over the recent decades, there have been some advances in academic research and drug development on growth factors. Particularly, IL-1b, IL-6 and CXCL12 are emerging as potential novel targets for drug development[9].

Platelet-derived growth factor (PDGF) is a crucial component of hematopoietic factors, yet its impact on leukemia remains unexplored. PDGF is a versatile cytokine that promotes the growth and activities of various primary tissues. This family consists of four polypeptide chains (PDGF-A, -B, -C, and -D), linked by disulfide bonds to form five distinct homodimers or heterodimers[10]. The PDGF receptors (PDGFR-a and -b) are part of the receptor tyrosine kinase type III family, encompassing c-KIT, FLT3, and the macrophage-colony-stimulating factor receptor[11]. These ligands interact with and activate their cognate receptors to stimulate the proliferation, survival and migration of mesenchymal cells and other cell types[12–14]. Several reports suggest that PDGF receptors can potentially regulate the functions of hematopoietic cells, while PDGF ligands secreted by hematopoietic cells are implicated in numerous physiological and pathological processes beyond the hematopoietic system[15]. Foss et al. demonstrated that externally sourced PDGF and PF-4, such as those secreted by platelets, can regulate leukemic hematopoiesis and potentially modify the activity of residual AML cells in peripheral blood stem cell transplants[16]. However, the existing studies only detected the expression of PDGF factor in leukemia cell lines in laboratory settings, lacking clinical sample data support, and few studies have focused on influence of the PDGF gene on the prognosis of leukemia patients, which is the focus of our research.

Our research delved deeply into investigating the significance of the PDGF family in AML, with a particular focus on PDGFD. We investigated the function of the PDGF family in AML utilizing public data, revealing an overexpression of PDGFD in AML patients with particular genetic abnormalities. Bioinformatics investigation revealed that increased PDGFD gene expression is associated with poor prognosis and diminishes patient responsiveness to chemotherapy. Clinical data analysis validated certain conclusions from the bioinformatics study. The findings highlight the potential importance of PDGFD in promoting the development and advancement of AML, suggesting its relevance as a prognostic indicator and therapeutic target for AML treatment.

Methods
Flow diagram
Figure 1 displays the flowchart that illustrates the entire study.

Database acquisition
The pan-cancer analysis encompassed 33 independent TCGA cancer cohorts, consisting of 9703 tumor samples obtained via the UCSC-Xena browser (https://xenabrowser.net/)[17]. The UCSC-Xena Shiny tool (https://github.com/openbiox/UCSCXenaShiny)[18], which offered RNA transcriptional datasets of healthy individuals from the Genotype-Tissue Expression (GTEx), provided data on RNA sequencing and clinical characteristics of 173 newly diagnosed AML patients from The Cancer Genome Atlas (TCGA) dataset.

The collection of tumor specimens and clinical information
The Ethics Committee of the First Affiliated Hospital of Soochow University provided approval for this study. The study collected bone marrow samples from 61 patients with primary acute myeloid leukemia and 18 healthy donors admitted to our hospital from 2017 to 2019. During the follow-up, we gathered comprehensive clinical data, encompassing age, gender, treatment protocol, and result (Supplementary Table S1). All experiments were performed according to regulations. Informed consent to participate in the study was obtained from patients.

Analysis of gene expression and survival prognosis
The expression differences of PDGF family members between AML patients and healthy individuals were analyzed based on the datasets: TCGA and GTEx (GEPIA2 project)[19], and Leukemia MILE study (Bloodspot project)[20]. The Kaplan-Meier Plotter database (KM-Plotter; https://kmplot.com/)[21] was commonly employed to assess the prognostic relevance of PDGFs in AML using information from TCGA.

Real-time quantitative polymerase chain reaction (RT-qPCR)
Bone marrow specimens were collected from 61 people diagnosed with primary AML and 18 volunteers. Total RNA was extracted via the Trizol reagent method, and the recovered RNA underwent reverse transcription utilizing the Reverse Transcription Reaction Kit (TaKaRa, Otsu, Japan) following the manufacturer's guidelines. The RT-qPCR assay was conducted using SYBR Green Master Mix (TaKaRa) under the following cycling parameters: the first cycle was at 95 °C for 30 s, and then there were 40 cycles of a two-step protocol (95 °C for 5 s and 60 °C for 30 s). The mRNA levels of the target genes were determined using the $2^{-\Delta Ct}$ method, with β-actin mRNA serving as the normalization standard. Supplementary Table S2 listed all primer sequences used.
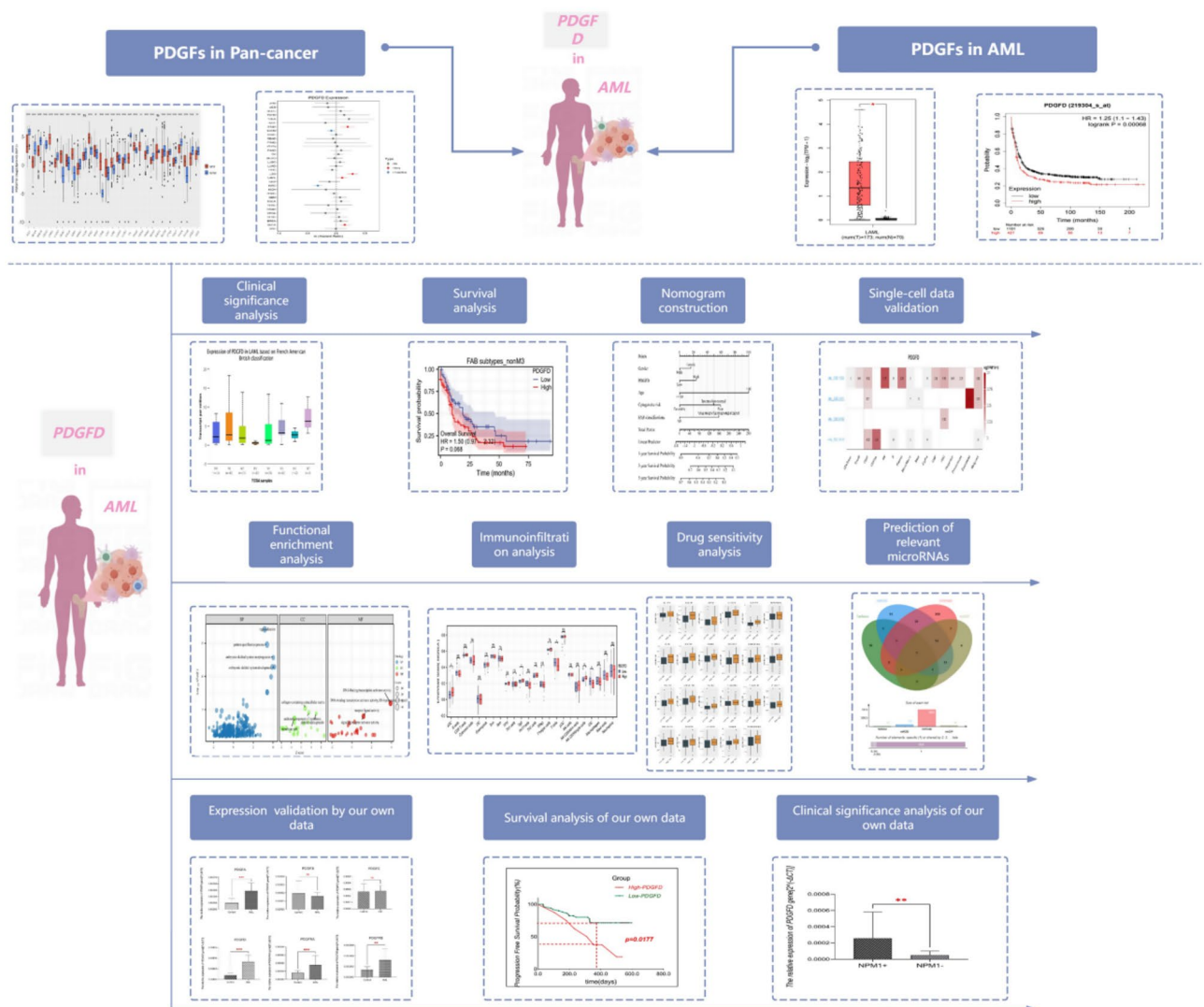
Fig. 1. The overall flow chart of our study. Step1: PDGFD was screened by pan-cancer analysis and analysis of the expression and prognostic significance in AML. Step2: Bioinformatics analysis of PDGFD, including clinical significance, immuno-infiltration, drug susceptibility, functional enrichment, single cell analysis, and prediction of target miRNA. Step3: Validation from clinical samples, including expression and clinical significance of PDGFD.

Correlation analysis of PDGFD with clinical factors in AML and construction of a nomogram

Clinical data from UALCAN (http://ualcan.path.uab.edu/analysis-prot.html)[22] and gene mutation data from the TCGA website were used to examine the potential correlation between the PDGFD gene and AML clinical characteristics as well as other gene mutations. The efficacy of PDGFD in diagnosing AML was assessed by the receiver operating characteristic (ROC) curve. The prognostic significance of the PDGFD gene in AML was evaluated by univariate and multivariate Cox analyses. The prognostic nomogram was developed by integrating relevant variables identified in the univariate Cox survival model. ROC and calibration curves were generated at various intervals to assess the predictive and calibration capabilities.

Identification of genes expressed in relation to PDGFD

Genes co-expressed with PDGFD in the TCGA-LAML cohort were found utilizing the LinkedOmics database (http://www.linkedomics.orglogin.php)[23]. Next, differentially expressed genes (DEGs) were evaluated between the PDGFD^{high} and PDGFD^{low} groups. The shared genes between the formers were identified utilizing the jVenn diagrams tool.

Functional analysis and gene set enrichment analysis

Functional enrichment analyses were conducted on the identified overlapping genes, utilizing tools such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)[24–26]. Gene Set Enrichment Analysis (GSEA) was employed to validate the functions linked to AML and investigate the potential signaling pathways

that contribute to malignant phenotypes in people with differing levels of PDGFD expression. In all GSEA assessments, the "c2.cgp.v2022.1.Hs.symbols.gmt" gene set from the MSigDB database (http://www.gseamsigdb)[27] was chosen as the standard reference set.

Analysis of tumor immune infiltration and prediction of immunotherapy efficacy

CIBERSORT method was employed to assess the proportions of 22 immune cell phenotypes in groups exhibiting high or low expression of PDGFD. Simultaneously, single sample gene set enrichment analysis (ssGSEA) was used to scrutinize the potential cancer phenotypes linked to PDGFD expression and to investigate the correlation between PDGFD and enrichment during specific phases of the anti-cancer immune cycle. The ESTIMATE algorithm was used to quantify the quantity of immunological and stromal components in each sample of the tumor microenvironment. The interaction between PDGFD and 10 immunological checkpoints (TIGIT, LAG3, HAVCR2, CTLA4, IGSF8, ITPRIPL1, SIGLEC15, PDCD1LG2, PDCD1, and CD274) was assessed through Spearman correlation analysis. The association between PDGFD and immune checkpoint genes was concurrently examined utilizing the CAMOIP database (https://www.camoip.net/)[28]. Moreover, ten other popular immune checkpoints targeting myeloid leukemia (CTLA-4, CD80, CD86, PDCD1, CD274, TIM3/HAVCR2, CD47, SIRPα, CD200, and CD200R) were retrieved from previous studies[29]. Correlation analysis was performed to determine the relationship between PDGFD expression and immune checkpoint genes. Somatic mutation data were utilized to calculate TMB scores and determine MSI scores for all samples. Spearman analysis was employed to investigate the relationship between PDGFD expression and TMB and MSI. The response to immune checkpoint inhibition was anticipated utilizing the TIDE (Tumor Immune Dysfunction and Exclusion) technique.

Single-cell analysis

We utilized single-cell datasets from the Tumor Immune Single Cell Hub (TISCH, http://tisch.comp-genomics.org/)[30] and the scCancerExplorer (https://bianlab.cn/scCancerExplorer)[31], specifically GSE116256, GSE135851, GSE147989, and GSE154109 to investigate the presence of potential tumor antigens in immune cells within the bone marrow.

Pharmacological sensitivity assessment

A drug sensitivity study was performed to forecast drug reactions using the "pRRophetic" software package within the R programming environment, leveraging data from the Genomics of Drug Sensitivity in Cancer (GDSC) repository (https://www.cancerrxgene.org/)[32].

Prediction of PDGFD-regulated MiRNAs

The target prediction databases Target Scan, miRDB, mirDIP, and miRWalk were utilized to ascertain the possible target miRNAs of PDGFD. The miRNAs identified through the predictions of these four databases were chosen for further examination.

Statistical analyses

Numerous online resources are available for providing extensive statistical support, including the use of R program (version 4.2.1) and GraphPad Prism 9 for statistical analysis and data visualization. All statistical evaluations were two-tailed, with significance determined at $P < 0.05$.

Results

Expression and prognosis of PDGFs in pan-cancers and AML

We evaluated the transcription levels of PDGFs in pan-cancer and AML alongside a preliminary investigation on prognosis (Supplementary Figs. S1, S2, and S3). The findings indicated that the expression of PDGFA and PDGFB was diminished in AML patients compared to healthy controls, while PDGFC and PDGFD (Fig. 2A and C) exhibited elevated transcription levels in AML (Supplementary Figs. S1A–D, S2A–D, and S3A–D). The datasets from TCGA project and Bloodspot both showed significantly up-regulated expression of PDGFRA and PDGFRB in AML (Supplementary Fig. S1E-F and S3E-F). Prognostic analysis showed that PDGFs are risky for most tumors (Supplementary Fig. S1H–M). However, in AML, only the PDGFD gene is a risky factor (Fig. 2B), while other PDGFs were either considered protective factors or exhibited no significant effect. Furthermore, the KM curve indicated that patients exhibiting PDGFD overexpression poorer survival (log-rank $P < 0.001$, Fig. 2D) and PDGFB underexpression experienced markedly similar results (log-rank $P = 0.0037$, Fig. S2I). Considering the findings above, we elected to prioritize the PDGFD gene as the subject of subsequent investigation.

Association of PDGFD expression with clinical attributes from the TCGA database

We further explored the clinical relevance of PDGFD in AML patients and employed the Wilcoxon rank sum test (Fig. 3A) to assess PDGFD expression across patients with varying clinical characteristics. PDGFD was markedly overexpressed in patients exhibiting high PB blasts (>70%; $P = 0.04$), FAB non-M3 subtype ($P < 0.05$), intermediate-poor cytogenetic risk ($P < 0.001$), positive NPM1 mutation ($P < 0.001$), positive FLT3 mutation ($P = 0.02$), and negative RAS mutation ($P < 0.05$). We then conducted a logistic analysis to investigate the relationship between clinical factors of AML and the high-low dichotomy of PDGFD. High expression of PDGFD demonstrated a substantial positive correlation with elevated PB blasts (>70%) (OR = 2.523; $P = 0.006$), non-M3 subtype (OR = 15.77; $P = 0.009$), intermediate-poor risk karyotype (OR = 21.149; $P < 0.001$), FLT3 mutation (OR = 2.348; $P = 0.021$), and NPM1 mutation (OR = 16.364; $P < 0.001$) (Table 1). Gender, age, white blood cell counts, bone marrow blast percentages, RAS mutation status, and IDH1 mutation status did not differ between the PDGFD^{high} and PDGFD^{low} groups ($P > 0.05$) (Table 1). Furthermore, the analysis of the mutational profile
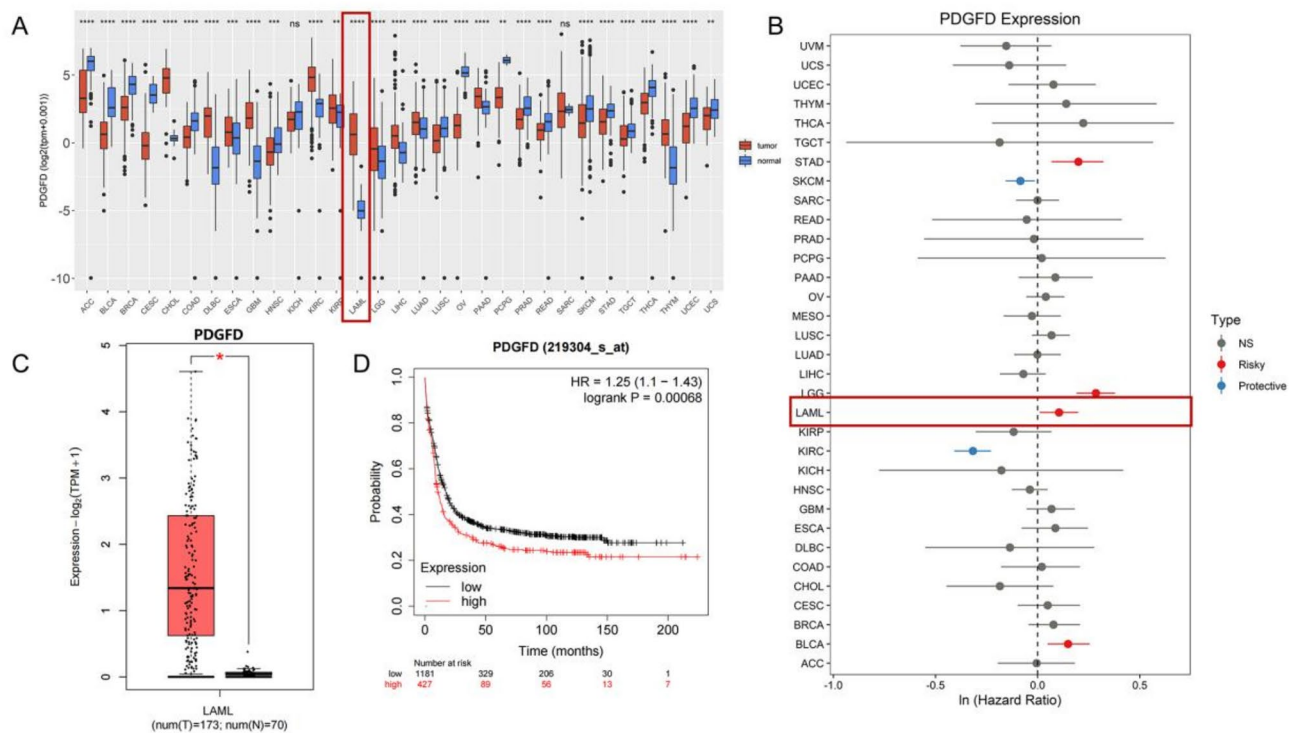
Fig. 2. The expression and prognostic significance of PDGFD in pan-cancers and AML. (**A**) The expression levels of PDGFD in pan-cancers. (**B**) A prognostic risk assessment of PDGFD in pan-cancers. (**C**) A comparative analysis of PDGFD expression in AML versus normal controls. (**D**) Kaplan–Meier survival curves of OS between PDGFD^{high} and PDGFD^{low} groups in AML.

revealed an increased frequency of NPM1 mutations in AML cases exhibiting elevated PDGFD expression ($P < 0.001$, Fig. 3B).

This study also examines the probable utility of PDGFD in distinguishing AML patients from healthy people. The analysis indicated the potential of PDGFD as a biomarker, evidenced by an AUC of 0.990 (Fig. 3C). And the investigation discovered that PDGFD demonstrated potential utility in identifying AML patients with NPM1 mutation (AUC = 0.841), non-M3 type AML patients (AUC = 0.811), and those with intermediate-high-risk karyotypes (AUC = 0.847).

Kaplan-Meier curves demonstrated that increased PDGFD expression in individuals aged ≤ 60 ($P = 0.021$), AML-M2 subtype ($P = 0.009$), RAS mutation-negative status ($P = 0.007$), NPM1 mutation-negative status ($P = 0.029$), IDH1-R132 mutation-negative status ($P = 0.004$), IDH1-R140 mutation-negative status ($P = 0.007$), and IDH1-R172 mutation-negative status ($P = 0.006$) correlated with unfavorable prognosis (Supplementary Fig. S4). Hereafter, Univariate Cox analysis revealed that, like cytogenetic risk (favorable vs. intermediate/poor, $P < 0.001$), FAB classification (M3 vs. non-M3, $P = 0.009$), and age (≤ 60 vs. >60, $P < 0.001$), PDGFD (high vs. low, $P = 0.011$) is a predictor of diminished overall survival (OS) (Table 2). Data suggests that elevated PDGFD gene expression is characteristic of high-risk AML and may correlate with unfavorable prognosis.

Nomogram construction for AML
Univariate Cox analysis suggested that age, karyotype, FAB subtype, and PDGFD expression level might be prognostic risk factors for AML ($P < 0.05$, Table 2). Therefore, we combined these factors to design a prognostic nomogram to improve the accuracy of AML patient survival prognostication (Supplementary Fig. S5A). Notably, the PDGFD predictive model effectively predicts survival outcomes among AML patients (Supplementary Fig. S5B–C).

Validation of expression pattern and clinical significance of PDGFD in AML from clinical data
We performed RT-qPCR experiments on all genes of the PDGF family. In comparison to 18 healthy donors, the expression levels of the PDGFA, PDGFD, PDGFRA, and PDGFRB genes were markedly elevated in 61 AML patients (all $P < 0.05$, Supplementary Fig. S6). We observed a significant increase in the expression of the PDGFD gene in non-M3 AML cases, prompting further investigation including 54 of these patients ($P < 0.001$, Supplementary Fig. S7).

We employed the Wilcoxon test and Spearman analysis to investigate the clinical relevance of PDGFD expression (Fig. 4). The correlation study demonstrated a positive association between PDGFD expression and WT1 fusion gene expression ($r = 0.334$, $P = 0.014$, Fig. 4A). The Wilcoxon rank-sum test indicated that PDGFD levels were significantly elevated in patients with negative MPO antigen ($P = 0.0196$), positive NPM1 mutation
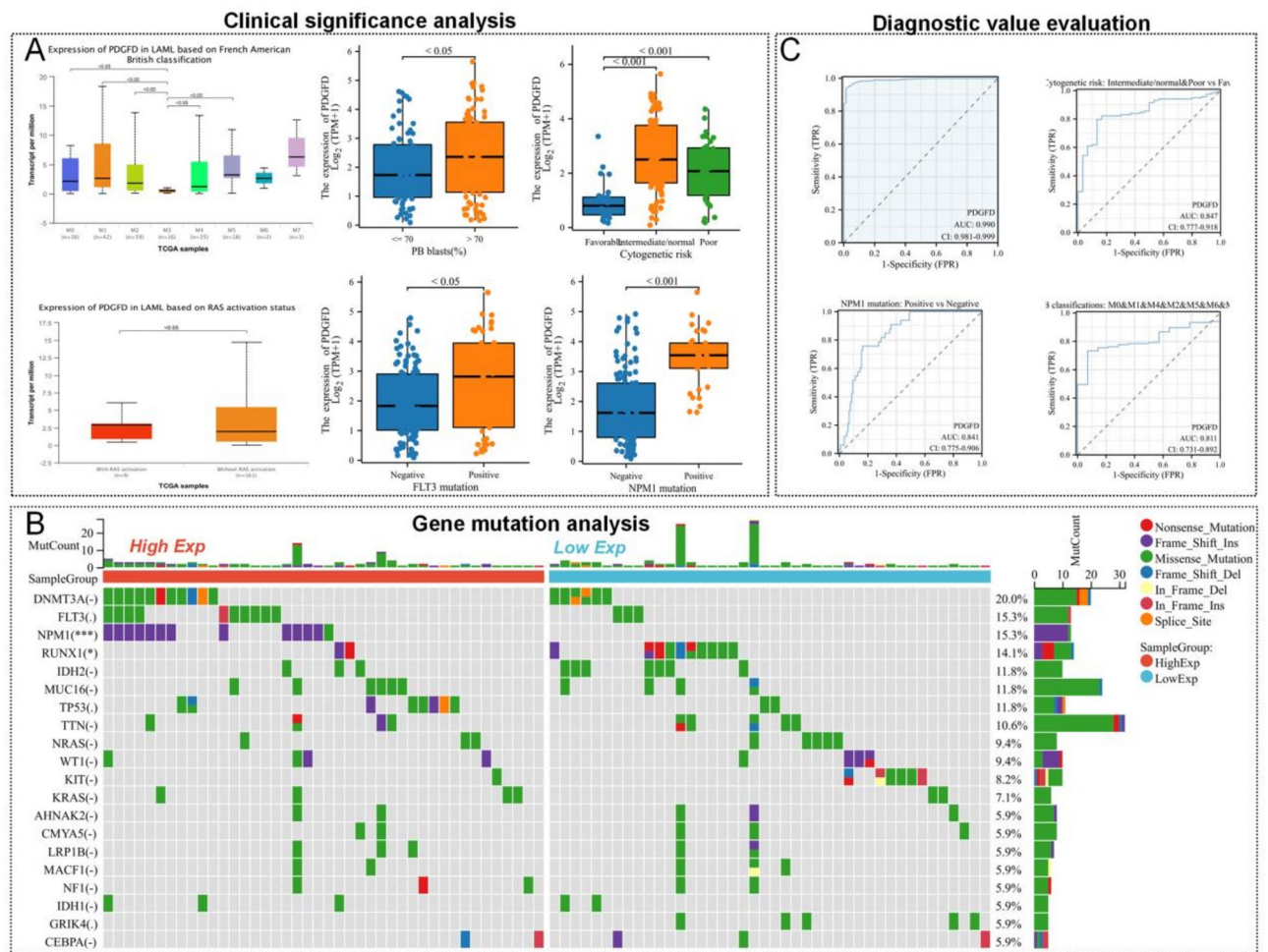
Fig. 3. Clinical correlation analysis and diagnostic value evaluation of PDGFD in AML. (**A**) Correlation between PDGFD expression level and clinical parameters. (**B**) The difference in gene mutation pattern between PDGFD^{high} and PDGFD^{low} group. (**C**) The value of PDGFD in diagnosing AML.

Characteristics	Total (*N*)	OR (95% CI)	*P* value
Gender (Male vs. Female)	150	1.055 (0.554–2.009)	0.870
Age (> 60 vs. <= 60)	150	1.315 (0.687–2.520)	0.409
WBC count(x10^9/L) (> 20 vs. <= 20)	149	1.583 (0.829–3.022)	0.164
BM blasts (%) (> 20 vs. <= 20)	150	1.183 (0.614–2.279)	0.616
PB blasts (%) (> 70 vs. <= 70)	150	2.523 (1.306–4.874)	*0.006*
FAB classifications (non-M3 vs. M3)	149	15.770 (2.006–123.982)	*0.009*
Cytogenetic risk (Intermediate & Poor vs. Favorable)	148	21.149 (4.809–93.011)	*< 0.001*
FLT3 mutation (Positive vs. Negative)	146	2.348 (1.136–4.853)	*0.021*
IDH1-R132 mutation (Positive vs. Negative)	148	2.423 (0.712–8.250)	0.157
IDH1-R140 mutation (Positive vs. Negative)	148	0.486 (0.140–1.688)	0.256
IDH1-R172 mutation (Positive vs. Negative)	148	1.028 (0.063–16.745)	0.985
RAS mutation (Positive vs. Negative)	149	1.739 (0.400–7.556)	0.460
NPM1 mutation (Positive vs. Negative)	149	16.364 (4.715–56.795)	*< 0.001*

Table 1. Association between PDGFD expression and clinical characteristics using logistic regression. Significance value in bold and italic indicates a *p* value < 0.05.

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Gender (Female vs. Male)	1.024 (0.671–1.564)	0.912		
Age (<=60 vs. > 60)	3.321 (2.156–5.116)	**< 0.001**	3.175 (1.950–5.169)	**< 0.001**
WBC count (x10^9/L) (<=20 vs. > 20)	1.156 (0.757–1.764)	0.503		
BM blasts (%) (<=20 vs. > 20)	1.159 (0.754–1.780)	0.502		
PB blasts (%) (<=70 vs. > 70)	1.224 (0.802–1.869)	0.349		
Cytogenetic risk (Favorable vs. Intermediate and Poor)	3.184 (1.637–6.193)	**< 0.001**	1.682 (0.741–3.820)	0.214
FAB classifications (M3vs. non-m3)	3.874 (1.408–10.658)	**0.009**	1.876 (0.569–6.188)	0.301
IDH1-R132 mutation (Negative vs. Positive)	1.706 (0.691–4.215)	0.247		
IDH1-R140 mutation (Negative vs. Positive)	0.886 (0.443–1.773)	0.733		
IDH1-R172 mutation (Negative vs. Positive)	1.644 (0.229–11.829)	0.621		
FLT3 mutation (Negative vs. Positive)	0.790 (0.498–1.253)	0.316		
RAS mutation (Negative vs. Positive)	1.558 (0.570–4.264)	0.388		
NPM1 mutation (Negative vs. Positive)	0.882 (0.548–1.421)	0.606		
PDGFD (Low vs. High)	1.733 (1.133–2.650)	**0.011**	1.289 (0.816–2.036)	0.277

Table 2. Univariate and multivariate Cox's regression analysis of factors associated with OS in AML. Significance value in bold and italic indicates a p value < 0.05.

($P = 0.0014$), positive FLT3 mutation ($P = 0.0436$), negative GATA2 mutation ($P = 0.0344$), negative CEBPA double mutation ($P = 0.0127$), and negative WT1 mutation ($P = 0.0256$) (Fig. 4B–G). Subsequently, we stratified AML patients into high- and low- groups according to PDGFD. A significant correlation existed between elevated PDGFD levels and increased WT1 fusion, FLT3 mutation ($P = 0.021$), and NPM1 mutation ($P < 0.001$) (Table 3). Nonetheless, the preceding analysis did not exhibit a significant correlation between PDGFD and gender, age, white blood cell count, hemoglobin levels, platelet count, bone marrow blast percentage, chromosomal karyotype, DNMT3A mutation, TET2 mutation, IDH2 mutation, NRAS mutation, c-Kit mutation, EZH2 mutation, and other common gene mutation statuses ($P > 0.05$, Table 3).

Subsequently, we examined the impact of PDGFD on the therapy outcome. We found elevated PDGFD levels in both the relapse group ($P < 0.05$, Fig. 4H) and the non-remission group ($P > 0.05$, Fig. 4I). We monitored AML patients for a median duration of 423 days. We categorized PDGFD based on the appropriate threshold and examined the disparities in OS, progression-free survival (PFS), and cumulative recurrence rate (CRR) between the PDGFD^{high} and PDGFD^{low} cohorts. The findings indicated that individuals exhibiting elevated PDGFD expression experienced reduced PFS ($P = 0.0177$, Fig. 4K). and elevated recurrence rates ($P = 0.0002$, Fig. 4L).

Functional enrichment analysis

Analysis of PDGFD co-expression genes in the TCGA-LAML cohort using the LinkedOmics database identified 2579 genes significantly associated with PDGFD in AML (|cor| > 0.2, FDR < 0.05) (Fig. 5A). 736 DEGs were found when comparing the high- and low- PDGFD groups in AML (Fig. 5B). An overlap of 339 PDGFD-related genes was identified between the co-expressed genes and DEGs (Fig. 5C). GO and KEGG analyses were conducted on the 339 shared genes to clarify the potential biological function of PDGFD in AML. The primary biological process (BP) identified was "regionalization", with "the collagen-containing extracellular matrix" being the predominant cellular component (CC). The molecular functions (MF) of PDGFD and its associated genes were mainly related to "the activity of DNA-binding transcriptional activator" (Fig. 5D). KEGG analysis (Fig. 5E) indicated that PDGFD and its related genes are predominantly involved in the "calcium signaling pathway". Furthermore, we analyzed the correlation between PDGFD-related genes and tumor pathways (Fig. 5F) and identified a significant enrichment in pathway linked to "NPM1-mutated AML". More enrichment pathways are available in the Supplementary Table S3-5.

We further analyzed the differential genes between NPM1 + AML and NPM1-AML. The results showed that 1820 genes were significantly associated with NPM1 mutation in AML (|log FC| > 1, pValue < 0.05) (Supplementary Fig. S8A), including 369 up-regulated genes and 1491 down-regulated genes. GO and KEGG analyses were conducted to clarify the potential biological function of PDGFD in NPM1 + AML. KEGG analysis (Supplementary Fig. S8B and Supplementary Table S6) indicated that PDGFD is predominantly involved in the "calcium signaling pathway". And GO analysis showed that the main molecular functions (MF) involved in PDGFD were "GO:0030546-signaling receptor activator activities". (Supplementary Fig. S8C and Supplementary Table S7).
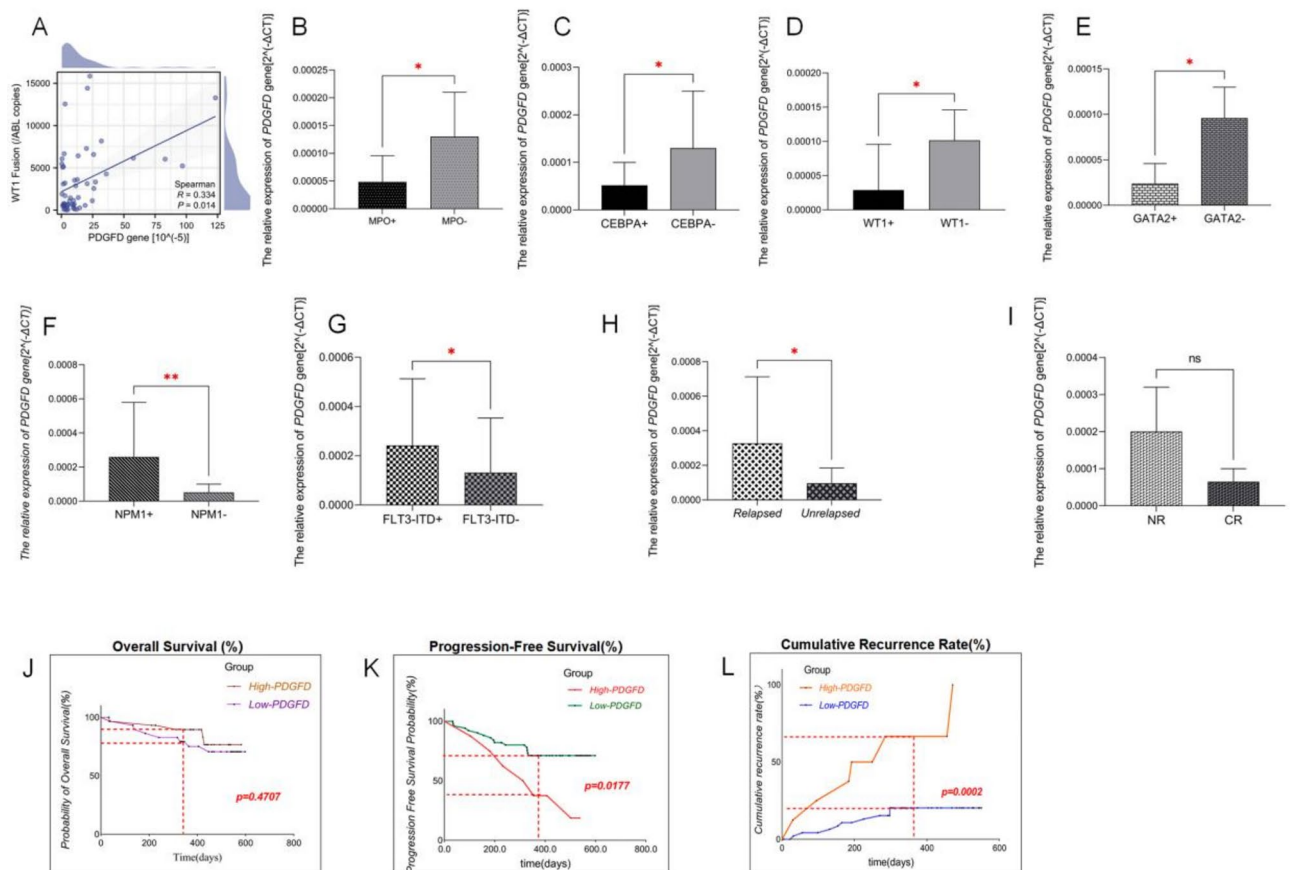
Fig. 4. Experimental validation of PDGFD expression and prognostic significance in AML from our clinical samples. (**A**) A positive correlation between the mRNA expression of PDGFD and the expression of the WT1 fusion gene. (**B**–**G**) Upregulated PDGFD in MPO-negative AML/ in CEBPA-negative AML/ in WT1-negative AML/ in GATA2-negative AML/ in NPM1-positive AML/ in FLT-ITD3-positive AML (all $P < 0.05$). (**H**) Upregulated PDGFD in the relapsed group ($P < 0.05$). (**I**) Hyper-expressive PDGFD in the non-remission group ($P > 0.05$). (**J**) The OS difference between PDGFD^{high} and PDGFD^{low} groups in AML($P > 0.05$). (**K**) The PFS difference between PDGFD^{high} and PDGFD^{low} groups in AML($P < 0.05$). (**L**) The CRR difference between PDGFD^{high} and PDGFD^{low} groups in AML($P < 0.05$).

Relationship between PDGFD and the immune landscape

Figure 6A-B illustrates that activated NK cells, resting mast cells, resting dendritic cells, and eosinophils were the principal immune cells influenced by PDGFD levels. A substantial increase in activated NK cells and eosinophils was strongly associated with the overexpression of PDGFD. Nonetheless, PDGFD overexpression exhibited a negative correlation with resting dendritic cells and resting mast cells (Fig. 6A). Afterwards, the ssGSEA results indicated a notable positive correlation between PDGFD expression levels and the infiltration of NK-CD56 (bright) cells, activated dendritic cells (aDCs), and T gamma delta cells (Tgd), alongside a negative correlation with T cells and immature dendritic cells (iDCs) (Fig. 6C). The ESTIMATE analysis demonstrated an inverse connection between PDGFD gene mRNA levels and both Stromal Score ($P < 0.05$) and ESTIMATE Score ($P < 0.05$) (Fig. 6D–E).

We analyzed the variations in gene expression of 10 immunological checkpoints at different PDGFD expression levels. Notably, the results showed a positive correlation only between PDCD1 and PDGFD expression (Fig. 6F). Utilizing the CAMOIP tool, we examined the association between PDGFD expression and multiple immunological markers, discovering a positive correlation with IL2RA, TGFB1, PDCD1, TM4SF, GPR146, CCL5, IL12A, among others. Conversely, we noted a negative connection between PDGFD expression and IMIGD2, along with CD244, BTLA, IL5, HS3ST2, VEGFA, ICAM1, MMP9, CD2, and additional factors (Supplementary Fig. 9A).

Moreover, we examined the association between PDGFD expression and ten other popular immune checkpoints targeting myeloid leukemia. The finding indicated that PDGFD was positively correlated with PDCD1 ($r = 0.178$, $P = 0.029$) and CD80 ($r = 0.224$, $P = 0.006$), whereas PDGFD was negatively correlated with CD200 ($r = -0.365$, $P < 0.001$) (Supplementary Fig. 9B-D).

The TMB and MSI scores for AML patients were computed to explore their association with PDGFD gene expression levels. No significant link was seen between gene expression and tumor microenvironment score (Supplementary Fig. S9E–F). Moreover, an assessment of the impact of the PDGFD gene on immunotherapy

Characteristics		High expression of PDGFD($n=27$)	Low expression of PDGFD($n=27$)	P value
Gender, n (%)	Female	15 (27.8%)	11 (20.4%)	0.276
	Male	12 (22.2%)	16 (29.6%)	
FAB classification, n (%)	AML-M0	0 (0%)	1 (1.9%)	0.089
	AML-M1	5 (9.3%)	1 (1.9%)	
	AML-M2	13 (24.1%)	20 (37%)	
	AML-M4	9 (16.7%)	4 (7.4%)	
	AML-M5	0 (0%)	1 (1.9%)	
Cytogenetics risk, n (%)	Favorable	2 (3.7%)	3 (5.6%)	0.497
	Intermediate	24 (44.4%)	21 (38.9%)	
	Poor	1 (1.9%)	3 (5.6%)	
NPM1 mutation, n (%)	Positive	8 (14.8%)	1 (1.9%)	**0.028**
	Negative	19 (35.2%)	26 (48.1%)	
FLT3-ITD mutation, n (%)	Positive	10 (18.5%)	3 (5.6%)	**0.026**
	Negative	17 (31.5%)	24 (44.4%)	
FLT3-TKD mutation, n (%)	Positive	4 (7.4%)	1 (1.9%)	0.348
	Negative	23 (42.6%)	26 (48.1%)	
DNMT3A mutation, n (%)	Positive	7 (13%)	2 (3.7%)	0.144
	Negative	20 (37%)	25 (46.3%)	
TET2 mutation, n (%)	Positive	3 (5.6%)	3 (5.6%)	1
	Negative	24 (44.4%)	24 (44.4%)	
CEBPA mutation, n (%)	Positive	8 (14.8%)	14 (25.9%)	0.097
	Negative	19 (35.2%)	13 (24.1%)	
WT1 mutation, n (%)	Positive	2 (3.7%)	7 (13%)	0.144
	Negative	25 (46.3%)	20 (37%)	
IDH2 mutation, n (%)	Positive	1 (1.9%)	3 (5.6%)	0.603
	Negative	26 (48.1%)	24 (44.4%)	
NRAS mutation, n (%)	Positive	6 (11.1%)	5 (9.3%)	0.735
	Negative	21 (38.9%)	22 (40.7%)	
c-Kit mutation, n (%)	Positive	1 (1.9%)	4 (7.4%)	0.348
	Negative	26 (48.1%)	23 (42.6%)	
GATA2 mutation, n (%)	Positive	0 (0%)	5 (9.3%)	0.06
	Negative	27 (50%)	22 (40.7%)	
EZH2 mutation, n (%)	Positive	4 (7.4%)	1 (1.9%)	0.348
	Negative	23 (42.6%)	26 (48.1%)	
Age, (mean ± sd)		42.074 ± 15.287	35.63 ± 12.314	0.094
WBC Count(*10^9/L), [median (IQR)]		28.19 (4.765, 64.365)	20.01 (10.735, 69.99)	0.918
Hemoglobin(g/L), (mean ± sd)		85.407 ± 22.517	90.333 ± 24.83	0.449
Platelet Count(*10^9/L), [median (IQR)]		44 (22, 71.5)	31 (19, 49)	0.268
BM blasts (%), (mean ± sd)		60.333 ± 20.012	57.337 ± 19.717	0.582
WT1 fusion, [median (IQR)]		3320.6 (915, 6287.5)	687 (550, 3145)	**0.030**
EVI1 fusion, [median (IQR)]		0 (0, 0)	0 (0, 0)	0.939

Table 3. Association between PDGFD expression and clinical features in AML from clinical data. Significance value in bold indicates a *p* value < 0.05.

response in AML patients revealed that the gene did not significantly alter patients' responses to immunotherapy ($P > 0.05$, Supplementary Fig. S10A).

Single-cell data validation of PDGFD gene

Single-cell data can more effectively depict the distribution of PDGFD in immune cells in the tumor microenvironment. The TISCH tool was employed to analyze the distribution of PDGFD gene expression across four AML single-cell datasets (GSE116256, GSE135851, GSE147989, and GSE154109, Fig. 7). The results showed an apparent elevation in PDGFD expression within tumor cells (GSE147989), NK cells (GSE116256), CD8 + Tex cells (GSE154109), and endothelial cells (GSE135851) adjacent to the tumor (Fig. 7B–C and Supplementary Fig. S11), in line with the findings of the immune infiltration analysis. When comparing NK cells to the control group, PDGFD-expressing NK cells in AML group decreased (Fig. 7D).
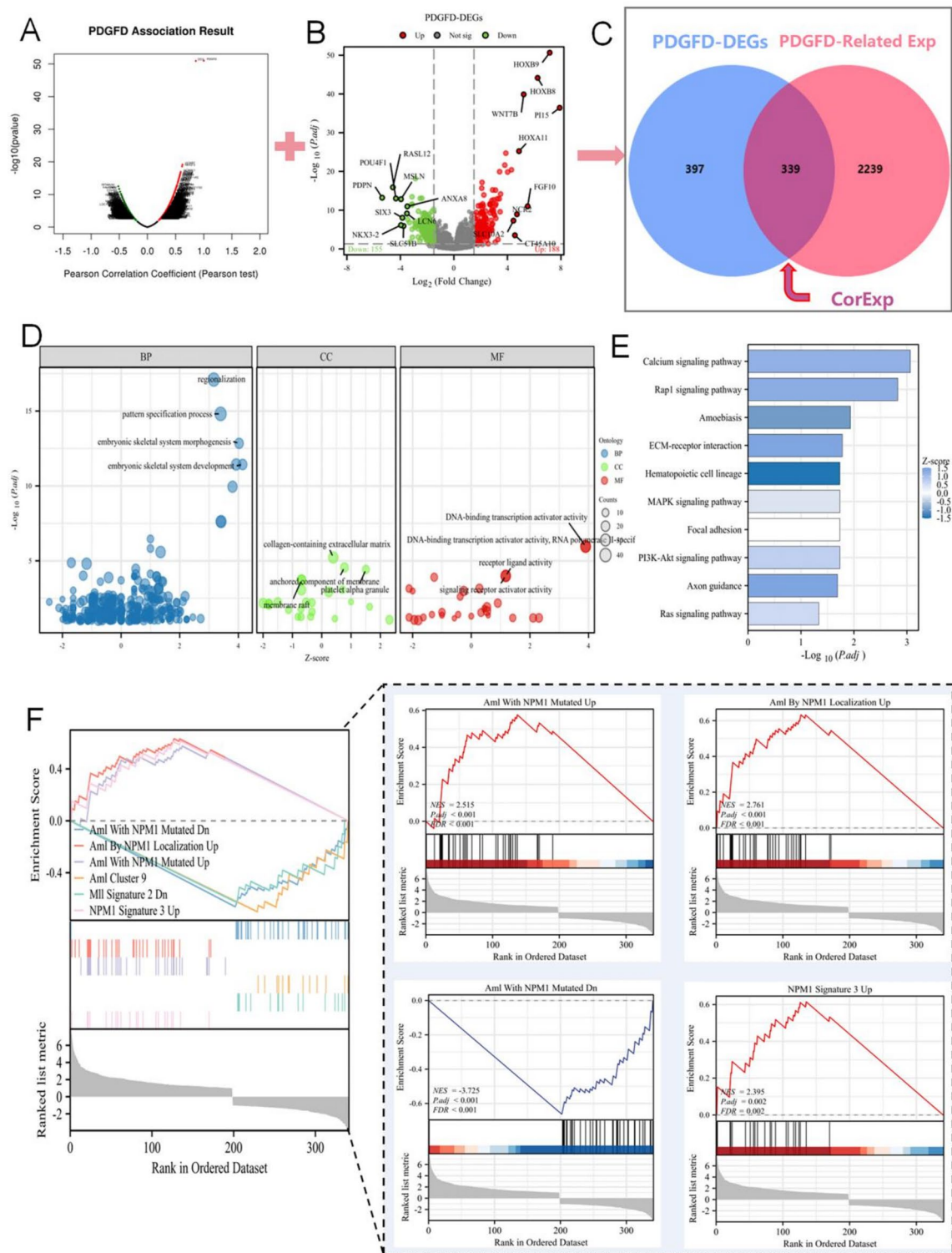
Fig. 5. Functional enrichment analysis of PDGFD gene. (**A**–**C**) Screening of genes related to PDGFD for functional enrichment analysis. (**D**) GO Terms. (**E**) KEGG pathways. (**F**) GSEA analysis.

Drug sensitivity analysis of PDGFD gene

An analysis of the drug susceptibility of PDGFD was undertaken, encompassing various frequently employed AML agents. The findings illustrated in Fig. 8 indicate a detrimental association between heightened PDGFD expression and drug sensitivity, implying an elevated likelihood of drug resistance or primary drug resistance among affected individuals. Our results indicate that patients with elevated PDGFD genes may exhibit increased resistance to BCL-2 inhibitors, as shown in Fig. 8A. BCL-2 inhibitors represent a representative class of drugs
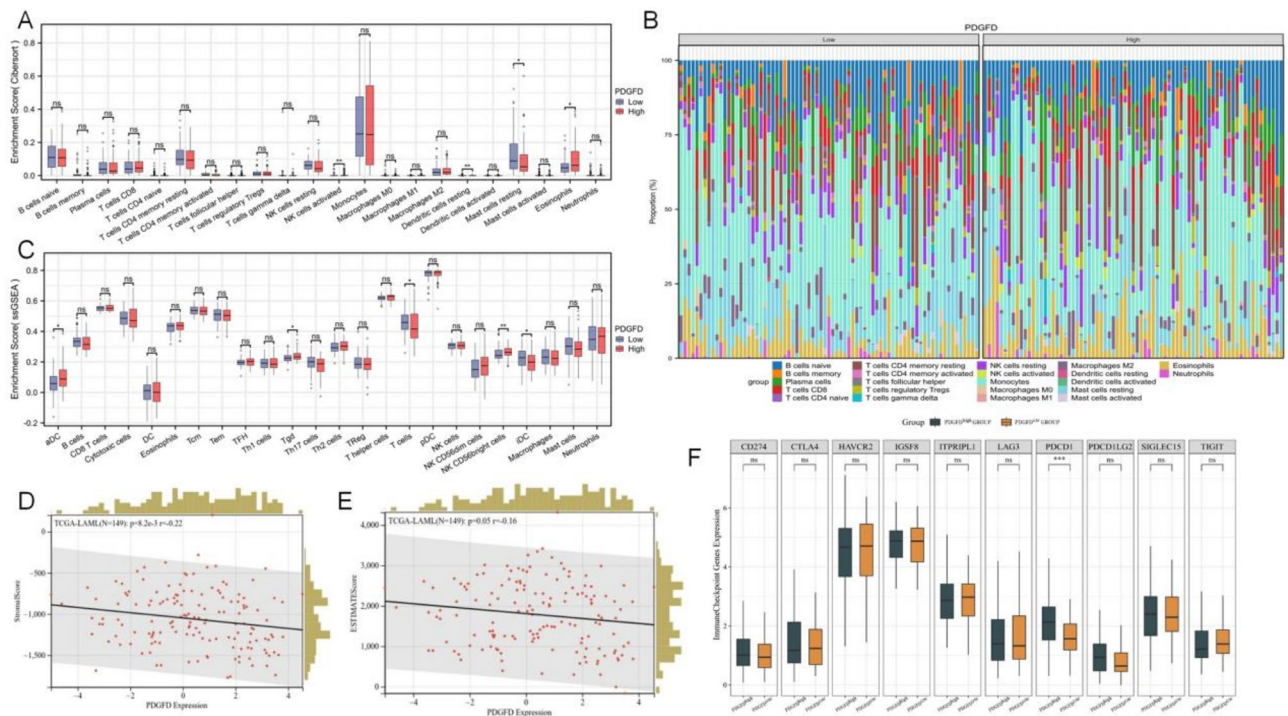
Fig. 6. Correlation analysis of PDGFD and Immune cell infiltration. (**A–B**) The CIBERSORT algorithm determined the differences between PDGFD^{high} and PDGFD^{low} groups. (**C**) ssGSEA analysis. (**D–E**) Stromal score and estimate score in PDGFD^{high} and PDGFD^{low} groups by ESTIMATE algorithm. (**F**) Association of PDGFD with immune checkpoints. P value < 0.05 indicates statistical significance. * $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$; ns, non-significant.

targeting apoptosis and have been widely used in newly treated and relapsed refractory AML patients in recent years.

Hsa-miR-203a-3p may act as an upstream regulator of PDGFD gene

An analysis was conducted on microRNAs that have the potential to modulate the genetic activity of the PDGFD gene. The investigation involved exploring miRNAs across four publicly available databases to identify potential target miRNAs. Two promising factors were pinpointed by examining data from Tarbase, miRDB, miRWalk, and mirDIP (Supplementary Fig. S12A) (Supplementary Fig. S12B). Then, expression correlation and prediction analyses were carried out (Supplementary Fig. S12C–F). The findings indicate that hsa-miR-203a-3p could act as an upstream regulator of the PDGFD gene.

Discussion

The PDGF signaling pathway is activated through the interaction of PDGFRs and PDGFs, which triggers the initiation of comparable signaling cascades. This pathway plays a significant role in the development of several diseases, such as organ fibrosis, atherosclerosis, and tumors[33–35]. Recent research focusing on the structural and functional aspects of PDGF has suggested that it can stimulate the growth of healthy hematopoietic progenitors and cells associated with chronic myelogenous leukemia, as well as modify the functional characteristics of stromal cell subsets[5]. However, the prognosis implications and biological significance of PDGF family members in AML have yet to be elucidated. It is hypothesized that certain PDGFs may contribute to the development of AML and hold prognostic value.

Our research initially investigated the expression levels and prognostic significance of the PDGF family in pan-cancer and AML. The findings indicated that the expression of PDGFA and PDGFB was diminished in AML patients compared to healthy controls, while PDGFC, PDGFD, PDGFRA and PDGFRB exhibited elevated transcription levels in AML. The hazard analysis showed that only PDGFD is a risky factor in AML, while the KM curve indicated that patients exhibiting PDGFD overexpression poorer survival and PDGFB underexpression experienced markedly similar results. This could be as a result of the fact that, despite belonging to the same PDGF family, PDGFB and PDGFD are controlled differently in the setting of illness. For instance, Michael et al. examined the functional enrichment pathways and expression patterns of the PDGF gene family in 16 solid tumors and discovered that, with the exception of GBM, the expression levels of PDGF subtypes varied among tumors[36]. Considering the findings above, we elected to prioritize the PDGFD gene as the subject of subsequent investigation.

Subsequent clinical factor correlation analysis showed that high expression of PDGFD in AML patients was associated with elevated peripheral blood (PB) cells, non-M3 subtypes, intermediate-high cytogenetic
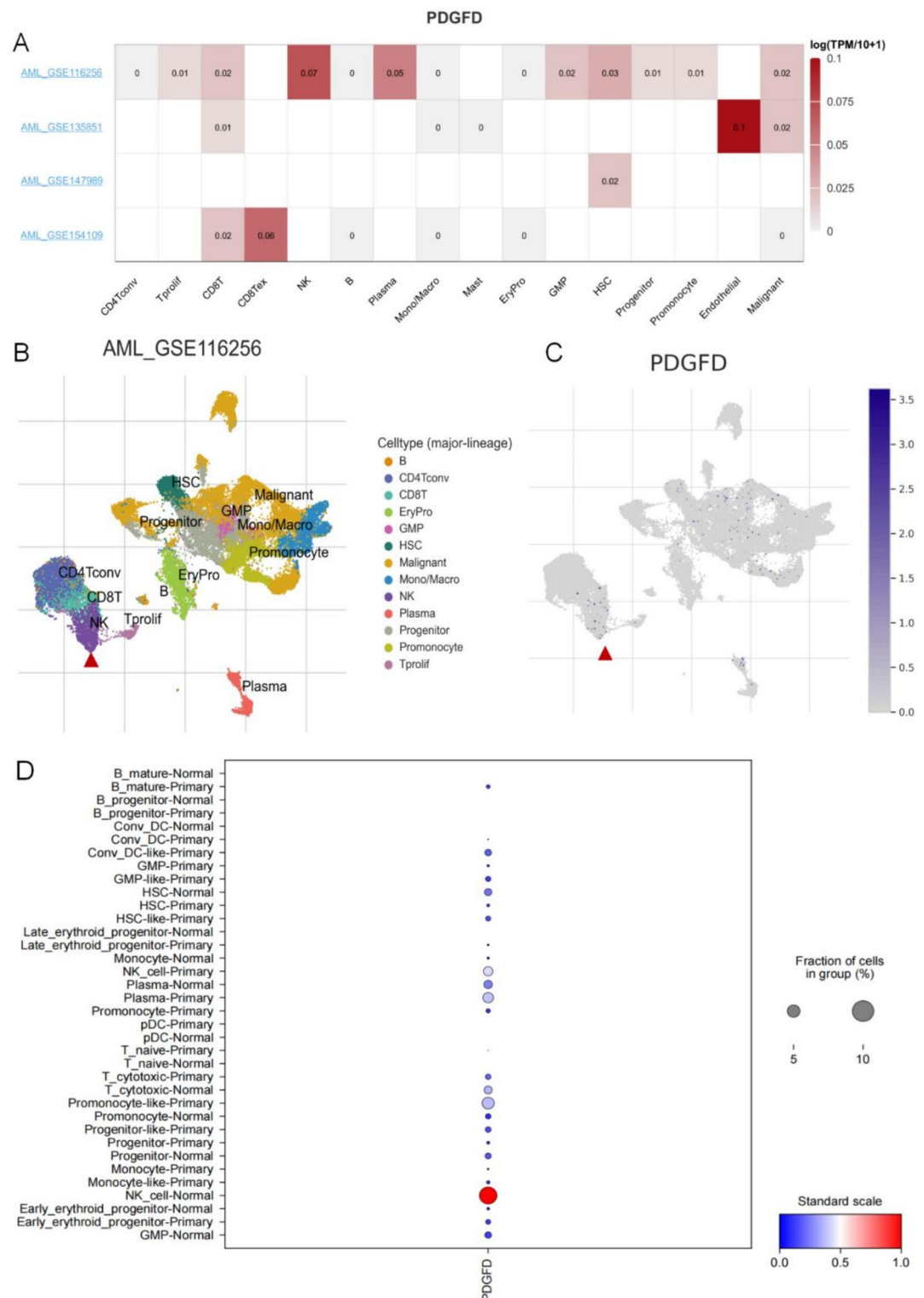
Fig. 7. Single-cell datasets validation of PDGFD expression levels in AML. (**A**) Total four single-cell datasets of AML (GSE116256, GSE135851, GSE147989, GSE154109) were enrolled. (**B–C**) High expressed PDGFD in NK cells anear tumor. (**D**) Expression of PDGFD in different cell types in the tumor microenvironment both in the control group and the AML group.

risk, NPM1 mutation, FLT3-ITD mutation, and poor prognosis, all of which are poor prognostic indicators of leukemia. The Kaplan-Meier curve for subgroups indicated that elevated PDGFD expression correlated with unfavorable prognosis in the age≤60 subgroup, AML-M2 subgroup, RAS mutation-negative subgroup, NPM1 mutation-negative subgroup, IDH1-R132 mutation-negative subgroup, IDH1-R140 mutation-negative

subgroup, and IDH1-R172 mutation-negative subgroup, respectively. These findings not only imply that PDGFD might be linked to high-risk leukemia behaviors, but they also imply that PDGFD might have negative effects on the group with a favorable prognosis. Notably, whereas 75% of newly diagnosed AML patients are over 60 years old[37], PDGFD predicts overall survival in individuals aged 60 or younger, a result that retains its significance. In the real world, patients in younger age groups tend to receive more aggressive treatments such as intensive chemotherapy or stem cell transplants, but even with many treatments, a proportion of patients become relapsed and refractory leukemia[38]. If PDGFD is a reliable predictor of survival in this group, it has the potential to serve as a biomarker to guide more aggressive or targeted treatment. While the current data suggest a lack of prognostic value in the older group based solely on PDGFD expression, this does not mean that PDGFD is not clinically relevant in this population. PDGFD may interact with other factors in older patients that were not considered in our original analysis. Future research could focus on exploring these potential interactions. Furthermore, PDGFD was found to be markedly upregulated in patients experiencing relapse, and drug sensitivity analysis indicated that individuals with high PDGFD expression levels exhibited reduced responsiveness to chemotherapy agents. These observations suggest that PDGFD may function as a negative prognostic indicator in AML.

NPM1 mutation is detected in roughly 30% of adult AML cases, making it one of the most common genetic abnormalities in AML and regarded as an initiating lesion, significantly linked to frank leukemia[39,40]. Typically, NPM1 mutations involve small insertions in the final exon of the gene, leading to the loss of a signal that directs the protein to the nucleolus and the creation of a new signal that directs it to the cell nucleus. Consequently, the normal movement of the NPM1 protein between the nucleus and cytoplasm is disrupted, resulting in abnormal accumulation of the protein in the cytoplasm of leukemic cells with NPM1 mutations[40,41]. Recent research using a mice model has shown that the mutated form of NPM1 (NPM1c) promotes the expression of HOX/MEIS1 genes, with HOX genes playing a role in sustaining the leukemic state in AML with NPM1 mutations[42]. However, the specific mechanisms linking the cytoplasmic localization of NPM1c and HOX gene expression require further investigation.

This study demonstrated that PDGFD expression was significantly elevated in NPM1-mutation-positive AML patients relative to those without the mutation. PDGFD expression levels have shown promise as a diagnostic marker for NPM1-mutated leukemia. Analysis of the mutational landscape revealed that frame-shift insertions in the NPM1 gene were more commonly observed in cases with increased PDGFD expression. Subsequent analysis of gene expression patterns indicated a close relationship between PDGFD and HOXB cluster genes. GSEA further confirmed a notable association between high PDGFD expression and the presence of NPM1 mutations.

Moreover, the biological enrichment analysis indicated that PDGFD was implicated in the calcium and Rap1 signaling pathways as the primary pathways. Disrupted calcium homeostasis plays a crucial role in the progression of various types of cancer[43]. Qiu et al. have documented that Rap1 GAP can enhance leukemia cells' differentiation and apoptosis while promoting leukemia cell invasion in vitro[44]. Hence, we hypothesize that PDGFD may contribute to initiating NPM1 mutations and the perpetuation of leukemia through interactions with HOXs. Further investigation is necessary to confirm the impact of elevated PDGFD expression on NPM1-mutated AML and to clarify the mechanisms involved.

MicroRNAs (miRNAs) are small noncoding RNAs capable of inducing mRNA degradation or suppressing translation, serving vital functions in various essential biological processes, such as cell proliferation, differentiation, migration, survival, and programmed cell death[45]. MiR-203a-3p has been documented to function as a tumor suppressor, with its expression downregulated in numerous cancers[46]. For instance, in inflammatory breast cancer[47], a significant decrease in miR-203a-3p expression has been noted, indicating its potential utility as a diagnostic biomarker for this disease. In colorectal cancer, miR-203a-3p modulates cancer cell proliferation, metastasis, and resistance to chemotherapy by targeting phosphodiesterase 4D (PDE4D) and the Wnt/β-catenin signaling pathways[48]. Furthermore, miR-203a-3p has been extensively investigated in other cancers like nasopharyngeal carcinoma and hepatocellular carcinoma[49]. Bioinformatics analysis showed that miR-203a-3p is inversely correlated with PDGFD and was downregulated in AML. Patients exhibiting high levels of miR-203a-3p tend to have a more favorable prognosis, suggesting a potential regulatory role of miR-203a-3p in modulating the oncogenic effect of PDGFD in AML.

There is mounting evidence indicating the significant impact of the bone immune micro-environment in immunotherapy, tumor progression, and prognosis in patients with AML[50]. Human Natural Killer (NK) cells constitute approximately 10–15% of circulating lymphocytes, with CD56(bright) and CD56(dim) NK cells being the primary subsets[51]. Traditionally, NK cells have been recognized for their importance in immunosurveillance and exerting anti-tumor effects[52]. Previous studies have confirmed that PDGFD can hinder tumor cell growth by interacting with NKp44, thereby prompting NK cells to release interferon (IFN-γ) and tumor necrosis factor (TNF)-α[53]. Recent studies emphasized the critical role of the PDGFD-PDGFRβ signaling pathway in regulating IL-5-mediated NK cell growth and survival[54]. Our examination of immune cell infiltration revealed a positive connection between PDGFD and the prevalence of several immune cells, including activated dendritic cells, gamma delta T cells, and CD56(bright) NK cells.

Furthermore, examinations at the single-cell level demonstrated heightened PDGFD gene expression in NK cells situated in the tumor microenvironment. Nevertheless, it is widely recognized that AML patients exhibit deficiencies in the number and cytotoxic activity of NK cells in the bone marrow, which are linked to the development, advancement, and relapses of AML. This insight may explain the challenges in predicting the efficacy of PDGFD immunotherapy. On a positive note, given the elevated expression of PDGFD in leukemia and its close relationship with NK cells, a potential strategy could involve selectively introducing the PDGF signal into NK cells during NK cell-based immunotherapy to promote the expansion, persistence, and enhancement of NK cell effector function.
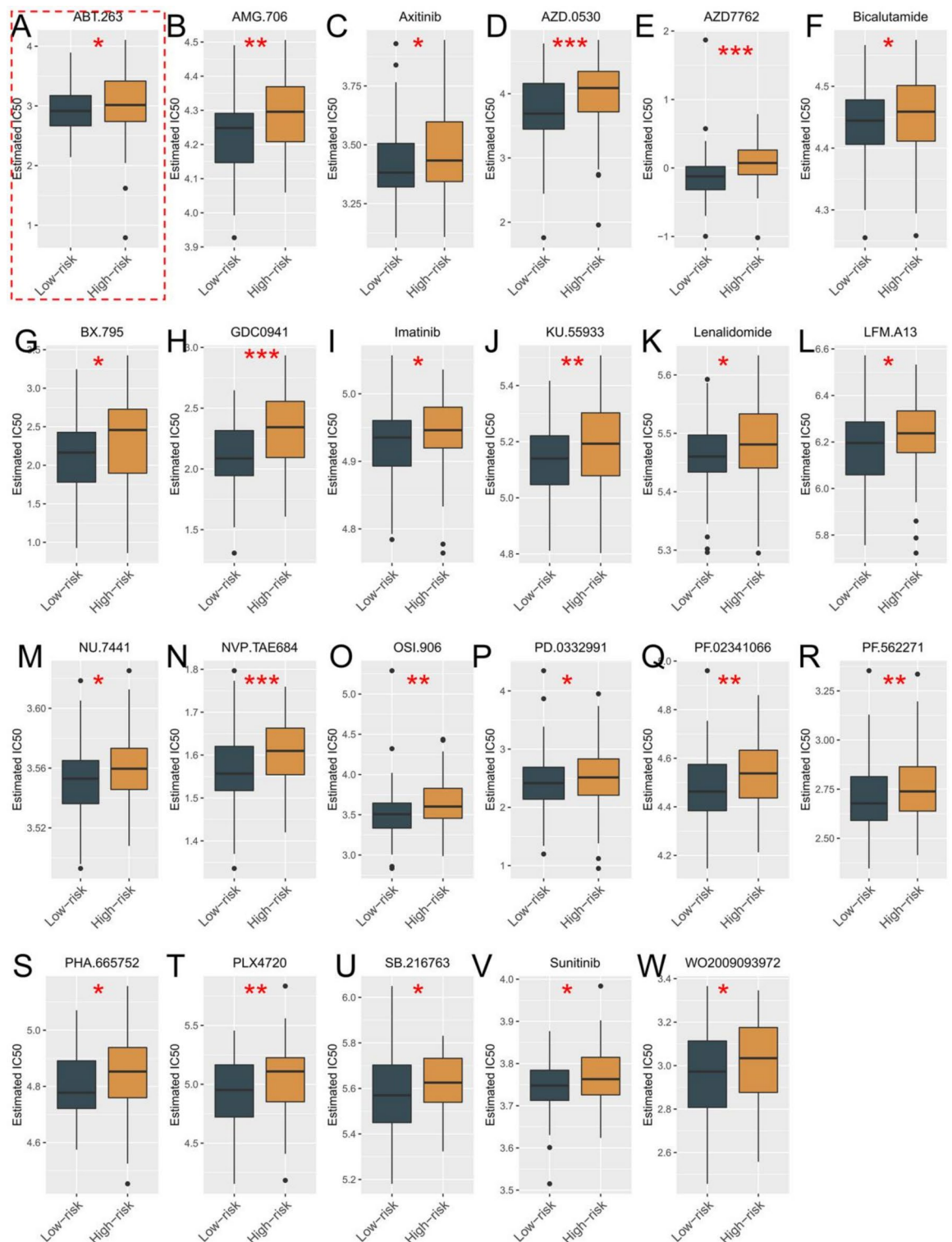
Although our analysis is based on solid data and methods, it is crucial to recognize specific limitations. Most of our research data is primarily sourced from public repositories, and it is recognized that bio-informatics analysis offers only a partial representation of physiological conditions within the body. Additionally, the survival data utilized in our study is derived from a clinical cohort established several decades ago, potentially limiting its applicability to current treatment practices for AML, which have evolved and improved over time. Furthermore, the clinical samples provided are from a single center and a small sample size, which may introduce bias to our conclusions. Consequently, the information presented in this study is primarily indicative and necessitates further validation through experimental studies.

In summary, this research has unveiled, for the first time, an increased expression of PDGFD in AML, which correlates with a poor prognosis. Furthermore, PDGFD is suggested to play a role in initiating and advancing NPM1-mutated leukemia through various mechanisms, such as the modulation of target genes and signaling

◀**Fig. 8**. Drug sensitivity analysis of PDGFD in AML. (**A**) ABT.263: Inhibitor of the Bcl-2 protein family, acting on Bcl-xL, Bcl-2 and Bcl-w; (**B**) AMG.706: ATP-competitive inhibitor of Vascular endothelial growth factor receptor (VEGFR)1/2/3; (**C**) Axitinib: Inhibitor of VEGFR; (**D**) AZD.0530: inhibitor of the Src family; (**E**) AZD7762: ATP-competitive checkpoint kinase (Chk) inhibitor; (**F**) Bicalutamide: non-steroidal androgen receptor (AR) antagonist; (**G**) BX.795: Inhibitor of PDK1; (**H**) GDC0941: Inhibitor of PI3Kα/δ; (**I**) Imatinib: Inhibitor of the tyrosine kinase; (**J**) KU.55,933: Inhibitor of the ATM; (**K**) Lenalidomide: Immunomodulator; (**L**) LFM.A13: Inhibitor of the BTK, JAK2, PLK; (**M**) NU.7441: Inhibitor of the DNA-PK; (**N**) NVP.TAE684: Inhibitor of the ALK; (**O**) OSI.906: Inhibitor of the insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R) kinases; (**P**) PD.0332991: Inhibitor of the cyclin D kinase 4/6; (**Q**) PF.02341066: Inhibitor of the ATP-competitive ALK and c-Met; (**R**) PF.562,271: ATP-competitive and reversible FAK and Pyk2 kinase inhibitor; (**S**) PHA.665,752: ATP-competitive, and active-site inhibitor of the catalytic activity of c-Met kinase; (**T**) PLX4720: Inhibitor of B-Raf-V600E; (**U**) SB.216,763: Inhibitor of the ATP-competitive GSK-3; (**V**) Sunitinib: Inhibitor of the tyrosine kinase; (**W**) WO2009093972: A potent HSP90 inhibitor; * $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$; ns, non-significant.

pathways. The sequestration of PDGFD by MIR-203-3p may demonstrate an anti-leukemic impact in adult AML. Our work together emphasizes the potential function of PDGFD in facilitating AML progression, highlighting its importance as a prognostic marker and a prospective therapeutic target in AML treatment.

Data availability

The publicly available data could be acquired from TCGA dataset (https://portal.gdc.cancer.gov/), GEO dataset (https://www.ncbi.nlm.nih.gov/geo/), and Bloodspot project (http://servers.binf.ku.dk/bloodspot/). The original contributions presented in the study are included in the article/Supplementary Material, Further inquiries can be directed to the corresponding authors.

References

1. De Kouchkovsky, I. & Abdul-Hay, M. Acute myeloid leukemia: a comprehensive review and 2016 update. *Blood Cancer J.* **6**, e441. https://doi.org/10.1038/bcj.2016.50 (2016).
2. Döhner, H., Weisdorf, D. J. & Bloomfield, C. D. Acute myeloid leukemia. *N Engl. J. Med.* **373**, 1136–1152. https://doi.org/10.1056/NEJMra1406184 (2015).
3. Long, N. A., Golla, U., Sharma, A. & Claxton, D. F. Acute myeloid leukemia stem cells: Origin, characteristics, and clinical implications. *Stem Cell. Rev.* **18**, 1211–1226. https://doi.org/10.1007/s12015-021-10308-6 (2022).
4. Schepers, K., Campbell, T. B. & Passegué, E. Normal and leukemic stem cell niches: insights and therapeutic opportunities. *Cell. Stem Cell.* **16**, 254–267. https://doi.org/10.1016/j.stem.2015.02.014 (2015).
5. Demoulin, J. B. & Montano-Almendras, C. P. Platelet-derived growth factors and their receptors in normal and malignant hematopoiesis. *Am. J. Blood Res.* **2**, 44–56 (2012).
6. Lotem, J. & Sachs, L. Cytokine control of developmental programs in normal hematopoiesis and leukemia. *Oncogene* **21**, 3284–3294. https://doi.org/10.1038/sj.onc.1205319 (2002).
7. Yamashita, M., Dellorusso, P. V., Olson, O. C. & Passegué, E. Dysregulated Haematopoietic stem cell behaviour in myeloid leukaemogenesis. *Nat. Rev. Cancer.* **20**, 365–382. https://doi.org/10.1038/s41568-020-0260-3 (2020).
8. Tsuzuki, M. et al. Proliferative effects of several hematopoietic growth factors on acute myelogenous leukemia cells and correlation with treatment outcome. *Leukemia* **11**, 2125–2130. https://doi.org/10.1038/sj.leu.2400870 (1997).
9. Binder, S., Luciano, M. & Horejs-Hoeck, J. The cytokine network in acute myeloid leukemia (AML): A focus on pro- and anti-inflammatory mediators. *Cytokine Growth Factor. Rev.* **43**, 8–15. https://doi.org/10.1016/j.cytogfr.2018.08.004 (2018).
10. Kazlauskas, A. PDGFs and their receptors. *Gene* **614**, 1–7. https://doi.org/10.1016/j.gene.2017.03.003 (2017).
11. Raica, M. & Cimpean, A. M. Platelet-derived growth factor (PDGF)/PDGF receptors (PDGFR) Axis as target for antitumor and antiangiogenic therapy. *Pharmaceuticals (Basel)* **3**, 572–599. https://doi.org/10.3390/ph3030572 (2010).
12. Fredriksson, L., Li, H. & Eriksson, U. The PDGF family: Four gene products form five dimeric isoforms. *Cytokine Growth Factor. Rev.* **15**, 197–204. https://doi.org/10.1016/j.cytogfr.2004.03.007 (2004).
13. Zou, X. et al. Targeting the PDGF/PDGFR signaling pathway for cancer therapy: A review. *Int. J. Biol. Macromol.* **202**, 539–557. https://doi.org/10.1016/j.ijbiomac.2022.01.113 (2022).
14. Gialeli, C. et al. PDGF/PDGFR signaling and targeting in cancer growth and progression: Focus on tumor microenvironment and cancer-associated fibroblasts. *Curr. Pharm. Des.* **20**, 2843–2848. https://doi.org/10.2174/13816128113199990592 (2014).
15. Su, R. J. et al. Platelet-derived growth factor promotes ex vivo expansion of CD34 + cells from human cord blood and enhances long-term culture-initiating cells, non-obese diabetic/severe combined immunodeficient repopulating cells and formation of adherent cells. *Br. J. Haematol.* **117**, 735–746. https://doi.org/10.1046/j.1365-2141.2002.03500.x (2002).
16. Foss, B., Ulvestad, E. & Bruserud, Ø. Platelet-derived growth factor (PDGF) in human acute myelogenous leukemia: PDGF receptor expression, endogenous PDGF release and responsiveness to exogenous PDGF isoforms by in vitro cultured acute myelogenous leukemia blasts. *Eur. J. Haematol.* **67**, 267–278. https://doi.org/10.1034/j.1600-0609.2001.0430a.x (2001).
17. Goldman, M. J. et al. Visualizing and interpreting cancer genomics data via the Xena platform. *Nat. Biotechnol.* **38**, 675–678. https://doi.org/10.1038/s41587-020-0546-8 (2020).
18. Wang, S. et al. UCSCXenaShiny: An R/CRAN package for interactive analysis of UCSC Xena data. *Bioinformatics* **38**, 527–529. https://doi.org/10.1093/bioinformatics/btab561 (2022).
19. Tang, Z., Kang, B., Li, C., Chen, T. & Zhang, Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res.* **47**, W556–w560. https://doi.org/10.1093/nar/gkz430 (2019).
20. Bagger, F. O., Kinalis, S. & Rapin, N. BloodSpot: a database of healthy and malignant haematopoiesis updated with purified and single cell mRNA sequencing profiles. *Nucleic Acids Res.* **47**, D881–d885. https://doi.org/10.1093/nar/gky1076 (2019).
21. Győrffy, B. Transcriptome-level discovery of survival-associated biomarkers and therapy targets in non-small-cell lung cancer. *Br. J. Pharmacol.* **181**, 362–374. https://doi.org/10.1111/bph.16257 (2024).

22. Chandrashekar, D. S. et al. UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia* **19**, 649–658. https://doi.org/10.1016/j.neo.2017.05.002 (2017).
23. Vasaikar, S. V., Straub, P., Wang, J. & Zhang, B. LinkedOmics: Analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Res.* **46**, D956–d963. https://doi.org/10.1093/nar/gkx1090 (2018).
24. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30. https://doi.org/10.1093/nar/28.1.27 (2000).
25. Kanehisa, M. Toward Understanding the origin and evolution of cellular organisms. *Protein Sci.* **28**, 1947–1951. https://doi.org/10.1002/pro.3715 (2019).
26. Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M. & Ishiguro-Watanabe, M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res.* **51**, D587–d592. https://doi.org/10.1093/nar/gkac963 (2023).
27. Liberzon, A. et al. The molecular signatures database (MSigDB) hallmark gene set collection. *Cell. Syst.* **1**, 417–425. https://doi.org/10.1016/j.cels.2015.12.004 (2015).
28. Lin, A. et al. CAMOIP: a web server for comprehensive analysis on multi-omics of immunotherapy in pan-cancer. *Brief. Bioinform.* **23** https://doi.org/10.1093/bib/bbac129 (2022).
29. Damiani, D. & Tiribelli, M. Present and future role of immune targets in acute myeloid leukemia. *Cancers (Basel).* **15** https://doi.org/10.3390/cancers15010253 (2022).
30. Sun, D. et al. TISCH: A comprehensive web resource enabling interactive single-cell transcriptome visualization of tumor microenvironment. *Nucleic Acids Res.* **49**, D1420–d1430. https://doi.org/10.1093/nar/gkaa1020 (2021).
31. Huang, C. et al. ScCancerExplorer: A comprehensive database for interactively exploring single-cell multi-omics data of human pan-cancer. *Nucleic Acids Res.* gkae1100. https://doi.org/10.1093/nar/gkae1100 (2024).
32. Yang, W. et al. Genomics of drug sensitivity in cancer (GDSC): A resource for therapeutic biomarker discovery in cancer cells. *Nucleic Acids Res.* **41**, D955–961. https://doi.org/10.1093/nar/gks1111 (2013).
33. Contreras, O., Córdova-Casanova, A. & Brandan, E. PDGF-PDGFR network differentially regulates the fate, migration, proliferation, and cell cycle progression of myogenic cells. *Cell. Signal.* **84**, 110036. https://doi.org/10.1016/j.cellsig.2021.110036 (2021).
34. Gallini, R., Lindblom, P., Bondjers, C., Betsholtz, C. & Andrae, J. PDGF-A and PDGF-B induces cardiac fibrosis in Transgenic mice. *Exp. Cell. Res.* **349**, 282–290. https://doi.org/10.1016/j.yexcr.2016.10.022 (2016).
35. Cao, Y. Multifarious functions of PDGFs and PDGFRs in tumor growth and metastasis. *Trends Mol. Med.* **19**, 460–473. https://doi.org/10.1016/j.molmed.2013.05.002 (2013).
36. Michael, B. & Kristian, P. PDGF family function and prognostic value in tumor biology. *Biochem. Biophys. Res. Commun.* **503**, 984–990. https://doi.org/10.1016/j.bbrc.2018.06.106
37. Tiong, I. S. et al. How comparable are patient outcomes in the real-world with populations studied in pivotal AML trials? *Blood Cancer J.* **14** https://doi.org/10.1038/s41408-024-00996-x (2024).
38. Martínez-Cuadrón, D. et al. Evolving treatment patterns and outcomes in older patients (≥60 years) with AML: Changing everything to change nothing? *Leukemia* **35**, 1571–1585. https://doi.org/10.1038/s41375-020-01058-4 (2021).
39. Weinberg, O. K. et al. The international consensus classification of acute myeloid leukemia. *Virchows Arch.* **482**, 27–37. https://doi.org/10.1007/s00428-022-03430-4 (2023).
40. Ranieri, R. et al. Current status and future perspectives in targeted therapy of NPM1-mutated AML. *Leukemia* **36**, 2351–2367. https://doi.org/10.1038/s41375-022-01666-2 (2022).
41. Hindley, A., Catherwood, M. A., McMullin, M. F. & Mills, K. I. Significance of NPM1 gene mutations in AML. *Int. J. Mol. Sci.* **22** https://doi.org/10.3390/ijms221810040 (2021).
42. Falini, B. NPM1-mutated acute myeloid leukemia: new pathogenetic and therapeutic insights and open questions. *Am. J. Hematol.* **98**, 1452–1464. https://doi.org/10.1002/ajh.26989 (2023).
43. Zheng, S., Wang, X., Zhao, D., Liu, H. & Hu, Y. Calcium homeostasis and cancer: insights from endoplasmic reticulum-centered organelle communications. *Trends Cell. Biol.* **33**, 312–323. https://doi.org/10.1016/j.tcb.2022.07.004 (2023).
44. Qiu, T., Qi, X., Cen, J. & Chen, Z. Rap1GAP alters leukemia cell differentiation, apoptosis and invasion in vitro. *Oncol. Rep.* **28**, 622–628. https://doi.org/10.3892/or.2012.1825 (2012).
45. Saliminejad, K., Khorshid, K., Soleymani Fard, H. R., Ghaffari, S. H. & S. & An overview of MicroRNAs: Biology, functions, therapeutics, and analysis methods. *J. Cell. Physiol.* **234**, 5451–5465. https://doi.org/10.1002/jcp.27486 (2019).
46. Labarrade, F., Botto, J. M. & Imbert, I. M. miR-203 represses keratinocyte stemness by targeting survivin. *J. Cosmet. Dermatol.* **21**, 6100–6108. https://doi.org/10.1111/jocd.15147 (2022).
47. Fahim, S. A. et al. Inflammatory breast carcinoma: elevated MicroRNA miR-181b-5p and reduced miR-200b-3p, miR-200c-3p, and miR-203a-3p expression as potential biomarkers with diagnostic value. *Biomolecules* **10** https://doi.org/10.3390/biom10071059 (2020).
48. Chen, L. et al. miR-203a-3p promotes colorectal cancer proliferation and migration by targeting PDE4D. *Am. J. Cancer Res.* **8**, 2387–2401 (2018).
49. Miao, R. et al. A novel long non-coding RNA XLOC_004787, is associated with migration and promotes cancer cell proliferation by downregulating mir-203a-3p in gastric cancer. *BMC Gastroenterol.* **23**, 280. https://doi.org/10.1186/s12876-023-02912-2 (2023).
50. Tang, L. et al. Characterization of immune dysfunction and identification of prognostic immune-related risk factors in acute myeloid leukemia. *Clin. Cancer Res.* **26**, 1763–1772. https://doi.org/10.1158/1078-0432.Ccr-19-3003 (2020).
51. Poli, A. et al. CD56bright natural killer (NK) cells: an important NK cell subset. *Immunology* **126**, 458–465. https://doi.org/10.1111/j.1365-2567.2008.03027.x (2009).
52. Aggarwal, N., Swerdlow, S. H., TenEyck, S. P., Boyiadzis, M. & Felgar, R. E. Natural killer cell (NK) subsets and NK-like T-cell populations in acute myeloid leukemias and myelodysplastic syndromes. *Cytometry B Clin. Cytom.* **90**, 349–357. https://doi.org/10.1002/cyto.b.21349 (2016).
53. Barrow, A. D. et al. Natural killer cells control tumor growth by sensing a growth factor. *Cell* **172**, 534–548e519. https://doi.org/10.1016/j.cell.2017.11.037 (2018).
54. Ma, S. et al. PDGF-D-PDGFRβ signaling enhances IL-15-mediated human natural killer cell survival. *Proc. Natl. Acad. Sci. U S A* **119** https://doi.org/10.1073/pnas.2114134119 (2022).

Acknowledgements

Author contributions

All authors contributed to the study conception and design: L.Y. and S.R. were responsible for design of experiment, statistical analysis, data collation, and interpretation. The RT-qPCR experiment was executed by R.Z. The first draft of this paper was written by L.Y., J.H., and Q.H. X.W. revised the manuscript. L.L. provided technical guidance. R.Z. and L.Y. offered funding for experimental projects. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Ethical approval
The Ethics Committee of the First Affiliated Hospital of Soochow University provided approval for this study.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-99038-0.

Correspondence and requests for materials should be addressed to X.W. or R.Z.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.