Identification of Prognostic Biomarker and Immune Infiltration of CDH23 in Acute Myeloid Leukemia

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Abstract

Background: Cadherin-23 (CDH23), which plays an important role in intercellular adhesion, is involved in the progression of several types of cancer. However, the biological functions and impact of CDH23 expression on the prognosis of patients with acute myeloid leukemia (AML) are yet to be explored. Herein, we aim to characterize the role and molecular functions of CDH23 in AML.

Methods: The expression level of CDH23 were assessed in patients with AML by Gene Expression Profiling Interactive Analysis (GEPIA). The prognostic value of CDH23 was analyzed via GEPIA and LinkedOmics. Correlation analysis and biology function analysis were conducted by LinkedOmics and GeneMANIA database. Relationship of CDH23 with immune infiltration level was detected by TIMER.

Results: In the present study, aberrant overexpression of CDH23 was first confirmed in patients with AML and contributed to poor prognosis. Notably, we observed a negative correlation between CDH23 mRNA expression and immune cell infiltration by calculating the ESTIMATE score. In addition, functional enrichment analysis confirmed that CDH23 plays a crucial role in tumor immunity.

Conclusions: Our findings indicate that upregulation of CDH23 expression corresponded to shortened overall survival of patients with AML. CDH23 may be involved in mediating tumor immunity, and this highlights the potential of CDH23 as a therapeutic target in AML.

1. background

Acute myeloid leukemia (AML) is an aggressive clonal hematopoietic neoplasm characterized by an accumulation of immature progenitor cells of the myeloid lineage in the bone marrow that leads to inhibition of normal hematopoietic cell proliferation.[1, 2, 3] Most AML patients can respond to standard chemotherapy. However, drug resistance is common, and the majority of patients die due to relapse of the disease and have a median overall survival (OS) of 3-7 months.[4, 5, 6, 7, 8, 9] In addition, the prognosis of older patients with AML is still dismal despite recent improvements.[10]

The cadherin family is a class of transmembrane proteins. They play roles in body development, tissue architecture, and signal transduction by regulating intercellular adhesion and cell-cell recognition in a Ca2+-dependent manner.[11, 12, 13, 14, 15] Cadherin-23 (CDH23) is classified as an atypical cadherin due to lack of classical cadherin features and a remarkably long EC domain. However, CDH23 is similar to classical cadherins in that it also uses trans and cis homologous interactions to adhere to adjacent cells. CDH23 expression has also been identified in multicellular organizations including the brain, heart, lungs, kidneys, nose, and eyes. The Human Protein Atlas has reported that CDH23 is expressed in almost all healthy epithelial tissues. Deregluation of CDH23 significantly disrupts homotypic and heterotypic adhesions and increases the metastatic potential of tumors. With regard to breast cancer, CDH23 was reported at the junction between MCF-7 and normal breast fibroblasts in vitro. Genome-wide association analysis showed that the genetic locus of CDH23 had a significant association with estimated glomerular filtration rate, and its downregulation in zebrafish embryo makes the kidney vulnerable to nephrotoxic
damage. These findings indicate a critical role for CDH23 in kidney development and function. Additionally, CDH23 protein levels are elevated in human breast cancer. The Cancer Genome Atlas (TCGA) shows the deregulation of CDH23 in various solid cancers and that it suppresses metastasis of sarcomas, adrenocortical carcinoma, and cervical cancer.\[16, 17, 18, 19, 20, 21\] However, the role of CDH23 in AML remains obscure. In this study, we comprehensively investigated the expression profile and biological functions of CDH23 in AML and further analyzed the association between CDH23 expression and the tumor immune microenvironment of AML by systematic bioinformatics analysis.

2. methods

2.1 GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) is an analysis tool that contains RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects.\[22\] In this study, we utilized the “Single Gene Analysis” module to perform a differential mRNA expression analysis and prognostic analysis of CDH23 expression in AML patients and healthy donors. The p-value cutoff was set at 0.05. Prognostic analysis was performed using a Kaplan-Meier curve.

2.2 LinkedOmics

LinkedOmics (http://www.linkedomics.org/) is a publicly available portal that includes multi-omics data from all 32 TCGA cancer types. The web application has three analytical modules: LinkFinder, LinkInterpreter, and LinkCompare. We used the “LinkFinder” module to investigate the transcriptional factor target enrichment of CDH23. The analysis results can be visualized using scatter plots, box plots, or Kaplan-Meier plots. To derive biological insights from the association results, the LinkInterpreter module performs gene set enrichment analysis based on Gene Ontology, biological pathways, network modules, and other functional categories.\[23\] We used the “clusterProfiler” R Package to conduct GO enrichment analysis, and the enriched pathways were finally visualized using the dotplot and emapplot functions. The R language software was used in this analysis. A two-tailed P value <0.05 was considered statistically significant.

2.3 TIMER

TIMER (https://cistrome.shinyapps.io/timer/) is a comprehensive resource that provides systematic analysis of immune infiltration.\[24\] In our study, we used the “Estimation” module to evaluate the correlation between CDH23 expression level and immune infiltration in the AML dataset from TCGA via several computational algorithms, such as TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, and XCELL. Immune cell scores were described by the immune and stromal scores. Different immune cell types were estimated, including B cells, macrophages, neutrophils, NK cells, CD8+ T cells, CD4+ T cells, and dendritic cells. The association of gene expression level with immune cell scores was considered significant and positive when P <0.05 and R >0.20.
2.4 GeneMANIA

GeneMANIA (http://www.genemania.org/) is a useful web resource that can explore the potential functions of selected genes and construct a protein-protein interaction network. Association data including protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity can be provided via GeneMANIA.[25]

3. Results

3.1 Aberrant expression and prognostic value of CDH23 in patients with AML

As shown in Figure 1A, we utilized the GEPIA dataset to analyze CDH23 expression level in AML. CDH23 mRNA expression was significantly higher in 173 AML tissues compared to the corresponding normal samples from TCGA and GTEx data. We then investigated whether CDH23 level was predictive of survival of patients with AML. Survival analysis performed using the GEPIA database demonstrated that high CDH23 mRNA levels were associated with shorter OS of patients with AML (HR=1.9, P=0.0019, Figure 1B, C). Analysis of the LinkedOmics database indicated that overexpression of CDH23 corresponded to poor prognosis (P=0.01). Notably, these results showed that high CDH23 expression was significantly related to poor OS in AML.

3.2 Correlated gene analysis of CDH23 in patients with AML

To illustrate the potential mechanisms and functions of CDH23 in AML, we used the LinkedOmics database for correlation analysis between CDH23 and various genes. The results in Figure 2A-C are presented as heat and volcano maps of the top 50 genes that are either positively or negatively correlated with CDH23 expression. CDH23 was positively and significantly correlated with PPM1M, C10orf105, TFEB, FGD2, and IGF2R. On the other hand, CDH23 had a significant negative correlation with CASP6, KDM5B, KCNQ5, and SCCPDH. We chose the most frequently altered neighboring genes (cor>0.5), including ITGAM (Spearman correlation: 0.6822, P=1.765e-24), TFEB (Spearman correlation: 0.7165, P=0), PPM1M (Spearman correlation: 0.7524, P=4.274e-32), and SLC8A1 (Spearman correlation: 0.6635, P=0), to conduct a correlation analysis via LinkedOmics (Figure 2D-G). As shown in Figure 3, we further analyzed the prognostic value of the four genes in AML. The results demonstrated that overexpression of ITGAM was highly associated with poor prognosis. Similarly, high TFEB, PPM1M, and SLC8A1 mRNA expression corresponded to poor OS.

3.3 Impact of CDH23 expression on immune cell infiltration and tumor microenvironment (TME) in patients with AML

Tumor-infiltrating immune cells are a major component of the TME and are involved in the occurrence, progression, and metastasis of cancer. Tumor-infiltrating lymphocyte grade is a powerful independent predictor of sentinel lymph node status and clinical survival in some cancer types.[26, 27] To explore the
relationship between CDH23 expression and immune cell infiltration, we performed analysis using the TIMER database based on sequencing data from AML cases in the TCGA database. The online database stratified patients with AML into high and low CDH23 expression groups. Subsequently, we used several computational algorithms to estimate the abundance of various tumor-infiltrating immune cell types (B cells, CD4+ T cells, CD8+ T cells, macrophages, monocytes, etc.) among the high and low CDH23 expression groups. As shown in Figure 4A, CDH23 expression was significantly related to the infiltration of various immune populations in AML including B cells (P<0.01), CD4+ T cells (P<0.01), CD8+ T cells (P<0.05), mast cells (P<0.01), monocytes (P<0.001), etc. Additionally, we used the TIMER database to further investigate the role of CDH23 in the TME. The analysis revealed that the ESTIMATE score (P<0.001) of the high CDH23 expression group was significantly higher than that of the low CDH23 expression group, and there was a statistically significant positive correlation between CDH23 expression and the immune and stromal scores (Figure 4B-D, P<0.001). All these results suggested that the expression level of CDH23 was highly correlated with tumor immune infiltration and the TME.

3.4 Functional enrichment analysis of CDH23 and related genes in patients with AML

To further study the molecular functions and biological processes of CDH23, we employed the LinkFinder module of the LinkedOmics database to analyze the mRNA sequencing data of patients with AML in the TCGA. The GO enrichment analysis data indicated that the expression of CDH23 and most of the related genes were correlated to pathways or biological processes of immune response and cytokine production such as neutrophil activation involved in immune response, neutrophil degranulation, positive regulation of cytokine production, phagocytosis, cellular response to biotic stimulus, cellular response to molecules of bacterial origin, cellular response to lipopolysaccharide, and so on (Figure 5A, B). Moreover, the significant transcription factor targets of CDH23 included PEA3, ELF1, IRF, PU.1, etc (Figure 5C). In addition, the top 10 pathways related to the functions of CDH23 expression level in AML were illustrated through KEGG analysis and included osteoclast differentiation, phagosome, lysosome, tuberculosis, chemokine signaling pathway, endocytosis, NOD-like receptor signaling pathway, regulation of actin cytoskeleton and others (Figure 5D). In conclusion, CDH23 had an extensive influence on the regulation of several pathways and processes involved in tumor immunity.

3.5 Protein-protein interaction network (PPI) analysis of CDH23 expression level via GeneMANIA

To further elucidate the mechanism of CDH23 in tumorigenesis, we constructed an integrated PPI network using GeneMANIA to screen for CDH23-binding proteins. As described in Figure 6, the interaction network showed that CDH23 is highly linked with USH1C, ABI1, NCKAP1, CYFIP1, WASF1, PCDH15, and other vital proteins. Among the interacting proteins, we observed enrichment of biological processes associated with actin-based cell projection, extrinsic components of the plasma membrane, neuromuscular processes, clusters of actin-based cell projections, retina homeostasis, stereocilium bundles, and extrinsic components of membranes. These results strongly support the hypothesis that CDH23 is involved in the tumorigenesis and pathogenesis of AML.
4. Discussion

It was reported that CDH23 was uniquely expressed at the cell-cell boundaries of various adjacent cells. [18] Although the role of CDH23 in cancer progression and prognosis has been illustrated by a few studies,[28, 29] systematic and comprehensive analysis of its role in AML via bioinformatics tools has not yet been carried out. Therefore, this is the first study to explore the influence of CDH23 mRNA expression level on immune infiltration and prognosis of AML.

In the past few years, a study on breast cancer found that CDH23 overexpression decreases heterotypic adhesion between breast cancer epithelial cells and fibroblasts and may contribute to early metastasis. [30] Reduction of the methylation of CDH23 is also associated with poor outcomes in diffuse large B-cell lymphoma (DLBCL).[28] Similarly, extensive data-mining from TCGA datasets revealed a reduction of CDH23 in most solid malignancies such as LUAD and ESCC.[18] In our present study, the GEPIA database revealed that the expression of CDH23 was higher in human AML than in normal counterpart samples. Moreover, we used GEPIA datasets to analyze the association of CDH23 expression with the clinical characteristics of the patients with AML, and the results showed that higher mRNA expression of CDH23 corresponded to reduced OS. This was in accordance with the results of LinkedOmics datasets. These findings raise the possibility that CDH23 may serve as a potential prognostic marker and a promising therapeutic target for AML in the future.

The TME is a complex system of cells (endothelial cells, fibroblasts, immune cells, etc.) and extracellular components (cytokines, growth factors, extracellular matrix, etc.). The TME provides strong support for tumor cells and has been demonstrated to play pivotal roles in tumor occurrence, progression, metastasis, and the efficacy of therapeutic interventions.[31, 32, 33] Among the cell types in the TME, immune cells such as macrophages, natural killer cells, neutrophils, dendritic cells, and myeloid derived suppressor cells, play significant roles in tumor progression.[34, 35, 36, 37, 38, 39, 40, 41, 42] In this study, we presented evidence of the potential correlation between CDH23 expression and tumor immune infiltration in AML. By utilizing various immune deconvolution methods, we first demonstrated a significant negative correlation between CDH23 expression and the infiltration level of various immune cells in AML. Notably, high CDH23 mRNA expression corresponded with a significantly higher infiltration level of monocytes. Accordingly, it has been reported that a higher frequency of monocytes in PBMCs is observed in immunotherapy responders compared to non-responders and allows for the prediction of responsiveness prior to the initiation of immunotherapy.[43, 44] These findings indicate that patients with AML who show high expression CDH23 may respond better to immunotherapy, and this may serve as a support for clinical decision making.

The functions of CDH23 and its significantly associated genes were prospectively predicted by analyzing gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) categories in the LinkedOmics datasets. Similarly, the PPI network was constructed using the GeneMANIA online tool. In brief, these findings suggest that CDH23 may be involved in tumor processes including cell apoptosis,
tumor invasion, phagocytosis, granulocyte activation, neutrophil-mediated immunity, interleukin-1 production, and heterotypic cell-cell adhesion.

Taken together, our bioinformatic analysis systematically disclosed statistical correlations of CDH23 expression with the prognosis and extent of immune cell infiltration of AML. We also comprehensively analyzed the functional pathways of CDH23, which provided bioinformatic and computational biology-based insights for further understanding of the role played by CDH23 in tumor processes.

5. Conclusions

In this study, we revealed that CDH23 was overexpressed in patients with AML. In addition, higher mRNA expression of CDH23 was found to be related to a reduction in the OS of these patients. Additionally, the expression of CDH23 may mediate immune infiltration of tumors. In summary, we have determined that CDH23 might be a potential prognostic biomarker and a promising therapeutic target in AML.

Abbreviations

AML: acute myeloid leukemia
DLBCL: diffuse large B-cell lymphoma
LUAD: lung adenocarcinoma
ESCC: esophageal squamous cell carcinoma
TME: tumor microenvironment
CDH23: cadherin-related 23
EC: extracellular
TCGA: The Cancer Genome Atlas
GO: gene ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes

Declarations

1. Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of the Qilu Hospital of Shandong University. All written informed consent was obtained in accordance with the Declaration of Helsinki. All datasets were
retrieved from the online databases, and it was confirmed that all informed written consent had already been obtained.

2. Consent for publication

Not applicable.

3. Availability of data and materials

The datasets supporting the conclusions of this article are available in the TCGA-LAML repository, http://cancergenome.nih.gov/.

4. Competing interests

The authors declare no competing interests.

5. Funding

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6. Authors’ contributions

F Lu and J Yang designed the experiments. GX Ma and J Yang performed the experiments. YH Pang, YN Zhao together with JJ Ye collected the samples and delivered them. J Yang wrote the manuscript and analyzed the data. T Sun, DX Ma together with CY Ji revised the manuscript. All authors read and approved the final manuscript.

7. Acknowledgements

Not applicable.

References


Figures

Figure 1
The transcription level and prognostic feature of CDH23 in AML. The expression status of CDH23 gene in AML patients and paired normal samples was analyzed by GEPIA (A). The overall survival curves comparing patients with high (red) and low (blue) CDH23 expression in AML were plotted by GEPIA (B) and LinkedOmics (C).

**Figure 2**
Correlated genes analysis of CDH23 in AML (LinkedOmics). (A) The volcano plots show the correlated genes of CDH23. (B, C) The heatmaps demonstrate positively and negatively differential expression genes, respectively. (D-G) The scatter plots show Spearman-correlation of CDH23 expression with ITGAM, TFEB, PPM1M, SLC8A1.

Figure 3
The prognostic value of genes significantly associated with CDH23 in AML (GEPIA). (A) The OS curves of high and low ITGAM expression in AML patients. (B) The OS curves of high and low TFEB expression in AML patients. (C) The OS curves of high and low PPM1M expression in AML patients. (D) The OS curves of high and low SLC8A1 expression in AML patients.

Figure 4

Correlation analysis between CDH23 expression in AML and tumor immune infiltration and tumor microenvironment (TIMER). Different algorithms were conducted to investigate the potential correlation between the expression level of CDH23 and the various immune cell infiltration level in AML. *significant correlation P<0.05, **significant correlation P<0.01, ***significant correlation P<0.001 (A). Correlation analysis between CDH23 expression in AML and immune score and stromal score (B-D).
Figure 5

Gene set enrichment analysis and transcription factor target of CDH23. Based on the LinkedOmics dataset, we supplied the dotplot (A) and emapplot (B) to perform the GO pathway analysis. We also analyze the transcription factor target (C) and KEGG pathways of CDH23 in AML (D).
Figure 6

PPI network analysis of CDH23. We used the GeneMANIA database to perform protein-protein interaction of CDH23. The colors of the line illustrate different type of their relationships. The distinct colors inside the gene dots indicate the biological function which these genes involved in, including actin-based cell projection, extrinsic component of plasma membrane, neuromuscular process, cluster of actin-based cell projections, retina homeostasis, stereocilium bundle and extrinsic component of membrane.