The Role of ARHGAP9: Clinical Implication and Potential Function in Acute Myeloid Leukemia

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Research

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Abstract

Background: Rho GTPase activating protein 9 (ARHGAP9) is expressed in many cancers and can inactivate Rho GTPases that are key regulators of cytoskeletal dynamics. However, the exact role of ARHGAP9 in acute myeloid leukemia (AML) is still unclear.

Methods: We compared the transcriptional expression, prognosis, differentially expressed genes, function enrichment, and hub genes in AML patients based on published data in UALCAN, GEPIA, Gene Expression Omnibus (GEO), the Human Protein Atlas (HPA), Cancer Cell Line Encyclopedia (CCLE), LinkedOmics, Metascape, and String databases. Data from the Cancer Genome Atlas (TCGA) database was used to evaluate the correlations between ARHGAP9 expression and various clinicopathological parameters as well as the significantly different genes associated with ARHGAP9 expression.

Results: We found that ARHGAP9 expression was higher in AML patient tissues and cell lines than the corresponding control tissues and other cancer types. Furthermore, ARHGAP9 over-expression was associated with shorter overall survival (OS) in AML patients. Compared with the ARHGAP9low group, ARHGAP9high patients received only chemotherapy showed the significantly worse OS and event-free survival (EFS), but no significant difference after treatment with autologous or allogeneic hematopoietic stem cell transplantation (auto/allo-HSCT). In addition, ARHGAP9high patients undergoing auto/allo-HSCT had significantly better prognosis in OS and EFS than those receiving only chemotherapy. Because most of the overlapping gene between the significantly different genes and co-expression genes were enriched in the immune functions, suggesting an immune regulation potential of ARHGAP9 in AML. Thirty-two hub genes were identified from the differently expressed genes, within which the KIF20A had significant prognostic value for AML.

Conclusions: Our results demonstrated that ARHGAP9 overexpression was associated with poor OS in AML patients and can be used as a prognosis biomarker. AML patients with ARHGAP9 over-expression could benefit from auto/allo-HSCT rather than chemotherapy.

Background

Acute myeloid leukemia (AML) is the second most common type of leukemia diagnosed in adults and children, which is caused by the malignant transformation of myeloid primordial cells. Despite the great progress made in risk stratification, supportive care, multiagent chemotherapy intensification, autologous or allogeneic hematopoietic stem cell transplantation (auto/allo-HSCT), the outcome of AML patients is still discouraging due to recurrence and refractory [1, 2]. Take, for example, approximately 10 to 40% of younger AML patients are refractory after standard chemotherapy, whereas the number is obviously higher for older patients above 60 years (40–60%) [3]. Therefore, identifying robust prognostic markers is important for providing optimal care to AML patients.

The Rho family of GTPases is a family of small (~ 21 kDa) signaling G proteins, which act as molecular switches and are tightly controlled by guanine nucleotide exchange factors (GEFs) and GTPase
activating proteins (GAPs) through generating active GTP-binding and inactive GDP-binding state, respectively [4, 5]. The activated Rho GTPases interact with their downstream effectors to regulate the cytoskeleton of the cell membrane or other cellular compartments [6, 7]. Considerable research has supported the importance of Rho GTPases in hematopoiesis and confirmed that Rho GTPases are related to cytoskeleton rearrangements including adhesion, cytokinesis, differentiation, migration, engraftment, aging, and self-renewal in the cellular process [8–14]. To date, 80 Rho family GAPs were identified, but less than half of them have been clearly investigated in cancers. Rho GTPase activating protein 9 (ARHGAP9) containing RhoGAP, SH3, WW, and PH domains, is a member of Rho GAPs family. Research indicates that it suppresses the adhesion of KG-1 (a human leukemia cell line) to fibronectin and collagen by the activation of cdc42 and Rac1 rather than RhoA [15]. ARHGAP9 is also considered as a novel MAP kinase docking protein and the WW domain of ARHGAP9 interacts with the CD domains of Erk2 and p38alpha leading to the inactivation of MAP kinases [16]. ARHGAP9 inhibits hepatocellular carcinoma cell migration and invasion by increasing the expression of FOXJ2/E-cadherin [17]. In contrast, silencing ARHGAP9 has been shown to reduce the proliferation, migration, and invasive of breast and gastric cancer cells in vitro [18, 19]. These phenomena indicated that ARHGAP9 may play distinct roles in various physiological conditions or in the various tissues and cells.

So far, no studies have been performed to access the expression profile and functions of ARHGAP9 in AML, though study has demonstrated the expression of ARHGAP9 in peripheral blood leukocytes [15]. In the present study, we investigated the expression of ARHGAP9 in human AML samples and cell lines and explored its associations with clinicopathological factors. Then we evaluated the prognostic significance of ARHGAP9. Moreover, we investigated the differentially expressed genes associated with ARHGAP9 expression and discussed their potential functions in AML.

**Materials And Methods**

**Patients and clinical samples**

A total of 151 AML patients with RNA-seq and clinical data from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov) were analyzed in our study [20]. Specifically, 67 patients received only chemotherapy and the remaining 84 patients underwent chemotherapy and autologous or allogeneic hematopoietic stem cell transplantation (auto/allo-HSCT). RNA-seq and clinical data are available on the TCGA website.

**ARHGAP9 expression analysis in AML patient tissues and cell lines**

The expression level of ARHGAP9 in various types of cancers and normal tissues were acquired from the GEPIA database (http://gepia.cancer-pku.cn) and UALCAN database (http://ualcan.path.uab.edu/) [21, 22]. ARHGAP9 expression profiles in various chromosome abnormalities were acquired from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo) database. Cancer Cell Line Encyclopedia
(CCLE, https://portals.broadinstitute.org/ccle) and Human Protein Atlas (HPA, https://www.proteinatlas.org/) were used to evaluate ARHGAP9 expression in multiple cell lines including various leukemia cell lines, lung cell lines, breast cell lines, brain cell lines and so on [23, 24].

**Significantly different genes and co-expression genes analysis**

We used the Limma package in R 3.3.3 to screen significantly different genes between ARHGAP9\(^\text{high}\) and ARHGAP9\(^\text{low}\) groups in AML patients. Adjusted P < 0.05 and |log1.5 FC| ≥ 1 were used as the cut-off value for identifying significantly different genes. Subsequently, co-expression genes in correlation with ARHGAP9 expression were analyzed using the LinkedOmics database (http://www.linkedomics.org/login.php) [25]. The LinkedOmics database including mRNA sequencing data from 151 AML patients in the TCGA was used to examine ARHGAP9 co-expression in AML. In the LinkFinder module of LinkedOmics, Pearson's correlation coefficient was calculated to analyze the data. The results were generated in volcano plots and heat maps. The overlapping genes between significantly different genes and co-expression genes were acquired by Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/), an online website.

**ARHGAP9 functional enrichment and protein-protein interaction (PPI) analysis**

Metascape (http://metascape.org/gp/index.html#/main/step1) is a free gene-list analysis tool for gene functional enrichment analysis [26]. The identified overlapping genes were inputted into the metascape database for the enriched Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, tissues, and disease enrichment analysis. We employed the String database (https://string-db.org/) to analyze the PPI network [27], which was visualization by the Cytoscape_v3.6.1 soft [28]. MCODE, a plugin in Cytoscape, was applied to screen the hub genes among the PPI. Also, the MCODE app sets the following parameters: degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. Depth = 100.

**Statistical analysis**

Statistical analysis was conducted using IBM SPSS 19.0.0. Pearson's chi-square and Fisher's exact tests were selected for comparing the categorical variables, such as sex, FAB classifications, and cytogenetics. Because the number of samples was far below 5000 in the two groups. Shapiro-Wilk test was used to explore whether the values in each group had normal distribution for the comparison of continuous variables (age, BM, WBC, and PB). two-sample Student's t-test was used if the values in each group had normal distribution; if not, Mann-Whitney’s U test was used. Except GEPIA and LinkedOmics were used to evaluate the outcome of overall survival (OS) of AML patients [29]. The prognostic effect of ARHGAP9 expression on event-free survival (EFS) and OS were analyzed with Logrank and Gehan-Breslow-Wilcoxon test in GraphPad Prism 7.0.
Results

ARHGAP9 overexpression in AML cell lines

The online databases CCLE and HPA were used to access ARHGAP9 expression in cell lines. In the HPA database, ARHGAP9 mRNA levels in myeloid such as HEL, HL60, NB4, and U937, were higher than that in lymphoid cell lines. While it was almost not expressed in the other cell lines including the brain, breast, lung and so on (Fig. 1a). Moreover, ARHGAP9 was the highest expressed in AML cell lines in CELL database (Fig. 1b).

ARHGAP9 overexpression in AML

By using the UALCAN and GEPIA databases, we analyzed the mRNA expression of ARHGAP9 in different human tumors. We found the mRNA expression levels of ARHGAP9 is the highest among all human cancers (Fig. 2a, b). Then different tumor tissues together with their adjacent normal tissues were analyzed by using the GEPIA database. The results showed that the expression level of ARHGAP9 in AML patients was significantly higher than normal tissues, and was highest among all cancer types (Fig. 2b, c). To further study whether ARHGAP9 expression is influenced by different chromosomal abnormalities. We retrieved two microarray data (GSE14468 and GSE13159) from the GEO database and evaluated the expression of ARHGAP9 among AML patients with major recurrent chromosomal translocations including inv(16), t(8;21), t(15;17), 11q23, and complex, as well as the normal karyotype. The analysis results of both data sets showed that t(15;17) AML patients had the lowest ARHGAP9 expression as compared with other cytogenetic abnormality groups (Fig. 2d). These results in the AML patient samples correspond with that in the cell lines.

Relationships between ARHGAP9 and clinicopathological characteristics of patients with AML

To investigate the association between ARHGAP9 expression and different clinicopathologic feathers, ARHGAP9 expression was divided into two groups (ARHGAP9\textsuperscript{low} and ARHGAP9\textsuperscript{high}) based on the median values of ARHGAP9 transcript using the Cancer Genome Atlas (TCGA) data. Seventy-five patients were identified as ARHGAP9\textsuperscript{high} group and seventy-five patients were identified as ARHGAP9\textsuperscript{low} group. There were no significant differences in age, sex, BM blasts, WBC, PB blasts (P > 0.05) between ARHGAP9\textsuperscript{low} and ARHGAP9\textsuperscript{high} (Table 1). The significant differences existed in the distribution of FAB classifications, cytogenetics, risk stratification, and gene mutations. High expression of ARHGAP9 was significantly correlated with FAB-M1 (p = 0.022), whereas low expression of ARHGAP9 was significantly correlated with FAB-M3 (p = 0), FAB-M4 (p = 0.010), and FAB-M5 (p = 0.005) in the distribution of FAB classifications. For Cytogenetics, ARHGAP9 overexpression was associated with cytogenetically normal AML (CN-AML) (p < 0.01), and low expression of ARHGAP9 was associated with the t(15;17) (p < 0.01) and complex (p = 0.044) subtypes. Furthermore, ARHGAP9\textsuperscript{low} cases tended to be associated with a good prognosis, whereas ARHGAP9\textsuperscript{high} cases were obviously correlated with intermediate risk. Among the
mutated genes, high expression of ARHGAP9 was only correlated with CEBPA (Additional file 1: Table S1).
Table 1
Correlations between ARHGAP9 expression and clinicopathological features in AML from TCGA cohort

| Patient's parameters          | ARHGAP9 expression |       |       |     |
|------------------------------|--------------------|-------|-------|-----|
|                              | Low (n = 75)       | High (n = 75) | p    |
| Sex, male/female             | 38/37              | 45/30  | 0.217|
| Median age, years (range)    | 53.5(21–77)        | 60(21–88) | 0.13 |
| Median BM blasts, % (range)  | 75(30–100)         | 69(30–99) | 0.063|
| Median WBC, × 10^9/L (range) | 13.1(0.4–137.2)    | 27.6(0.6–223.8) | 0.131|
| Median PB blasts, % (range)  | 39.5(0–97)         | 39(0–96) | 0.465|
| FAB classifications           |                    |       |       |     |
| M0                           | 6                  | 9     | 0.414|
| M1                           | 12                 | 24    | 0.022|
| M2                           | 13                 | 23    | 0.056|
| M3                           | 14                 | 0     | 0     |
| M4                           | 16                 | 5     | 0.010|
| M5                           | 10                 | 1     | 0.005|
| M6                           | 1                  | 0     | 0     |
| M7                           | 1                  | 0     | 1     |
| NA                           | 1                  | 0     | 1     |
| Cytogenetics                 |                    |       |       |     |
| Normal                       | 23                 | 37    | 0.000|
| t(8;21)                      | 3                  | 4     | 0.719|
| t(15;17)                     | 14                 | 0     | 0     |
| inv.(16)                     | 7                  | 3     | 0.327|
| +8                           | 5                  | 3     | 0.719|
| 11q23                        | 7                  | 1     | 0.063|
| -7/del(7)                    | 2                  | 4     | 0.442|
| Complex                      | 5                  | 13    | 0.044|

n, number of patients; FAB, French–American–British subtypes; BM-blast, bone marrow blast; PB-blast, peripheral blood blast; WBC, white blood cell
The prognostic values of ARHGAP9 in AML

We investigated whether the expression of ARHGAP9 was associated with prognosis in AML patients. The OS against ARHGAP9 expression was evaluated using the GEPIA and LinkedOmics databases. As the results from GEPIA and LinkedOmics databases shown, high expression of ARHGAP9 was correlated with the poor OS for AML patients (Fig. 3a, b). We further analyzed the survival data from TCGA and found ARHGAP9 overexpression was related to shorter event-free survival (EFS) (Fig. 3c), albeit statistical significance was not achieved.

Among CN-AML patients, there was no significant difference in OS (Log-rank p = 0.8016) and EFS (p = 0.6696) between ARHGAP9\textsuperscript{low} and ARHGAP9\textsuperscript{high} groups in CN-AML (Fig. 4a, b). To investigate whether AML patients with high expression of ARHGAP9 could benefit from chemotherapy or auto/allo-HSCT, we divided the tested AML patients into two groups according to their treatment regimens. Significantly shorter OS (Log-rank p = 0.018) and EFS (Log-rank p = 0.0288) were found in AML patients with only chemotherapy (Fig. 4c, d). Among patients who received auto/allo-HSCT, no significant differences were found in OS (Log-rank p = 0.7782) and EFS (Log-rank p = 0.8022) between ARHGAP9\textsuperscript{low} and ARHGAP9\textsuperscript{high} groups (Fig. 4e, f). Patients undergoing auto/allo-HSCT had a better prognosis OS (p = 0.005) and EFS (p = 0.0425) than those who received only chemotherapy (Fig. 4g, h). Acute promyelocytic leukemia (FAB M3) is a unique subtype of AML associated with peculiar clinical features and treatment strategies and the prognosis of APL is good. After the exclusion of FAB M3 from the AML cases, the prognostic values of ARHGAP9 expression in CN-AML, chemotherapy, and auto/allo-HSCT were the same as that without the exclusion (Additional file 2: Figure S1). These findings indicated that the above results were not subject to the effect of FAB M3.

Taken together, ARHGAP9 expression is a poor factor for AML rather than CN-AML. Moreover, chemotherapy alone did not show any prognostic influence in ARHGAP9\textsuperscript{high} cases. AML patients with
high expression of ARHGAP9 could benefit from auto/allo-HSCT.

**ARHGAP9-associated gene analysis between ARHGAP9\textsuperscript{high} and ARHGAP9\textsuperscript{low} in AML patients**

To further explore the role of ARHGAP9 in AML, we conducted differently expressed genes associated ARHGAP9 expression profiles by the overlapping genes of the significantly different genes between ARHGAP9\textsuperscript{low} and ARHGAP9\textsuperscript{high} groups and the co-expression genes associated with ARHGAP9 expression.

Firstly, the transcriptomes of ARHGAP9\textsuperscript{low} and ARHGAP9\textsuperscript{high} groups were compared by using the TCGA database. In total, 3,094 genes were significantly different between ARHGAP9\textsuperscript{low} and ARHGAP9\textsuperscript{high} groups (p < 0.05, |log1.5 FC| > 1, Fig. 5a, Additional file 1: Table S2), in which 1,185 marked by red circles and 1,909 marked by green circles significantly up-expressed and down-expressed in the ARHGAP9 high group, respectively.

Subsequently, we analyzed the co-expressed in conjunction with ARHGAP9 genes using the linkedomics database. As shown in Fig. 5A, 3310 genes represented by dark red dots were positive correlations with ARHGAP9 and 4268 genes represented by dark green dots were negative correlations with ARHGAP9 in AML (Fig. 5b). And 3,322 co-expression genes were significantly negative and positive correlations with ARHGAP9 in AML (False discovery rate, FDR < 0.05 and p < 0.05, |cor.|>0.3) (Additional file 1: Table S3).

By comparing the significantly different genes and co-expressed genes, total 864 overlapping genes were obtained. These 864 genes contained 456 positively up-regulated genes and 276 negatively down-regulated genes (Fig. 5a, b).

**Functional analysis of the overlapping genes**

Finally, we sought to investigate the possible biological function of ARHGAP9 in patients with AML. These 864 genes were further analyzed by using the metascape tool to study KEGG and GO annotation. The top 20 clusters of enriched sets were showed in Fig. 6a. we found that ARHGAP9 mediated the function of Rho GTPases, including regulation of small GTPases mediated signal transduction, signaling by Rho GTPases, regulation of cell adhesion, actin cytoskeleton organization, regulation of cell shape, microtubule cytoskeleton organization, negative regulation of cellular component organization, regulation of vesicle-mediated transport, organelle localization, cell division. Interestingly, six clusters belonged to the immune system including leukocyte activation involved in immune response, signaling by interleukins, adaptive immune system, regulation of leukocyte mediated immunity, response to interferon-gamma, and positive regulation of innate immune response. ARHGAP9 was also associated with protein autophosphorylation and regulation of phosphatidylinositol 3-kinase signaling. What is more, the overlapping genes enrich in blood, spleen, and bone marrow (Fig. 6b), further suggesting immunological functions of ARHGAP9 in leukemogenesis. Moreover, among different diseases, the overlapping genes participate primarily in acute promyelocytic leukemia (APL) (Fig. 6c).
Validation of hub genes

The PPI was constructed by using the STRING online website and the figures were generated by Cytoscape (MCODE plug-in) software. A total of 864 common genes were imported into the PPI network. We obtained 855 nodes and 4233 edges (Fig. 7a). The most significant module (MCODE score = 29.802) contained 32 genes were identified in Fig. 7b. Subsequently, OS analysis of the hub genes was performed using the GEPIA database. Of 32 hub genes, 31 genes had no no effect on OS of AML patients (Additional file 2: Fig. S2). Only KIF20A was significantly related to OS of AML (p = 0.02, Fig. 7c). Compared to normal tissues, KIF20A in AML samples was significantly lower (Fig. 7d).

Discussion

Rho GAPs inactivate Rho GTPases by the conservative GAP domain that promotes the GTP hydrolysis and accelerates the intrinsic GTPase activity, limiting the duration of the regulated reaction [29, 30]. The abnormal expression of Rho GAPs was observed in various tissues from patients with cancer and immunological diseases [31–34]. The expression of Rho GAPs was found to be heterogeneous among different tumors. ARHGAP30, for instance, was downregulated in lung cancer and colorectal cancer, whereas over-expressed in pancreatic cancer [35–37]. A low expression of ARHGAP9 was found in hepatocellular carcinoma and bladder cancer, whereas a high expression was observed in breast cancer [17, 19, 38]. In this study, we found that ARHGAP9 is over-expressed in both AML samples and cell lines compared with the normal counterparts [19]. Furthermore, the low expression of ARHGAP9 was significantly correlated with t(15;17) AML.

Some studies have been explored the role of Rho GAPs in cancer and found that most of Rho GAPs were associated with good outcomes in many kinds of solid tumors [33, 36, 37, 39]. ARHGAP9 also showed a good prognosis in bladder cancer and gastric cancer [18, 38]. However, in our survival analysis study, we found ARHGAP9 overexpression was associated with a poor prognosis in AML. These results indicated that ARHGAP9 may play different roles in various cancers. Meanwhile, DOCK2 belonging to the Rho GEFs family activates GTPase which has the opposite function compared with Rho GAPs. Also, it was an independent favorable prognostic factor for both EFS and OS in AML [40]. Thus, in addition to being associated with GTPase, some regulators of Rho GTPase may have other functions in cancer. Despite the high expression of ARHGAP9 was related to CN-AML, we did not find any relationship between ARHGAP9 expression and prognosis in CN-AML. In the other study, some genes such as NCALD, IL2RA, and BCL2 are associated with prognosis in AML patients with auto/allo-HSCT and/or chemotherapy [41–43]. In the present study, ARHGAP9\textsuperscript{high} groups had poor prognosis in post-chemotherapy AML patients, whereas no significant difference were found in OS and EFS between ARHGAP9\textsuperscript{high} group and ARHGAP9\textsuperscript{low} group in patients after auto/allo-HSCT, suggesting that the effects of ARHGAP9 over-expression could be eliminated by auto/allo-HSCT, instead of chemotherapy.

Previous studies showed that some of Rho GAPs expressed mainly in hematopoietic cells [8, 44–46]. Costa and his colleagues have shown that lacking ARHGAP15 leads to enhanced chemotactic responses,
straighter directional migration, amplified reactive oxygen species production, increased phagocytosis as well as improved bacterial killing in neutrophils [44]. ARHGAP25 negatively regulates leukocyte transendothelial migration in mice and phagocytosis acting in human neutrophilic granulocytes [45, 47]. Studies of both in vitro and in vivo indicated that ARHGAP21 knockdown could impair the function of T cells, reduced erythroid commitment and differentiation, and enhanced RhoC activity [12]. In T lymphocytes, ARHGAP19 was shown to affect the stiffness and shape of lymphocytes by regulating cytokinesis and chromosome segregation [8]. Taken together, Rho GAPs may play an important role in hematopoietic cells and regulate cell motility, cell cycle, adhesion, phagocytosis, NADPH oxidase, auto/allo-HSCT development, inflammatory responses, and neutrophil chemotaxis. This hypothesis was well supported by the functional analysis of KEGG and GO enriched by the overlapping genes in our study.

Upon immunization, Rho GAPs are critical for innate immunity and adaptive immunity [48–50]. While more than 11 members of Rho GAPs take part in various neutrophil functions that belong to the adaptive immunity [51]. We speculated that ARHGAP9 probably also takes part in immune response in the AML because the overlapping genes were enriched in the immune system and the immune tissues. Our study showed the differently expressed genes associated with ARHGAP19 expression were enriched in APL. While 95% of APL is the abnormalities of t(15;17) which encoded the PML-RARA fusion protein [52]. What is more, the expression of ARHGAP9 is the lowest in t(15;17) AML compared with the other chromosome abnormalities in AML, and all patients with t(15;17) were in the ARHGAP9 low group in our study. Therefore, the expression of ARHGAP9 may be suppressed by the PML-RARA fusion protein. We should further investigate ARHGAP9 physiological role in APL.

Conclusions

In brief, our study found that ARHGAP9 was high expression in AML tissues and cells, and elevated ARHGAP9 was significantly correlated with poor outcome in whole AML. auto/allo-HSCT can overcome the adverse outcomes related to high ARHGAP9 expression, rather than chemotherapy. The function analysis of differently expressed genes between ARHGAP9 high and low expression showed ARHGAP9 could serve multiple functions in AML and it is necessary to further study the physiological role of ARHGAP9 in AML. Besides, ARHGAP9 was down-expression in the t(15;17) patients and differently expressed genes associated with ARHGAP9 expression were enriched in acute promyelocytic leukemia. PML-RARA may act as negative regulators of ARHGAP9 expression in AML.

Abbreviations

ARHGAP9: Rho GTPase activating protein 9; AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; CN-AML: cytogenetic normal acute myeloid leukemia; TCGA: The Cancer Genome Atlas; EFS: event-free survival; OS: overall survival; FAB: French-American-British subtypes; BM-blast: bone marrow blast; PB-blast: peripheral blood blast; WBC: white blood cell; allo-HSCT: allogeneic hematopoietic stem cell transplantation; GEO: Gene Expression Omnibus; CCLE: cancer cell cine encyclopedia; HPA: Human
Declarations

Ethics approval and consent to participate

The written informed consent of all patients in this study was consistent with the Helsinki Declaration.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available. Please contact the author to get the datasets.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

CH wrote the manuscript and prepared tables and figures. SH, RW, and WH analyzed the data. QQ and XM performed statistical analyses. XG revised the manuscript critically. LY and YL contributed to study design and editing. All authors contributed to the article and approved the final version.

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