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ORIGINAL RESEARCH

HES1 is an independent prognostic factor for acute myeloid leukemia

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Abstract: HES1 is the target of Notch signaling which is reported to affect cell differentiation and maintain the cells in G0 phase in various tissues including the hematopoietic tissue. HES1 expression appears to be an independent prognostic factor for survival in a heterogeneous group of acute myeloid leukemia (AML) patients. To better assess its significance, we analyzed HES1 expression in a group of non-core binding factor AML patients and correlated its expression with the overall survival and relapse-free survival of AML patients. First, we detected the messenger RNA expression of HES1 in 40 patients with AML by real-time polymerase chain reaction. The top 50% of AML cases with the high HES1 expression were compared with the rest of the AML cohort. Overall survival was calculated from the date of diagnosis until the date of death from any cause or until the date of final follow-up. Relapse-free survival was determined for responders from the time of diagnosis until relapse or death from any cause. We showed that the lower-expression group had a shorter overall survival time and shorter relapse-free survival time compared with those of the high-expression group (37.6±1.6 versus 54.0±1.3 months, 28.6±1.8 months versus 44.8±2.1 months, respectively, $P<0.05$), and Cox regression showed that HES1 was an independent prognostic factor. In all, we conclude that expression of HES1 is a useful prognostic factor for patients with non-core binding factor AML.

Keywords: acute myeloid leukemia, HES1, prognostic factor

Introduction

Acute myeloid leukemia (AML) is a clonal disorder involving a hierarchy of leukemic cells.[1] With investigation of the mechanism of AML, many epigenetically-regulated genes including *Flt3, NPM1, DNMT3A, IDH1, IDH2* and *TET2*, have been approved as new prognostic factors for AML.[2–6]

It is well known that HES1 is the target of Notch signaling which is reported to affect cell differentiation and maintain the cells in G0 phase in various tissues including the hematopoietic tissue.[7,8] Our previous studies reported that over-expression of HES1 inhibited cycling of hematopoietic stem and progenitor cells in vitro and cell expansion in vivo.[9] Similarly, other studies reported that HES1 could suppress the proliferation of AML cells. All of these confirmed that HES1 functioned as a suppressor of cell cycle. In this study, we found that the expression of HES1 in AML bone marrow mononuclear cells (BMNCs), the majority of which were leukemic blasts, was downregulated compared to that in normal BMNCs which suggested that HES1 may be a prognostic factor of AML. And, we showed that the expression of HES1 was an independent prognostic factor for overall survival (OS) and relapse-free survival (RFS) in patients with non-core binding factor (CBF) AML.

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Materials and methods

Patient samples

The BMNCs of 40 newly diagnosed AML patients between August 2008 and January 2014 were collected with Ficoll after approval of the ethics committee and informed consent. All of these patients completed follow-up. Baseline morphology, cytogenetics, and cell surface antigen analysis were performed as part of the routine clinical evaluation of the patients. All of the 40 patients completed follow-up. The characteristics of patients are shown in Table 1.

Real-time reverse-transcription polymerase chain reaction (RT-PCR) analyses

Total ribonucleic acid (RNA) of AML BMNCs was extracted with Trizol (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription was achieved using QuantiTect Reverse Transcription Kit (Qiagen NV, Venlo, the Netherlands). Real-time PCR was performed using ABI-Prism 7500 Sequence Detector (Thermo Fisher Scientific) and Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, number 4367659). The parameters for the thermal cycling of PCR were as follows: 15 seconds at 95°C and 60 seconds at 60°C, 45 cycles. The sequences of HES1 primers were upper: 5-GCAGATGACGGCTGCGCTGA-3, lower: 5-AAGCGGGTCACCTCGTTCATGC-3. GAPDH was used as housekeeper. The sequences of GAPDH primers were upper: 5-CGGAGTCAACGGATTTGGTCGTAT-3, lower: 5-AGCCTTCTCCATGGTGGTGAAGAC-3.

Table 1 Clinical characteristics of the HES1 high-expression and low-expression groups

	Low-expression group	High-expression group
Number of patients	20	20
Male	8	11
Female	12	9
Prognostic marker		
Age	55 (12–75)	52 (20–77)
FAB		
M1	4	4
M2	7	4
M3	2	5
M4	5	2
M5	2	5
Karyotype		
Intermediate	10	13
Unfavorable	10	7
Median HGB, g/L (range)	80 (50–114)	88 (45–129)
Median white blood cell count, *10^9/L (range)	30 (7.8–60)	22 (5.6–50)
Median platelet count, *10^9/L (range)	84 (15–248)	90 (30–276)

Statistical analysis

Statistical analysis was performed using SPSS. For Kaplan–Meier estimates graphs, GraphPad Prism version 3.0 (GraphPad Software, Inc., La Jolla, CA, USA) software package for Windows was used. OS was calculated from the date of diagnosis until the date of death from any cause or until the date of final follow-up. RFS was determined for responders from the time of diagnosis until relapse or death from any cause. The significance of difference between survival curves was calculated by the log-rank test. Groupwise comparisons of the distributions of variables were performed with the generalized Wilcoxon test. The Cox proportional hazards regression model was used in multivariate analysis to compare the factors proven to be statistically significant or to demonstrate a trend in the univariate analysis. A P-value <0.05 was considered significant.

Results

Low HES1 expression is a poor prognostic factor for OS and RFS

To investigate whether HES1 expression levels are associated with the prognosis of AML patients, we correlated results from real-time PCR data with clinical outcome of 40 patients with AML. Because CBF-AML has a good prognostic risk profile, we excluded AML cases belonging to this group. First, we compared the expression of HES1 in AML BMNCs to that in normal donor BMNCs. Results of agarose gel and Tm curve to quality PCR specificity is shown in Figure S1. Results showed that the average expression of HES1 in AML BMNCs was 0.7±0.1, which was lower than that in normal BMNCs (1.7±0.2, $P<0.05$, Figure 1A). The top 50% of AML cases with the high HES1 expression (>0.7, n=20) were compared with the rest of the AML cohort (<0.7, n=20, Figure 1B). The results of western are shown in Figure 1C. The OS time in the low-expression group was significantly shorter than that in the high-expression group. The median survival of HES1-low group was 37.6±1.6 months, while that of HES1-high group was 54±1.3 months ($P<0.05$, Figure 2A). The RFS time in the low-expression group was also significantly shorter than that in the high-expression group (28.6±1.8 months versus [vs] 44.8±2.1 months, $P<0.05$, Figure 2B). The possible predictive factors of OS are summarized in Table 2. Sex, age, white blood cell (WBC) counts, unfavorable karyotype types, the International Prognostic Scoring System risk category, marrow blast percentage and HES1 expression were correlated with a poor OS. We found a statistically significant association ($P<0.05$)

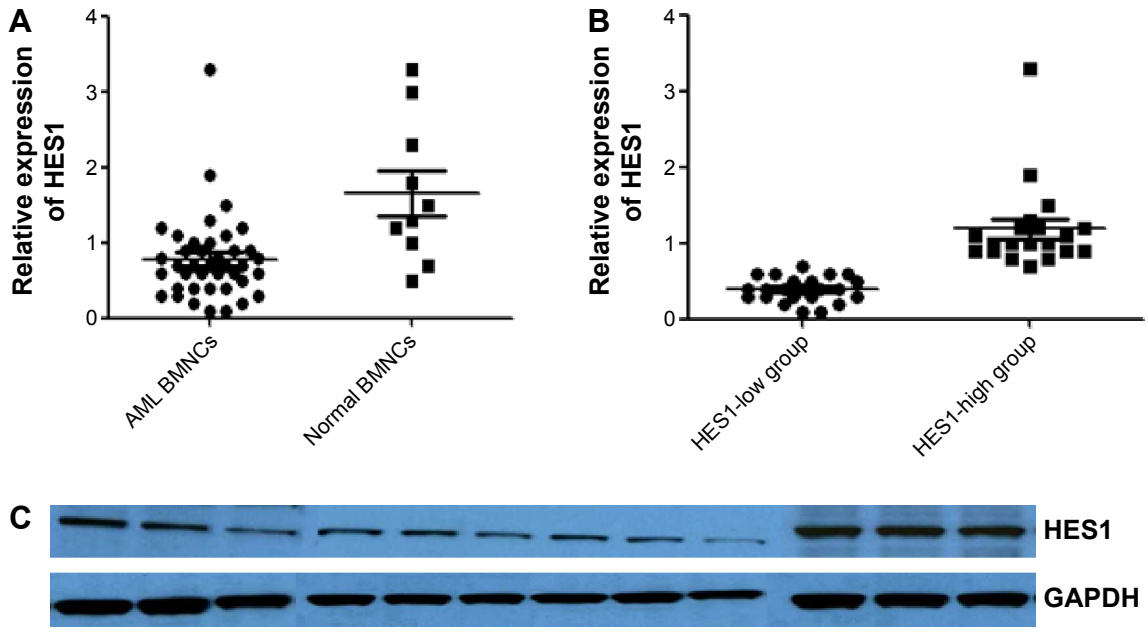

Figure 1 Expression of HES1 in AML was analyzed by real-time PCR and wester.
Notes: (**A**) Relative expression of HES1 in AML BMNCs and normal BMNCs by real-time PCR. (**B**) Different expression of HES1 in HES1-high and -low group respectively. (**C**) Western blot results of HES1 protein expression in AML cases.
Abbreviations: AML, acute myeloid leukemia; PCR, polymerase chain reaction; BMNCs, bone marrow mononuclear cells.

between OS and WBC count, unfavorable karyotype types and HES1 expression.

HES1 is an independent prognostic factor

In univariate analysis, we found that sex, age, WBC counts, unfavorable karyotypes, and marrow blast percentage were prognostic factors for patients with AML. Interestingly, HES1 expression was also a prognostic factor for AML patients. Importantly, multivariate Cox regression analysis showed that the expression of HES1 had a positive effect on OS and RFS ($P<0.001$; hazard ratio =1.134 and $P<0.001$, hazard ratio =1.102, respectively), indicating that HES1 expression is an independent risk factor for death and relapse.

Discussion

AML is a group of cytogenetically and molecularly heterogeneous diseases. It has been well accepted that cytogenesis and molecular factors are independent prognostic predictors to stratify AML patients into favorable, intermediate-risk, and unfavorable groups.[10]

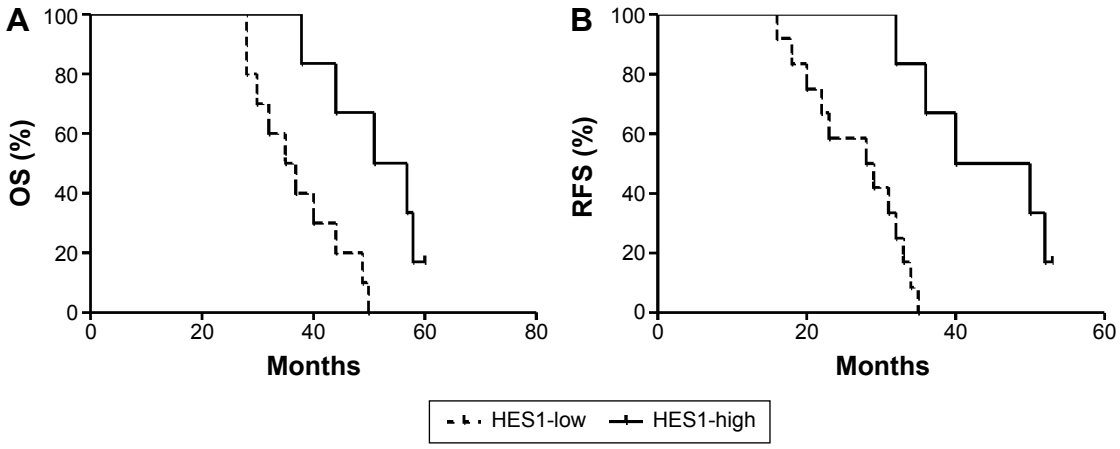

Figure 2 Overall survival (OS) and relapse-free survival (RFS) analysis of HES1-high and -low groups.
Notes: (**A**) Effect of HES1 expression on OS in non-CBF AML patients. The OS time of the high-expression group is significantly longer than that of the low-expression group (37.6±1.6 months versus 54.0±1.3 months, $P<0.05$). (**B**) Effect of HES1 expression on RFS in non-CBF AML patients. The RFS time of the high-expression group was longer than that of the low-expression group (28.6±1.8 months versus 44.8±2.1 months, $P<0.05$).
Abbreviations: AML, acute myeloid leukemia; CBF, core binding factor.

Table 2 *P*-values of all prognostic markers for overall survival as determined by the log-rank test and Gehan–Breslow–Wilcoxon test

Prognostic maker	*P*, log-rank	*P*, Gehan–Breslow–Wilcoxon
HES1	<0.0001	<0.0001
Sex	<0.05	<0.05
Age	<0.05	<0.05
White blood cell count	<0.05	<0.05
Karyotype	<0.0001	<0.0001

HES1, the downstream effector of Notch pathway, is a member of basic helix-loop-helix transcription factors which belongs to the Hes family.[11–13] Its roles in embryogenesis, chronic myelogenous leukemia, development of perinatal T cells, normal hematopoiesis have been reported.[14–16] HES proteins generally act as repressors of transcription.[17] It has been reported that HES1 was involved in cell cycle, and maintained multipotent precursor cells in an undifferentiated state in several tissues during development and adulthood.[18] Interestingly, HES1 expression can be used as a marker for poor prognosis for medulloblastoma and T cell acute lymphoblastic leukemia.[19,20] However, the role of HES1 in the prognosis of AML has not been well demonstrated. To investigate the clinical significance, we analyzed the HES1 expression in 40 patients with non-CBF AML by quantitative real-time RT-PCR.

According to the PCR results, these patients were then divided into the high-expression group and low-expression group. We then evaluated the expression of HES1 as a prognostic factor for non-CBF AML patients by Kaplan–Meier analysis. We showed that the high-expression group had a longer OS time and RFS time compared with those of the low-expression group. Cox regression showed that HES1 was an independent prognostic factor. However, the analyzed number of cases was low and the patients were non-CBF AML.

It was the limitation that we could not be sure whether HES1 was a reliable predictor for OS and RFS in other or larger cohorts. It is reported that many parameters such as new cytogenetic risk categories, and immunophenotypes of myeloid progenitor cells and epigenetically-regulated genes including *DNMT3A, Flt3, IDH1, IDH2*, and *TET2*, have been approved as new prognostic factors for AML.[2–6] Until now, the prognosis of epigenetics-regulated genes became more and more important while the prognosis of sex, age, and WBC count for AML gradually weaken. HES1 is a newly epigenetically-regulated gene found to be a prognostic factor for AML.

In conclusion, our results indicated that the expression of HES1 can be used as a poor and independent prognostic factor for patients with non-CBF AML.

Acknowledgment

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Author contributions

Chen Tian did all the experiments; Yingjun Tang, Tengteng Wang, Yong Yu, Yafei Wang, Xiaofang Wang provided clinical samples and helped to revise the manuscript; Chen Tian and Yizhuo Zhang designed experiments, interpreted data, and wrote the manuscript.

Disclosure

The authors have no conflicts of interest to disclose.

References

1. Haferlach C. Genes break barrier between MDS and AML. *Blood*. 2015;125(1):9–10.
2. Lerch E, Espeli V, Zucca E, et al. Prognosis of acute myeloid leukemia in the general population: data from southern Switzerland. *Tumori*. 2009;95(3):303–310.
3. Metzeler K, Maharry K, Radmacher M, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2011;29(10):1373–1381.
4. Gaidzik V, Bullinger L, Schlenk R, et al. RUNX1 mutations in acute myeloid leukemia: results from a comprehensive genetic and clinical analysis from the AML study group. *J Clin Oncol*. 2011;29(10):1364–1372.
5. Van Vlierberghe P, Patel J, Abdel-Wahab O, et al. PHF6 mutations in adult acute myeloid leukemia. *Leukemia*. 2011;25(1):130–134.
6. Patel J, Gonen M, Figueroa M, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079–1089.
7. Kato T, Sakata-Yanagimoto M, Nishikii H, et al. HES1 suppresses acute myeloid leukemia development through FLT3 repression. *Leukemia*. 2015;29(3):576–585.
8. Kannan S, Sutphin RM, Hall MG, et al. Notch activation inhibits AML growth and survival: a potential therapeutic approach. *J Exp Med*. 2013;210(2):321–337.
9. Tian C, Zheng G, Cao Z, et al. HES1 mediates the different responses of hematopoietic stem and progenitor cells to T cell leukemic environment. *Cell Cycle*. 2013;12(2):322–331.
10. Harrison H, Farnie G, Howell SJ, et al. Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Res*. 2010;70(2):709–718.
11. Hughes DP. How the NOTCH pathway contributes to the ability of osteosarcoma cells to metastasize. *Cancer Treat Res*. 2010;152:479–496.
12. Kanamori E, Itoh M, Tojo N, Koyama T, Nara N, Tohda S. Flow cytometric analysis of Notch1 and Jagged1 expression in normal blood cells and leukemia cells. *Exp Ther Med*. 2012;4(3):397–400.
13. Vo TT, Ryan J, Carrasco R, et al. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. *Cell*. 2012;151(2):344–355.

14. Del Giudice I, Rossi D, Chiaretti S, et al. NOTCH1 mutations in +12 chronic lymphocytic leukemia (CLL) confer an unfavorable prognosis, induce a distinctive transcriptional profiling and refine the intermediate prognosis of CLL. *Haematologica*. 2012;97(3):437–441.
15. Klinakis A, Lobry C, Abdel-Wahab O, et al. A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. *Nature*. 2011; 473(7346):230–233.
16. Yu X, Alder JK, Chun JH, et al. HES1 inhibits cycling of hematopoietic progenitor cells via DNA binding. *Stem Cells*. 2006;24(4): 876–888.
17. Nwabo Kamdje AH, Mosna F, Bifari F, et al. Notch-3 and Notch-4 signaling rescue from apoptosis human B-ALL cells in contact with human bone marrow-derived mesenchymal stromal cells. *Blood*. 2011;118(2): 380–389.
18. Dahlberg A, Delaney C, Bernstein ID. Ex vivo expansion of human hematopoietic stem and progenitor cells. *Blood*. 2011;117(23): 6083–6090.
19. Larson Gedman A, Chen Q, Kugel Desmoulin S, et al. The impact of NOTCH1, FBW7 and PTEN mutations on prognosis and downstream signaling in pediatric T-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Leukemia*. 2009;23(8): 1417–1425.
20. Fiaschetti G, Abela L, Nonoguchi N, et al. Epigenetic silencing of miRNA-9 is associated with HES1 oncogenic activity and poor prognosis of medulloblastoma. *Br J Cancer*. 2014;110(3): 636–647.

Supplementary material

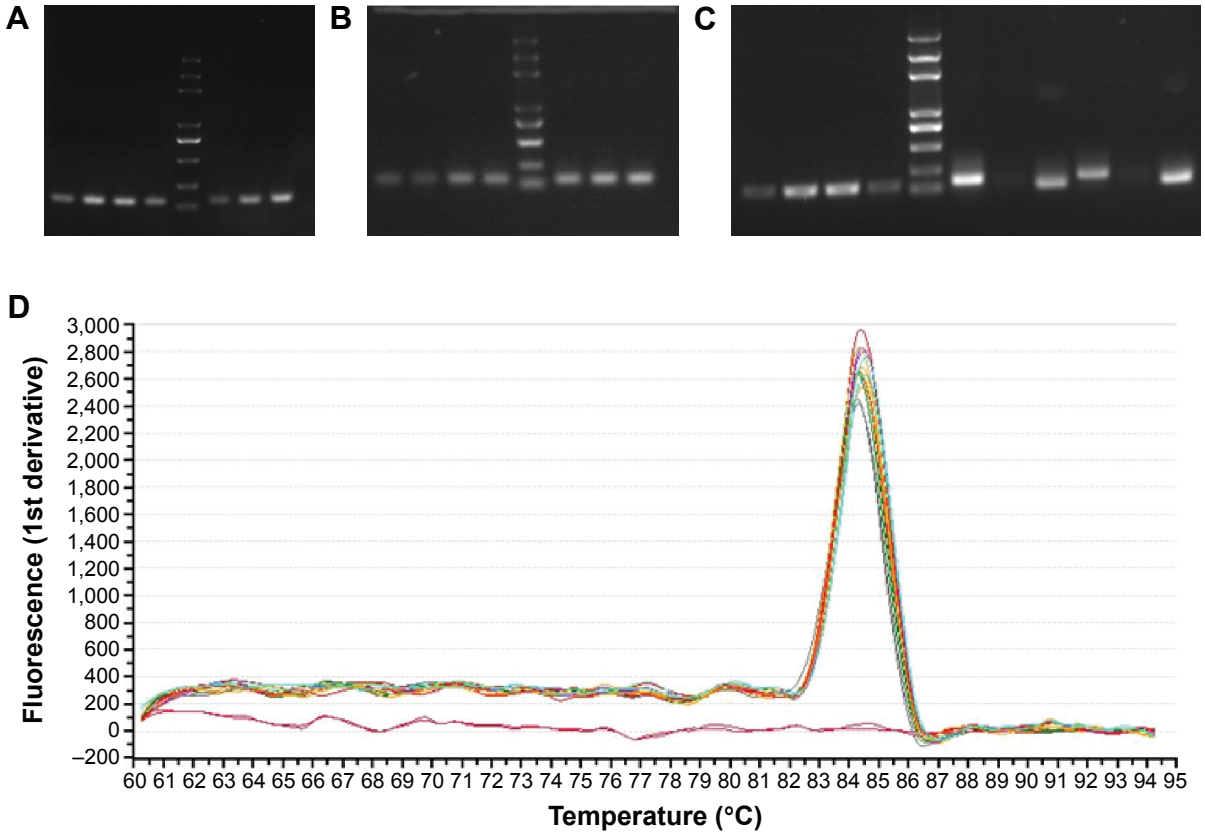

Figure S1 The specificity of Hes1 primers.
Note: The results of agarose gel (**A**–**C**) and Tm curve (**D**) to verify the specificity of Hes1.