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Activation of Notch signal pathway is associated with a poorer prognosis in acute myeloid leukemia

Xin Xu · Yu Zhao · Maozhong Xu · Qiuxin Dai · Wenjun Meng · Jiangang Yang · Rujuan Qin

Abstract *Objective* Notch signal pathway plays a fundamental role in regulating haematopoietic development. It is also an important mediator of growth and survival in several cancer types, with Notch pathway genes functioning as oncogenes or tumor suppressors in different cancers. However, the clinical role of Notch signal pathway in acute myeloid leukemia (AML) remains unclear. *Methods* To address this problem, we investigated the gene expression levels of Notch signal pathway members (Notch1, Jagged1 and Delta1) in bone marrow mononuclear cells by real-time quantitative PCR in a cohort of 100 patients with newly diagnosed de novo AML and normal marrow donors. The prognostic values of the three molecules in AML were also analyzed. *Results* Comparing with the normal controls, we show the transcriptional up-regulation of Notch1, Jagged1 and Delta1 in the bone marrow of AML patients with statistic differences ($P = 0.008$, 0.01 and 0.01, respectively). In addition, univariate analysis of factors associated with relapse-free survival and overall survival showed a significantly shorter survival in the patients with unfavorable karyotype, higher Notch1 expression, higher Jagged1 expression, or higher Delta1 expression. Moreover, Cox proportional hazards multivariate analysis of the univariate predictors identified karyotype and gene expression levels of Notch1, Jagged1 and Delta1 as independent prognostic factors for relapse-free survival and overall survival. Furthermore, the prognostic significance of Notch1, Jagged1 and Delta1 expression was more obvious in the subgroup of patients with intermediate-risk cytogenetics. *Conclusion* Taken together, our data suggest for the first time that the activation of Notch pathway may indicate a poor prognosis in AML. Especially, Notch1, Jagged1 and Delta1 expression may be relevant prognostic markers in intermediate risk AML.

Keywords Acute myeloid leukemia · Notch1 · Jagged1 · Delta1 · Prognosis

Introduction

Acute myeloid leukemia (AML) is a hematologic cancer characterized by the uncontrolled proliferation of myeloid cells that accumulate at various stages of development, where their further differentiation appears to be blocked [1]. It could be considered as a heterogeneous group of diseases, which often present with different morphological, immunophenotypic and cytogenetic patterns. Many clinical features are associated with a poor outcome and include advanced age, high leukocyte count, extramedullary mass and a history of proceeding hematologic disorders such as myelodysplastic syndrome [2, 3]. In addition, cytogenesis is also recognized as an important prognostic parameter that is used to determine the response to therapy in AML. The good prognostic group of AML is associated with t(8, 21) (q22; q22), t(15, 17) (q22; q11-12) or inv (16) (p13; q22). Conversely, AML associated with -5, del (5q), or -7 belongs to the poor prognostic group. The remaining group, the intermediate prognostic group includes AML associated with a normal karyotype and rare chromosome aberration [4]. Recent studies have provided evidence that patients with a normal karyotype have an intermediate risk with a 35–45% 5-year overall survival, but the clinical outcome varies greatly [5, 6]. As a result, additional

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markers with prognostic significance are needed to identify clinical characteristics of AML patients for a better prognostic evaluation and for a more appropriate therapeutic approach.

The Notch signaling cascade influences several key aspects of normal development by regulating differentiation, proliferation and apoptosis [7]. This pathway includes Notch ligands, receptors, negative and positive modifiers and Notch target transcription factors. The Notch genes (Notch1–Notch4), originally identified by homology to a single Notch gene from Drosophila, encode highly conserved cell surface receptors [8]. After activation by ligand binding, the Notch proteins are proteolytically cleaved in two steps by ADAM10 and γ-secretase, after which the intracellular domain of Notch (ICN) is translocated to the nucleus. Nuclear ICN interacts with the transcription factor CSL, also known as RBP-Jκ, and the mastermind-like (MAML) protein, which leads to transcriptional activation of CSL target genes. These include the basic helix-loop-helix (bHLH) transcription factors of the Hes family and Hes-related repressor proteins (Herp), e.g. HES1, HES5, HERP2, Hes and Herp family proteins are transcriptional repressors. Independent of CSL activity, intracellular Notch receptors can interact with the Notch target protein DELTEX1, which modulates Notch-mediated transcription and promotes feedback inhibition of Notch pathway signaling [9, 10].

Notch signaling is aberrantly activated in a variety of human cancers. Its association with human cancers is firmly established in T-cell acute lymphoblastic leukemia, where point mutations or chromosomal translocations lead to constitutive signaling [11]. However, the clinical role of Notch signal pathway in AML remains unclear. To address this problem, we in the present study investigated the gene expression levels of Notch signal pathway members (Notch1, Jagged1 and Delta1) in bone marrow mononuclear cells by real-time quantitative PCR in a cohort of 100 patients with newly diagnosed de novo AML and normal marrow donors. The prognostic values of the three molecules in AML were also analyzed.

Materials and methods

Patients and tissue samples

Prior informed consent was obtained from the patients for the collection of specimens in accordance with the guidelines of the Affiliated Jiangyin Hospital of Southeast University Medical College, China, and the study protocols were approved by the Affiliated Jiangyin Hospital of Southeast University Medical College Ethics Committee.

Table 1 Characteristics of the 62 patients with acute myeloid leukemia

Clinical variables	No. of patients (%)
Gender	
Male	62 (62.0)
Female	38 (38.0)
Age (year)[a]	66.3 (39–86)
FAB classification	
M0	3 (3.0)
M1	32 (32.0)
M2	28 (28.0)
M4	20 (20.0)
M5	12 (12.0)
M6	5 (5.0)
Karyotype[b]	
Favorable	35 (35.0)
Intermediate	52 (52.0)
Unfavorable	13 (13.0)
WHO classification	
AML with recurrent genetic abnormalities	6
AML with myelodysplasia-related changes	31
Acute myeloid leukemia, not otherwise specified	25

FAB French–American–British classification, *WHO* World Health Organization classification

[a] Median (range)

[b] Favorable, t(8;21), inv(16); unfavorable, −7, del(7q), −5, del(5q), 3q abnormality, complex; intermediate, normal karyotype and other abnormalities

All specimens were handled and made anonymous according to the ethical and legal standards.

One hundred adult patients with untreated primary AML were included in this study. They were selected from the files of the Department of Pathology, the Affiliated Jiangyin Hospital of Southeast University Medical College, China. The patients (62 men and 38 women) ranged in age from 39 to 86 years (median 66). All the cases were de novo adult AML cases, which were classified as M0–6 according to the French–American–British (FAB) criteria. In addition, using the 2008 WHO criteria [12] resulted in the distribution of AML subcategories listed in Table 1. All patients were treated with standard induction chemotherapy (3 days of an anthracycline [idarubicin or daunorubicin] and 7 days of cytarabine) and received consolidation therapy with high-dose cytarabine with or without the anthracycline after achieving complete remission. The control group consists of 30 adult patients (30–80 years) with various diseases, but with normal bone marrow morphology as demonstrated by cytological and histological analyses. Follow-up data were available for all patients. The median follow-up duration was 35 months.

RNA isolation and cDNA synthesis

Mononucleated cells were separated by Ficoll Hypaque density gradient centrifugation of 2 ml bone marrow samples in EDTA from newly diagnosed patients and control samples. Total RNA was extracted from 10^6 mononucleated cells with Ultraspect tm RNA (Biotexc Laboratories, TX). About 1 µg total RNA was reverse transcribed with 100 U MMLVreverse transcriptase (Promega, Madison, WI), using 100 µM random hexamer primers in accordance with the manufacture instructions.

Expression analysis of Notch1, Jagged1 and Delta1 by qPCR

Expression of Notch1, Jagged1 and Delta1 genes in every individual sample was performed by qPCR using the LightCycler technology (Roche Applied of Science, Mannheim, Germany) with SYBR-Green I dye. RPLP0 were used as control genes. The sequences of primers and probes of different genes are listed in Table 2. PCR was performed in a 10-µl reaction volume, using 1 µl of LightCycler Fast Start Master Sybergreen (Roche Applied of Science, Mannheim, Germany), with 0.5 µM of forward and reverse primer each, 3–4 mM of $MgCl_2$ and 1 µl cDNA sample. Running conditions for the specific genes were for RPLP0, 40 cycles (95°C5 s/60°C10 s/72°C12 s/84°C0 s); for Notch1, 45 cycles (95°C 5 s/55°C10 s/72°C10 s/84°C 5 s); for Jagged1, 35 cycles (95°C 5 s/55°C10 s/72°C10 s/84°C5 s); and for Delta1, 32 cycles (95 C5 s/55°C10 s/72°C15 s/84°C5 s). LightCycler data were analyzed using the LightCycler 3.5 software and the second derivative maximum method. Positive and negative controls were included in all tests, and the problem samples were conducted at least in duplicate and in two different runs to confirm the results. Specificity of the desired products was documented with melting curves analysis and by electrophoresis on a 2% agarose minigel.

Quantification was done with the QGene relative quantification software [13]. A standard curve was produced for every target with a respective purified RT–PCR product using a series of fivefold dilutions, which were used in every test. To assess the quality and quantity of the isolated

RNA as well as the efficiency of cDNA synthesis, each sample was normalized against the expression of RPLP0. Relative quantification was performed by calculating the ratios of the target gene in a standard curve in relation to the control gene. All expression ratios are given as target gene/RPLP0.

Statistical analysis

The software of SPSS version 16.0 for Windows (SPSS Inc, IL, USA) and SAS 9.1 (SAS Institute, Cary, NC) was used for statistical analysis. With regard to the correlation of leukemia clinical features with the gene expression levels of Notch1, Jagged1 and Delta1, intergroup comparisons were performed using one-way analysis of variance (ANOVA). When the equal variance test or normality test failed, the Kruskall–Wallis non-parametric test was applied. To address the problem of multiple comparisons, these tests (ANOVA, Kruskall–Wallis) were followed by a post hoc Bonferroni test. Spearman's correlation test was used for the correlation between the individual expression of the genes studied (Notch1, Jagged1, and Delta1). The Kaplan–Meier survival curves were used to determine any significant relationship between the gene expression levels of the investigated markers and the status of the patients with respect to relapse-free survival (RFS) or overall survival (OS). Differences were considered statistically significant when p was less than 0.05.

Results

Notch1, Jagged1 and Delta1 expression in AML patients

Notch1, Jagged1 and Delta1 expression was detected in bone marrow from patients with AML and normal controls. RPLP0 were used as control genes, to exclude any possible heterogeneous expression of RPLP0 in the different AML subtypes [14]. Expression of the different genes studied was normalized with RPLP0, and the values obtained were compared. In spite of the wide range of individual values of the target genes, median levels of Notch1 ($P = 0.008$),

Table 2 Sequences of primer pairs and probes of different target genes

| Target gene	Forward (5′–3′)	Reverse (5′–3′)	Probe (5′–3′)
Notch1	CAGACTGCAGAGCAGACCAGAA	CTCTAGCTTGTAGGTGGATAATGAATTC	ACCCAGGTACTAAATCA
Jagged1	CGAGGGCCTGGAGTGTGT	CCGCATAATCTGCATGGTGAT	CCCACTGAGGAGTCC
Delta1	CTGTGAAGGGCGAGTTCGA	TGGTAGGAAGGAAGCTTGTTGAC	CAATCAGGATACGAACCATGA
RPLP0	AAGAGATCAAGGCCTACTGTGACA	TCCTCACGTCGCTGAATAATTG	CCGCCTCCTCCAGCT
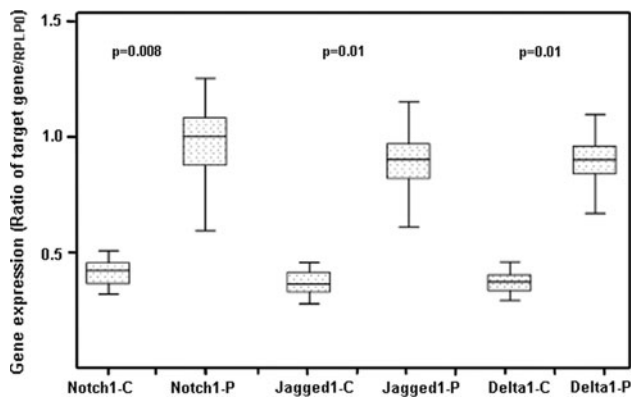
Fig. 1 Expression of Notch1, Jagged1 and Delta1 in bone marrow from patients with acute myeloid leukemia (*P*), compared with that in healthy controls (*C*). *P* values were calculated using the Mann–Whitney U test

Jagged1 ($P = 0.01$) and Delta1 ($P = 0.01$) were significantly higher in AML patients than in normal donors (Fig. 1).

Positive correlations were observed between the individual expression of the genes studied (Notch1, Jagged1, and Delta1): Notch1 and Jagged1 ($P = 0.005$); Notch1 and Delta1 ($P = 0.003$); and Jagged1 and Delta1 ($P = 0.009$).

Notch1, Jagged1 and Delta1 expression and clinical characteristics of AML patients

Assessment of correlation between gene expression levels of Notch1, Jagged1 and Delta1 and FAB subtypes, peripheral white blood cell (WBC) count, blast count, hemoglobin value, platelet count, serum lactate dehydrogenase (LDH) level, age and sex found only a positive correlation of Notch1, Jagged1 and Delta1 expression with

absolute peripheral blast count ($P = 0.01$, 0.03 and 0.03, respectively) and a positive relationship of Notch1 with higher WBC counts ($P = 0.02$).

Cytogenetic analysis was performed in all AML patients. No differences in the expression of Notch1, Jagged1 and Delta1 associated with the presence of known recurrent karyotype abnormalities were found nor in with those with normal karyotypes.

Notch1, Jagged1 and Delta1 expression and clinical outcome of AML patients

Patients were divided into a low group (expression of target genes below the average) and a high group (above the average). Univariate analysis of factors associated with RFS showed a significantly shorter survival in the patients with unfavorable karyotype, higher Notch1 expression, higher Jagged1 expression or higher Delta1 expression (Table 3). Parameters, such as sex, age, WBC counts, hemoglobin level, platelet counts and WHO subtype had no impact. On the other hand, the variables that were associated with poor OS on univariate analysis were also unfavorable karyotype, higher Notch1 expression, higher Jagged1 expression or higher Delta1 expression. Cox proportional hazards multivariate analysis of the univariate predictors identified karyotype and gene expression levels of Notch1, Jagged1 and Delta1 as independent prognostic factors for RFS and OS (Please see the detail in Table 4). The Kaplan–Meier curves for RFS and OS stratified according to Notch1, Jagged1 and Delta1 expression in bone marrow from patients with AML are shown in Fig. 2.

To further illustrate the impact of Notch1, Jagged1 and Delta1 expression on OS, Kaplan–Meier survival curves of three cytogenetic-risk groups stratified for their expression

Table 3 Univariate analysis of the impact of variables on relapse-free survival and overall survival in AML patients

Variable	No. of patients	Relapse-free survival		Overall survival	
		Median (months ± SD)	P	Median (months ± SD)	*P*
Karyotype					
Favorable	35	Not reached	<0.001	Not reached	0.001
Intermediate	52	12.8 ± 1.3		21.3 ± 2.6	
Unfavorable	13	3.6 ± 0.9		10.5 ± 2.9	
Notch1 expression					
Low	42	13.8 ± 2.5	0.009	38.7 ± 3.3	0.006
High	58	8.3 ± 1.9		22.8 ± 2.6	
Jagged1 expression					
Low	45	13.2 ± 1.7	0.01	38.5 ± 2.8	0.008
High	55	9.8 ± 1.3		24.6 ± 3.5	
Delta1 expression					
Low	46	13.6 ± 2.1	0.01	38.6 ± 3.2	0.008
High	54	9.9 ± 1.0		24.1 ± 3.6	

Table 4 Multivariate analysis of the impact of variables on relapse-free survival and overall survival in AML patients

Variable	P	Odds ratio
Relapse-free survival		
Karyotype		
Unfavorable versus favorable/intermediate	0.009	4.09
Notch1 expression		
High versus low	0.01	3.52
Jagged1 expression		
High versus low	0.01	3.56
Delta1 expression		
High versus low	0.02	3.49
Overall survival		
Karyotype		
Unfavorable versus favorable/intermediate	0.02	3.36
Notch1 expression		
High versus low	0.03	3.01
Jagged1 expression		
High versus Low	0.04	2.67
Delta1 expression		
High versus Low	0.04	2.59

were analyzed. Notch1 ($P = 0.009$), Jagged1 ($P = 0.02$) and Delta1 ($P = 0.02$) expression had an even more obvious impact on OS in patients with intermediate-risk karyotype. However, there was no survival difference between patients with low and high expression levels of Notch1, Jagged1 and Delta1 in the favorable-risk group (all $P > 0.05$) and unfavorable-risk group (all $P > 0.05$).

Discussion

Although an increasing amount of recent studies have pointed to the usefulness of dividing AML in subclasses according to gene expression profiles, little is known regarding the involvement of Notch signal pathway in the progression and prognosis of AML. Here, we demonstrated that Notch receptor (Notch1) and ligands (Jagged1, Delta1) expression was elevated in the bone marrow from patients with newly diagnosed AML, compared with normal controls. In addition, both univariate and multivariate analysis revealed that the expression levels of Notch1, Jagged1 and Delta1 were predictors of shorter RFS and OS, independent of cytogenetics. Furthermore, the prognostic relevance of the three genes expression became even more pronounced in the patients with intermediate-risk karyotype. In other words, Notch1, Jagged1 and Delta1 expression may serve as potential biomarkers for prognosis prediction, especially in the intermediate-risk cytogenetic group.

Notch signal pathway consists of Notch (Notch1-4 in mammals), a family of transmembrane receptors, that undergo proteolytic activation in response to ligand (Delta-like1, 3, 4 and Jagged1-2 in mammals) binding to release the intracellular domain of Notch [15, 16]. Notch signaling is normally activated by ligand-receptor binding between two neighboring cells. This interaction induces a conformational change in the receptor, exposing a cleavage site, S2, in its extracellular domain. After cleavage by the metalloprotease TACE and/or Kuzbanian, Notch receptor undergoes intramembrane proteolysis at cleavage site S3. This cleavage, mediated by the γ-secretase complex, liberates the Notch intracellular domain (N-ICD), which then translocates into the nucleus to activate Notch target genes. Inhibiting γ-secretase function prevents the final cleavage of the Notch receptor, blocking Notch signal transduction [17]. Aberrant Notch signaling contributes to the genesis of diverse cancers. As the normal effect of Notch signaling during development differs from cell type to cell type, the tumorigenic effect of Notch is varied and depends on the tissue in which the tumor develops. The Notch signaling pathway has been implicated in the development of several leukemia and lymphoma. In T-cell leukemias, constitutively active Notch1 transforms cells in vitro [18] and causes mice to develop leukemia [19]. In contrast, Notch is a tumor suppressor in certain epithelial cancers where its normal function is to promote terminal differentiation [20]. Mice with Notch1-deficient epithelia develop spontaneous basal cell carcinoma-like tumors [21]. In AML, reciprocal Notch signaling might be necessary for the proliferation and survival of the HL60 human promyelocytic leukemia cell line, possibly through the maintenance of the expression of c-Myc and Bcl2, as well as the phosphorylation of the Rb protein [22]. In addition, Delta1-induced Notch activation activated the NF-kappaB pathway in AML cell lines [23]. Moreover, Yan group also demonstrated that Notch signaling pathway-related genes may contribute to the drug resistance of AML [24].

In the present study, we show that the high levels of Notch1 gene expression represent an important feature of AML patients. Patients with high Notch1 gene expression (values above the average) showed several adverse prognostic parameters such as a trend towards higher absolute peripheral blast counts and WBC counts. A possible role for Notch ligands in inducing high level of pathway activity was assessed through the analysis of Jagged1 and Delta1 transcription levels in the bone marrow from patients with newly diagnosed AML. Both ligands were selected since Jagged1 overexpression has been found in acute leukemia blood samples [25]. Moreover, Jagged1 and Delta1 expression have been detected in bone marrow stromal cells, and thymic epithelial cells seem to drive haematopoietic differentiation by interacting with Notch1
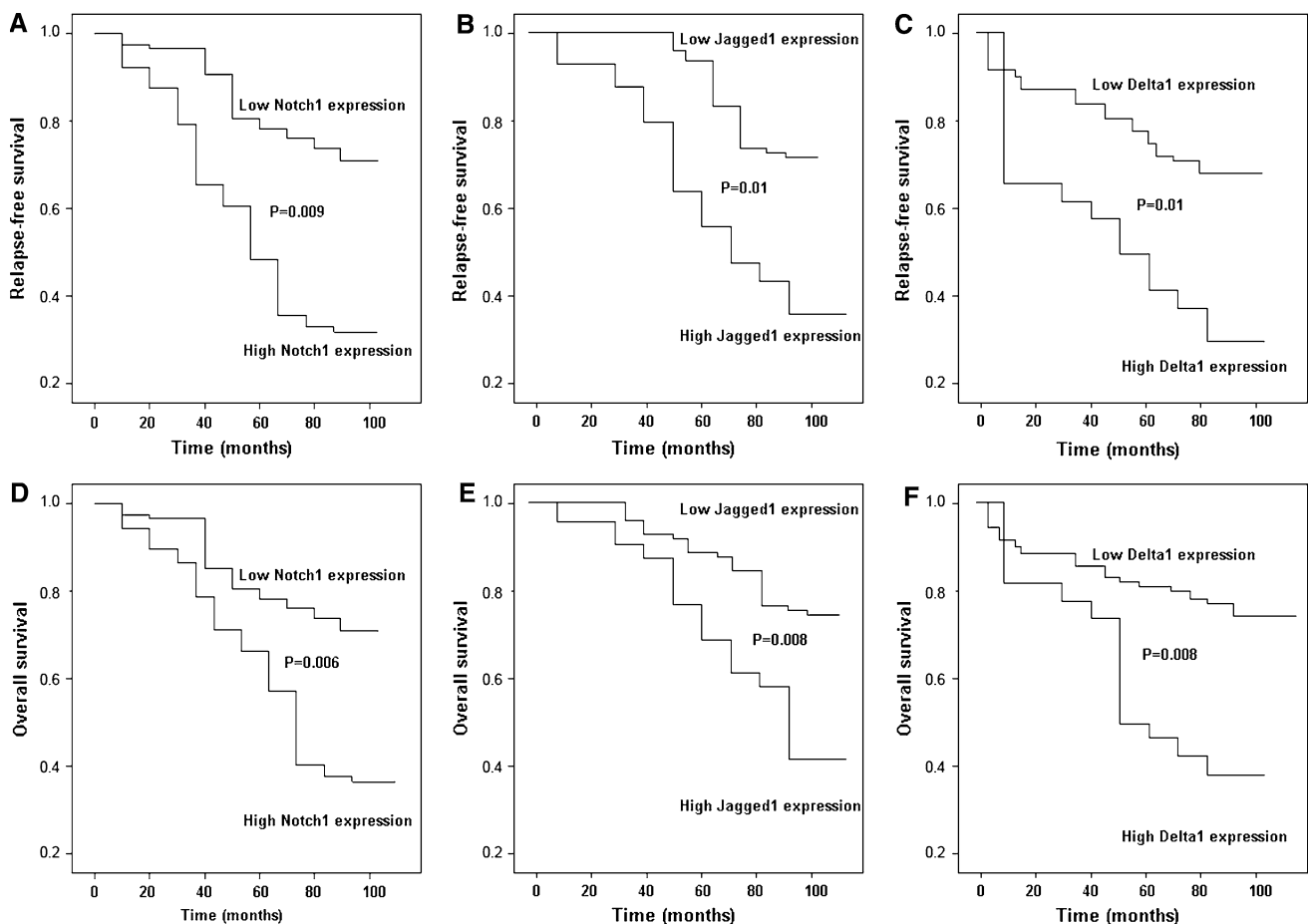
Fig. 2 (a–c) Kaplan–Meier curves of relapse-free survival of patients with newly diagnosed acute myeloid leukemia stratified by the level of Notch1, Jagged1 and Delta1 expression. (d–f) Kaplan–Meier curves of overall survival of patients with newly diagnosed acute myeloid leukemia stratified by the level of Notch1, Jagged1 and Delta1 expression

in haematopoietic progenitors [26]. Finally, a possible role for Jagged1 has been suggested for T-cell-derived anaplastic large cell lymphoma [27] and acute myelogenous leukemia [28]. We have demonstrated that Jagged1 and Delta1 are highly expressed in AML samples, thus confirming a selective role of these ligands in AML.

Notch pathway activation in tumor cells has been associated with clinical outcome in solid cancers, such as papillary bladder transitional cell carcinoma [29], breast [30], prostate cancer [31], lung [32] and glioma [33]. Recently, Santagata et al. reported that high Jagged1 expression in a subset of patients with prostate cancer was significantly associated with recurrence and survival, independent of other clinical parameters [31]. In this study, our observation that there was an inverse relationship between the expression levels of Notch signal pathway members (Notch1, Jagged1 and Delta1) and survival time in AML patients was consistent with this finding. However, some previous studies showed the opposite. For example, in the report of Shi et al. demonstrated that the Notch family expression pattern in papillary bladder transitional cell carcinoma is different from that in invasive bladder transitional cell carcinoma. Low expression of Notch1 as well as Jagged1 is potentially a useful marker for survival in patients with papillary bladder transitional cell carcinoma [29]. The reasons for these differences between our study and their study remain unknown, but they suggest that Notch pathway plays different roles in different tumors.

In conclusion, our data for the first time show that the activation of Notch signal pathway in the bone marrow indicates an unfavorable prognosis in AML, and the prognostic significance of Notch1, Jagged1 and Delta1 expression was more obvious in the subgroup of patients with intermediate-risk cytogenetics. To explain the different findings between our study and those reported previously, further more comprehensive studies are needed.

References

1. Gröschel S, Lugthart S, Schlenk RF, et al. High EVI1 expression predicts outcome in younger adult patients with acute myeloid

leukemia and is associated with distinct cytogenetic abnormalities. J Clin Oncol. 2010; in press.

2. Verhaak RG, Valk PJ. Genes predictive of outcome and novel molecular classification schemes in adult acute myeloid leukemia. Cancer Treat Res. 2009;145:67–83.

3. Stirewalt DL, Meshinchi S. Receptor tyrosine kinase alterations in AML—biology and therapy. Cancer Treat Res. 2009;145:85–108.

4. Park MH, Cho SA, Yoo KH, et al. Gene expression profile related to prognosis of acute myeloid leukemia. Oncol Rep. 2007;18:1395–402.

5. El Kholy NM, Sallam MM, Ahmed MB, et al. Expression of indoleamine 2,3-dioxygenase in acute myeloid leukemia and the effect of its inhibition on cultured leukemia blast cells. Med Oncol. 2010; in press.

6. Zhou FL, Zhang WG, Wei YC, et al. Involvement of oxidative stress in the relapse of acute myeloid leukemia. J Biol Chem. 2010; in press.

7. Hughes DP. How the NOTCH pathway contributes to the ability of osteosarcoma cells to metastasize. Cancer Treat Res. 2010; 152:479–96.

8. Mehta K, Osipo C. Trastuzumab resistance: role for Notch signaling. ScientificWorldJournal. 2009;9:1438–48.

9. Hirose H, Ishii H, Mimori K, et al. Notch pathway as candidate therapeutic target in Her2/Neu/ErbB2 receptor-negative breast tumors. Oncol Rep. 2010;23:35–43.

10. Fan X, Khaki L, Zhu TS, et al. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. Stem Cells. 2010;28:5–16.

11. Ellisen LW, Bird J, West DC. TAN-1, the human homolog of the Drosophila Notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell. 1991;66:649–61.

12. Swerdlow SH, Campo E, Harris NL, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008. p. 109–38.

13. Muller PY, Janovjak H, Miseret A, Dobbie Z. Processing of gene expression data generated by quantitative real time RT-PCR. Biotechniques. 2002;6:2–7.

14. Roumier C, Fenaux P, Lafage M. New mechanisms of AML1 gene alteration in hematological malignancies. Leukemia. 2003; 17:9–16.

15. Siar CH, Nakano K, Han PP, Nagatsuka H, Ng KH, Kawakami T. Differential expression of Notch receptors and their ligands in desmoplastic ameloblastoma. J Oral Pathol Med. 2010; in press.

16. Zhang P, Yang Y, Zweidler-McKay PA, Hughes DP. Critical role of Notch signaling in osteosarcoma invasion and metastasis. Clin Cancer Res. 2008;14:2962–9.

17. Wang M, Xue L, Cao Q, et al. Expression of Notch1, Jagged1 and beta-catenin and their clinicopathological significance in hepatocellular carcinoma. Neoplasma. 2009;56:533–41.

18. Pui JC, Allman D, Xu L. Notch1 expression in early lymphopoiesis influences B versus T lineage determination. Immunity. 1999;11:299–308.

19. Radtke F, Wilson A, Stark G. Deficient T cell fate specification in mice with an induced inactivation of Notch1. Immunity. 1999;10:547–58.

20. Gestblom C. The basic helix-loop-helix transcription factor dHAND, a marker gene for the developing human sympathetic nervous system, is expressed in both high- and low-stage neuroblastomas. Lab Invest. 1999;79:67–79.

21. Grynfeld A, Pahlman S, Axelson H. Induced neuroblastoma cell differentiation, associated with transient HES-1 activity and reduced HASH-1 expression, is inhibited by notch1. Int J Cancer. 2000;88:401–10.

22. Li GH, Fan YZ, Liu XW, et al. Notch signaling maintains proliferation and survival of the HL60 human promyelocytic leukemia cell line and promotes the phosphorylation of the Rb protein. Mol Cell Biochem. 2010; in press.

23. Itoh M, Fu L, Tohda S. NF-kappaB activation induced by Notch ligand stimulation in acute myeloid leukemia cells. Oncol Rep. 2009;22:631–4.

24. Yan S, Ma D, Ji M, et al. Expression profile of Notch-related genes in multidrug resistant K562/A02 cells compared with parental K562 cells. Int J Lab Hematol. 2009; in press.

25. Chiaramonte R, Basile A, Tassi E, et al. A wide role for NOTCH1 signaling in acute leukemia. Cancer Lett. 2005;219:113–20.

26. Li L, Milner LA, Deng Y. The human homolog of rat Jagged1 expressed by marrow stroma inhibits differentiation of 32D cells through interaction with Notch1. Immunity. 1998;8:43–5.

27. Jundt F, Anagnostopoulos I, Forster R, Mathas S, Stein H, Dorken B. Activated Notch1 signaling promotes tumor cell proliferation and survival in Hodgkin and anaplastic large cell lymphoma. Blood. 2002;99:3398–403.

28. Tohda S, Nara N. Expression of Notch1 and Jagged1 proteins in acute myeloid leukemia cells. Leuk Lymphoma. 2001;42:467–72.

29. Shi TP, Xu H, Wei JF, et al. Association of low expression of Notch-1 and Jagged-1 in human papillary bladder cancer and shorter survival. J Urol. 2008;180:361–6.

30. Jubb AM, Soilleux EJ, Turley H, et al. Expression of vascular notch ligand delta-like 4 and inflammatory markers in breast cancer. Am J Pathol. 2010;176:2019–28.

31. Santagata S, Demichelis F, Riva A, et al. JAGGED1 expression is associated with prostate cancer metastasis and recurrence. Cancer Res. 2004;64:6854–7.

32. Eliasz S, Liang S, Chen Y, et al. Notch-1 stimulates survival of lung adenocarcinoma cells during hypoxia by activating the IGF-1R pathway. Oncogene. 2010;in press.

33. Shih AH, Holland EC. Notch signaling enhances nestin expression in gliomas. Neoplasia. 2006;8:1072–82.