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Significance of Notch Expression in Acute Myeloid Leukemia

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Background: *Notch* is a gene family encoding receptors to transduce intercellular signals involved in cell-fate determination. Although several lines of evidence indicate that abnormal Notch signaling may contribute to neoplastic transformation, little is known regarding the role of Notch in the pathogenesis of leukemia.

Methods: To explore the functional significance of Notch1 in acute myeloid leukemia (AML), the expression of Notch1 and its association with survivin and p27Kip1 expression was examined in 50 patients with *de novo* AML.

Results: Notch1 transcripts were expressed in 40 (80%) cases with a variable degree of expression, and the fraction of AML cells in the G_0/G_1 phase was higher in Notch1-positive cases than in Notch1-negative cases. Survivin was shown to be present in 38 (76.0%) cases, and Notch1 expression highly correlated with survivin mRNA expression (r=0.7170, $P < 0.001$). p27Kip1 was present in 40 (80.0%) cases of AML and p27Kip1 expression was significantly associated with Notch1 expression (r=0.8770, $P < 0.001$). Except for one case, the simultaneous expression of survivin and p27Kip1 was not seen in all cases that were negative for Notch1 expression. There were no differences in clinical outcomes according to Notch1 expression.

Conclusion: Notch1 expression was a frequent event and has functional significance in the alteration of the cell cycle in AML cells. Notch1 expression was also significantly associated with survivin and p27kip1 expression in AML cells. To evaluate the clinical significanceand functional role of Notch1 expression in the aberrant regulation of survivin and p27Kip1 expression in *de novo* AML, a further study with a larger number of patients is necessary. ***(Korean J Hematol 2008;43:9-18.)***

INTRODUCTION

The evolutionary conserved Notch transmembrane receptor family has been found to be a fundamental cell-fate controlling mechanism that involves signals between neighboring precursor cells.[1] Notch is not associated with the transmission of these signals, but appears to control fates by modulating a precursor cell's ability to

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respond to the signals.[2] The unique role of Notch as a regulator of cell fate decisions stems from its capacity to engage in a variety of molecular interactions. It integrates inductive signals to influence gene expression in a way that reflects the precise developmental context of a particular cell.[1] Since the initial demonstration that Notch1 is expressed in normal bone marrow (BM) hematopoietic precursors,[3] considerable evidence has accumulated that supports a conserved role for Notch in the mediation of cell fate decision and self-renewal of progenitors during hematopoiesis.[4] In general, Notch activation leads to the transcriptional suppression of lineage-specific genes, thus inhibiting differentiation as a response to inductive signals.[5] Notch signaling limits the number of cells that adopt a particular fate and leaves some progenitors uncommitted but competent to adopt alternative fates.[6] Although several lines of evidence indicated that abnormal Notch signaling may contribute to neoplastic transformation,[3] little is known regarding the role of Notch signaling in the pathogenesis of leukemia. Considerable interest has recently been focused on the role of dysregulated apoptosis in leukemia and its ability to aberrantly prolong cell viability, facilitate the accumulation of mutations and also promote drug resistance.[7] Progression of the cell cycle and control of apoptosis are thought to be intimately linked processes.[4] The candidate molecules for the interface between apoptosis and cell cycle are survivin and p27Kip1.

Survivin is a recently identified inhibitor of the apoptosis protein (IAP) family of proteins.[8] Its expression is detected specifically in tumor cells and in cells during their embryonic development.[8] Survivin plays an important role in both cell cycle regulation and inhibition of apoptosis. In proliferating cells expression of survivin is cell cycle-dependent with an upregulation in the G_2/M phase and rapid decline in the G_1 phase.[4] Survivin may counteract a default induction of apoptosis in the G_2/M phase.[4] Survivin can bind to caspase-3 and caspase-7 and the overexpression of survivin blocks apoptosis induced by caspase-3 and caspase-7, growth factor deprivation and the microtubule-stabilizing anticancer drugs.[4,8] Several studies have reported that survivin is expressed as high levels in human cancers.[8] Survivin expression was detected in a substantial proportion of AML cases,[9] and was significantly associated with a lower white blood cell count, cytogenetics, and survival.[9]

Positive control of cell cycle progression in eukaryotic cells is provided by cyclin-dependent kinases (Cdk), while negative control is provided by two families of Cdk inhibitors. The first family, INK4, includes p15, p16, p18 and p19; the second family, Cip/Kip, includes p21, p27 and p57.[10] Members of the Cip/Kip family bind to all G_1 and G_1/S Cdk complexes, but preferentially inhibit those containing Cdk2.[10] p27Kip1 can produce cell cycle arrest in response to such inhibitory stimuli as TGF-β or cAMP, lack of adhesion, and cell contact inhibition.[10] Deregulated expression of cell cycle genes plays a significant role in oncogenesis.[11] Deregulation of p27Kip1 protein expression has recently been demonstrated in several human neoplasms. Low levels of p27Kip1 within these neoplasms correlates with poor clinical outcomes.[12] AML patients with high p27 expression had a significantly increased disease-free survival.[13]

It has been suggested that there is a potential link of Notch effects on hemopoiesis by means of its effects on cell cycle regulation.[5] Notch interacts directly or indirectly with the regulators of the cell-cycle machinery, as has been suggested for other transcription factors involved in proliferation and differentiation commitment.[5,14] In the present study, Notch1 expression was demonstrated to be highly associated with p27Kip1 and survivin expression in AML cells. These results suggest that the role of Notch signaling in regulating the apoptosis, or proliferation of leukemic cells is potentially complex.

MATERIALS AND METHODS

1. Patients

Fifty patients with a diagnosis of *de novo* AML in Severance Hospital from 2001 to 2006 were studied. The median age of patients was 44 years (range, 16~72). Patients with acute promyelocytic leukemia or with a history of preceding myelodysplasia were excluded from this study. All of the patients received identical induction chemotherapy containing idarubicin ($12mg/m^2$/ day, days 1~3) and cytosine arabinoside ($100mg/m^2$/day by continuous infusion, days 1~7). Complete remission (CR) was defined as the presence of <5% blast cells in a standardized bone marrow study. Patients who achieved CR received two courses of high-dose consolidation therapy consisting of intermediate-dose cytosine arabinoside ($1,000mg/m^2$ every 12 hours by i.v. infusion on day 1~4), mitoxantrone ($10mg/m^2$ by i.v. infusion on day 1~3) and VP-16 ($100mg/m^2$ i.v. on day 1~2).

2. Isolation of leukemic cells

Bone marrow samples were obtained after informed consent was obtained before chemotherapy was started. Mononuclear cells were separated by density gradient centrifugation on Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden). After washing with phosphate buffered saline (PBS), the monocytes were removed by 2-hour adherence to plastic dish. T-lymphocytes were then depleted by using the miniMACS column (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the directions of the manufacturer. These preparations contained at least 95% leukemic blasts as judged by morphological criteria using May-Grunwald-Giemsa staining.

3. Flow cytometric analysis of Notch1 expression

In order to measure Notch1 expression, AML cells were washed twice and made permeably by incubation with PermeaFix (Ortho, Raritan, NJ, USA), washed twice, and then incubated at room temperature with a mouse anti-human Notch1 monoclonal antibody (Clone A6; Neomarkers, Lab Vision, Washington, USA) for 20 minutes. After washing twice with PBS, cells were incubated at $4^{\circ}C$ with phycoerythrin-conjugated anti-mouse IgG antibody (Becton Dickinson, San Jose, CA, USA). Control staining was measured using an isotype matched directly labeled or unlabeled antibody of irrelevant specificity. Cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). CD45/SSC gating was used for immunofluorescence analysis of leukemic blasts.

4. Cell cycle analysis

Cells (5×10^5) were washed twice in PBS, and resuspended in $300 \mu L$ PBS containing 3% fetal calf serum (FCS; GIBCO-BRL, Gaithersberg, MD, USA). $700 \mu L$ cold ethanol was added slowly and the cells were then fixed at $-20^{\circ}C$ for 1 hour. After washing twice, the cells were treated with $0.5mg/mL$ RNaseA (Sigma, St. Louis, MO, USA) in PBS for 30 minutes. Cells were centrifuged and resuspended in PBS containing 3% FCS and $5 \mu g/mL$ propidium iodide (PI). Measurement of fluorescence caused by the PI at 488nm was determined by flow cytometry. The DNA content profiles were analyzed using Cell Quest software (Becton Dickinson).
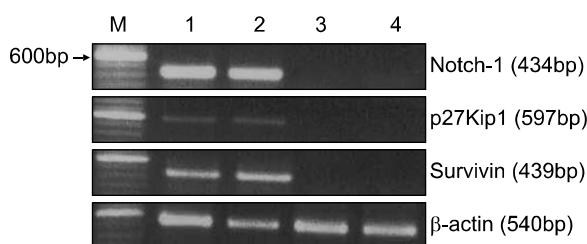
5. Cytogenetics

Chromosome analysis was performed on metaphases from direct preparations, as well as from 24 hour and 48 hour cultures of bone marrow samples. The cytogenetic preparations and G-banding were carried out according to routine laboratory procedures.[15]

6. Reverse transcription-polymerase chain reaction (RT-PCR)

RNA extraction was carried out with the RNeasy mini kit (Quiagen, Hilden, Germany) following the manufacturer's instructions. Reverse

transcription (RT) was performed at 37°C in 20 μL volumes using Moloney murine leukemia virus reverse transcriptase following the manufacturer's instructions (MMLV-RT, PERKIN-ELMER, New Jersey, USA); 1 μg of total RNA and 2.5 μM random hexamer primers were used per RT reaction. Each PCR reaction was performed in a 50 μL reaction mixture containing: 1mM MgCl₂; 0.25mM dNTP; 20pmole of each upstream and downstream primer; 1 μL of the RT reaction described previously and 2.5U of Taq DNA polymerase (PERKIN-ELMER). The primer sequences are as follows: Notch1, forward 5'-GAT GCC AAC ATC CAG GAC AAC ATG GG-3' and reverse 5'-GGC AGG CGG TCC ATA TGA TCC GTG AT-3'; Survivin, forward 5'- GGC ATG GGT GCC CCG ACG TT-3' and reverse 5'- AGA GGC CTC AAT CCA TGG CA-3'; p27Kip1, forward 5'-ATG TCA AAC GTG CGA GTG TCT-3' and reverse 5'-TTA CGT TTG ACG TCT TCT GA-3'; β-actin, forward 5'-GTG GGG CGC CCC AGG CAC CA-3' and reverse 5'-CTC CTT AAT GTC ACG CAC GAT TTC-3'. The PCR amplification was performed in a PERKIN ELMER 9600 as follows: initial denaturation at 94°C for 5 minutes; followed by cycles of denaturation at 94°C for 1 minute; primer annealing at 60°C for 1 minute for

Notch1, survivin, and β-actin, and 58°C for 1 minute for p27Kip1. Thirty cycles were used for p27Kip1 and β-actin, and 35 cycles were used for Notch1, and survivin. The PCR products were visualized by gel electrophoresis of 10 μL of the PCR reaction on an agarose gel which was polymerized with ethidium bromide. We analyzed the levels of Notch-1, survivin and p27Kip1 expression using densitometer scanning.

7. Statistics

The statistical analysis was performed using the Chi-square for binary variables and the Mann-Whitney test for continuous variables. Correlation coefficiency was determined using the Spearman's test. The survival curves were plotted according to the Kaplan-Meier method and checked by a log-rank test. A P-value <0.05 was considered to indicate statistical significance. Data were analyzed with the SPSS statistical soft-

Fig. 1. RT-PCR analysis of Notch1, survivin, and p27Kip1 expression in representative AML samples. RT-PCR was performed with specific primers described in Materials and Methods. PCR products derived from 1 μg of total RNA were applied to each lane. Lane 1, 100-bp molecular weight marker. Cases expressing Notch1 transcript (lanes 2∼3) demonstrate both of survivin and p27Kip1 transcripts. Survivin and p27Kip1 transcripts are not expressed in two cases that do not express Notch1 transcript (lanes 4∼5).

Table 1. Characteristics of AML patients according to Notch1 expression

	Notch1 (−) n=10	Notch1 (+) n=40	P value
Median age (years)	44	42	NS
WBC (10⁹/L)*	17.2±3.2	16.9±4.5	NS
FAB			NS
M0	1	2	
M1/M2	1/4	3/21	
M4/M5	2/1	10/3	
M6	1	1	
Cytogenetics			NS
Favorable	1	5	
Intermediate	7	26	
Unfavorable	2	9	

Notch1 expression in AML cells was evaluated by RT-PCR analysis. Notch1 (−), cases did not express Notch1 transcript; Notch1 (+), cases expressed Notch1 transcript. Abbreviations: AML, acute myeloid leukemia; WBC, white blood cell; NS, not significant. Favorable cytogenetics include t(8;21), and inv (16). Intermediate cytogenetics include normal 46XX or 46XY. Unfavorable cytogenetics include 5-, 7-, 5q-, 7q- or of exaggerated hyperdiploidy, trisomy 8, t(6;9), trisomy 11, & multiple chromosomal abnormalities. *Data represent the mean value±standard deviations.

ware package (SPSS Inc., Chicago, IL).

1. Notch1 expression in AML

To evaluate the expression of Notch1, RT-PCR was performed using the specific primers as described in Materials and Methods. RT-PCR performed in the absence of RNA was used as a negative control. Notch1 transcript was expressed in 40 (80.0%) of 50 AML cases. As shown in Fig. 1, the levels of Notch1 mRNA expression were variable according to the patients. Notch1 mRNA expression was not correlated with age, white blood cell count, cytogenetics, or FAB subtypes (Table 1). The intracellular staining of Notch1 was analyzed by flow cytometry in 14 cases. Two representative experiments are shown in Fig. 2, which demonstrate a negative staining of Notch1 in one case (Fig. 2A), and an intense intracellular staining of Notch1 in another case (Fig. 2B). Most cases showing Notch1 transcript (10 out of 12) demonstrated Notch1 intracellular staining as measured by flow cytometry (data not shown).

2. Cell cycle distribution according to Notch1 expression

The fraction of AML cells in the G_0/G_1 phase in the Notch1-positive cases was higher compared to that in the Notch1-negative cases ($86.1 \pm 5.4\%$ vs. $74.2 \pm 5.9\%$, $P < 0.05$). The fraction of AML cells in the S phase or the G_2/M phase was also significantly different between the Notch1-positive cases and the Notch1-negative cases (Table 2).

3. Survivin expression in AML

To evaluate the expression of survivin, RT-PCR was performed using the specific primers as

Table 2. Cell-cycle distribution according to Notch1 expression

	G_0/G_1 phase	S phase	G_2/M phase
Notch1 (+) (n=40)	86.1 ± 5.4	7.3 ± 2.1	6.4 ± 2.9
Notch1 (−) (n=10)	74.2 ± 5.9	15.6 ± 3.7	10.2 ± 4.5
P value	< 0.05	< 0.05	< 0.05

Cell cycle analysis was done using flow cytometry. Data represent the mean value±standard deviation. Differences are calculated with the Mann-Whitney test.
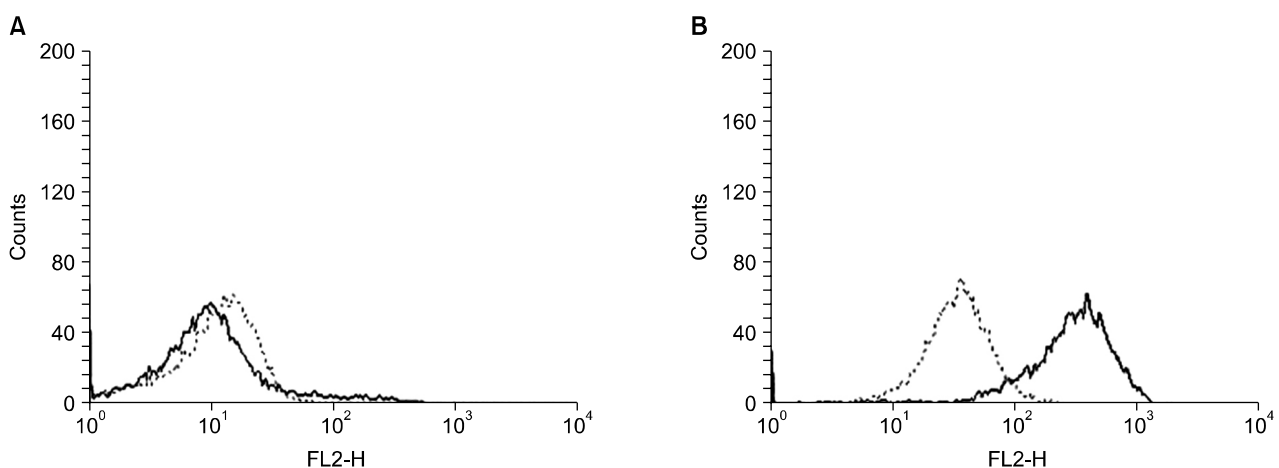
Fig. 2. Expression of Notch1 by AML cells. Fluorescence histograms of permeabilized AML cells show no shift in the fluorescent intensity after staining with Notch1 antibody in a representative case, indicating absence of Notch1 (A), and a homogenous shift in the fluorescent intensity curve in another case, indicating a high expression of Notch1 (B). The y-axis represents cell number, and the x-axis represents log fluorescence intensity. The solid line indicates staining with mouse anti-human Notch1 monoclonal antibody, and the dashed line represents staining with an isotype-matched control antibody of irrelevant specificity.

described in Materials and Methods. Survivin transcript was expressed in 38 (76.0%) of AML samples (Table 3). The levels of survivin transcripts were variable according to the patients, and were not correlated with age, white blood cell count, cytogenetics, or FAB subtypes (data not shown). Expression of survivin mRNA was detected in 36 (90.0%) of 40 Notch1-positive AML samples (Table 3). Most of Notch1-negative AML cases did not express survivin transcript as shown in Fig. 1. The expression of Notch1 mRNA was highly correlated with survivin mRNA expression in AML samples (r=0.7170, $P < 0.001$).

4. p27Kip1 expression in AML

To evaluate the expression of p27Kip1, RT-PCR was performed using the specific primers as described in Materials and Methods. In 40 (80%)

**Table 3.** Expression of survivin, or p27Kip1 according to Notch1 expression

	Notch1 (−) n=10	Notch1 (+) n=40	Correlation efficiency with Notch1 expression	P value
Survivin			r=0.7170	<0.001
Positive	2	36		
Negative	8	4		
p27Kip1			r=0.8440	<0.001
Positive	0	40		
Negative	10	0		
Survivin & p27Kip1			r=0.8770	<0.001
Positive	0	36		
Negative	8	0		

Numerical data represent the number of cases showing the respective pattern of expression. Survivin & p27Kip1 positive, cases coexpressing survivin and p27Kip1 mRNA; Survivin & p27Kip1 negative, cases lacking both of survivin and p27Kip1 mRNA. Correlation coefficiency between Notch1 and survivin expression, Notch1 and p27Kip1 expression, Notch1 and coexpression of survivin/p27Kip1 was calculated using the Spearman's test.

Table 4. Clinical outcomes according to Notch1, survivin, or p27 mRNA expression

	Complete remission (%)	P value
Notch1 mRNA		NS
Positive (n=40)	33 (82.6)	
Negative (n=10)	7 (70.0)	
Survivin mRNA		NS
Positive (n=38)	30 (78.9)	
Negative (n=12)	10 (83.3)	
p27Kip1 mRNA		NS
Positive (n=40)	33 (82.6)	
Negative (n=10)	7 (70.0)	

Statistical analysis was done using the Mann-Whitney test. Abbreviation: NS, not significant.
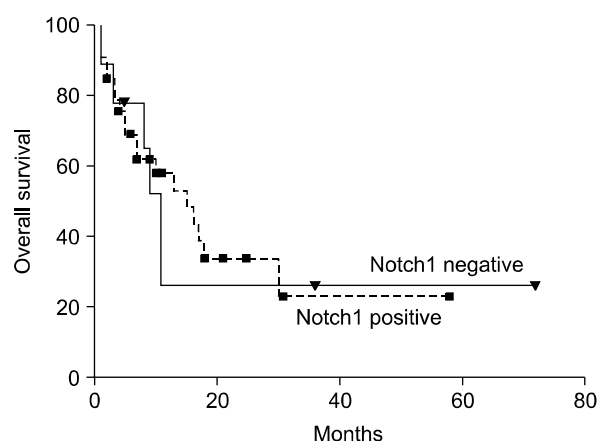
Fig. 3. Kaplan-Meier survival curves for AML patients according to the Notch1 expression. The dotted line represents AML cases that expressed Notch1, and the solid line represents AML cases that did not express Notch1. Squares and triangles represent censored patients. The difference between the curves is not statistically significant ($P = .83$ for overall survival by log-rank analysis).

of AML samples, the p27Kip1 transcript was detected (Table 3). The levels of p27Kip1 transcripts were variable according to the patients, and were not correlated with age, white blood cell count, cytogenetics, or FAB subtypes (data not shown). The expression of p27Kip1 was significantly correlated with Notch1 mRNA expression (r=0.8770, $P<0.01$) (Table 3).

All the AML samples expressing Notch1 mRNA also expressed p27Kip1 mRNA as shown in Fig. 1. In contrast, no p27Kip1 transcript was detected in the Notch1-negative AML samples (Table 3). Simultaneous expression of p27Kip1 and survivin transcript was shown in 36 (90%) of Notch1-positive AML cases (Table 3; Fig. 1).

5. Clinical outcomes

Eighty percent of AML patients reached CR criteria after the induction chemotherapy. The CR rate was not significantly different between patients having Notch1-positive AML cells and patients having Notch1-negative AML cells (82.6% and 70.0%, respectively) (Table 4). The overall survival of the patients was not different according to the expression of Notch1 in AML cells (Fig. 3). There was no difference in the CR rate according to the expression of survivin or p27Kip1 (Table 4).

DISCUSSION

In this study, we have shown that Notch1 expression is a frequent event in *de novo* AML, having been detected in 80% of all patients who were analyzed. The levels of Notch1 expression were variable according to the patients. The finding that substantial proportion of AML cases was positive for Notch1 raises the possibility that the wild-type Notch1 receptor could potentially play an important role in the pathogenesis of leukemic disorders. However, the functional significance of Notch expression in leukemia is still not clear.

Dysregulated Notch signaling may prove to be a frequent occurrence in malignant transfor-

mation.[16] The importance of Notch signaling in leukemia was illustrated by the finding that the $t(7;9)$ translocation associated with T-cell acute lymphoblastic leukemia resulted in the production of a truncated Notch1 protein.[17] Up to 50% of the mice transplanted with genetically engineered mouse bone marrow cells that expressed truncated human Notch1 developed clonal T-cell leukemia.[18] Aberrant expression of Notch activity might contribute to malignant transformation by inhibiting normal differentiation and permitting the continued proliferation of undifferentiated cells.[19] Identifying the molecular consequences induced by Notch1 signaling will be a critical step in resolving the various issues of aberrant, malignant hematopoiesis in acute leukemia. One distinct possibility is that Notch interacts directly or indirectly with the cell-cycle machinery, or cell cycle-dependent intracellular molecules similar to that which has been suggested for other transcription factors involved in proliferation and differentiation commitment.[5,14] In this study, the fraction of leukemic cells in the G_0/G_1 phase was higher in the Notch1-positive AML compared to the Notch1-negative AML. Notch1-positive AML showed a significantly lower proportion of cells in the S phase, and in the G_2/M phase compared with the Notch1-negative AML. These results suggest that Notch1 expression has a functional significance in the alteration of cell cycle in AML cells, and could affect the expression of those cell cycle-dependent molecules that are critically important in the regulation of proliferation, or apoptosis of AML cells.

Survivin plays an important role in both cell cycle regulation and inhibition of apoptosis. In proliferating cells the expression of survivin is cell cycle-dependent with upregulation in the G_2/M phase and a rapid decline in the G_1 phase.[4] At the molecular level, survivin was determined to be the first apoptosis regulator to be expressed in mitosis in a cell cycle-dependent manner and to localize mitotic spindle microtubules in a reaction required for apoptosis inhibition.[4] Overex-

pression of survivin resulted in an accelerated S phase shift, resistance to G_1 arrest, and an activated Cdk2/Cyclin E complex leading to Rb phosphorylation.[20] Survivin was detected in a substantial proportion of AML samples.[9] Survivin expression was demonstrated to be an independent negative prognostic factor for survival in AML.[9] By RT-PCR analysis, survivin transcript was detected in the majority of AML cases of this study. Interestingly, the expression of survivin mRNA was highly correlated with Notch1 mRNA expression in our AML samples. However, because the expression of surviving was generally shown to decline in G_1 phase,[4] it is not clear why this survivin expression was significantly correlated with Notch1 expression, of which fraction in G_1 phase increased, in AML cells. One of possible hypothesis is that there is the involvement of the ubiquitin-proteasome pathway in posttranslational regulation of surviving.[21] The c-IAP (inhibitor of apoptosis-1) and XIAP (X-linked inhibitor of apoptosis) inhibitors of apoptosis were selectively lost in glucocorticoid- or etoposide-treated thymocytes in a proteasome-dependent manner before death.[21] It was also shown that proteasome inhibitors could block survivin degradation during the G_1 phase.[8] Recently the interaction between the Notch signaling and proteasomes was suggested.[22] Consequently, it can be considered that Notch1 possibly affects the expression or downregulation of survivin by its interactions with the ubiquitin/proteasome pathway.

This study also demonstrated that the expression of p27Kip1, which is a Cdk inhibitory protein, was significantly associated with Notch1 in AML. p27Kip1 plays a pivotal role in the control of cell proliferation.[10] Deregulation of p27Kip1 protein expression has recently been demonstrated in several human neoplasms. Low levels of p27Kip1 within these neoplasms correlates with poor clinical outcomes.[12] AML patients with high p27Kip1 expression had a significantly increased disease-free survival.[13] Transition from

the G_1 to the S phase is promoted by G_1 cyclin/Cdk complexes, such as cyclin D/Cdk4 and 6 and cyclin E/Cdk2. p27Kip1 inhibits the activities of these directly by binding to them.[23] The elimination of p27Kip1 during the late G_1 phase is required for G_1 cyclin/Cdk complex activation and cell cycle progression from the G_1 to the S phase in various cell lines.[24] Consistent with this idea is that the forced expression of p27Kip1 blocks cell cycle progression during the G_1 phase. Selective expression of p27Kip1 in Notch1- positive AML cases in this study strongly suggests a critical role for Notch in the expression of p27Kip1 by means of cell cycle regulation. Our finding that p27Kip1 was not expressed in Notch1-negative AML, and the fraction of cells in the S phase was increased in Notch1-negative AML could support this hypothesis. As for survivin, posttranslational machinery exists that controls the p27Kip1 expression levels.[10] The ubiquitin-proteasome pathway was suggested to be involved in p27Kip1 degradation in mammals.[25] In parallel with its ubiquitin-dependent degradation, p27Kip1 was processed rapidly by an ubiquitination-independent but an adenosine triphosphate (ATP)-dependent mechanism at a higher activity during the S, rather than the G_0/G_1 phase.[25] Therefore it is tempting to consider that the regulatory function of Notch1 is closely related to ubiquitin/proteasome-mediated, or ATP-dependent degradation of p27Kip1 in AML cells.

In the following, we evaluated the clinical impact of Notch1 expression in *de novo* AML patients. Because the relatively small number of Notch1-negative patients (n=10), we could not find any significant differences in clinical outcomes such as CR or survival rate according the Notch1 expression. However, further studies with larger number of patients must be needed to decide the clinical significance of Notch1 expression in AML.

How the Notch1 signaling regulates the proliferation, differentiation and induction of apoptosis of leukemic progenitor cells requires further

investigation. The contribution of Notch, if any, to the pathogenesis of AML should become apparent as the role of Notch in malignant hemopoiesis and its integration with other signaling pathways is more clearly elucidated.

요   약

배경: Notch는 세포의 운명을 결정하는 세포 내 신호전달체계에 관여하는 수용체를 형성하는 유전자군이다. 그간 세포 내 Notch 신호전달체계의 이상이 여러 악성종양에서 관찰되며 종양의 발생과 연관되어 있다는 다양한 보고가 있어왔으나 대표적 혈액종양인 백혈병에서의 Notch 신호전달체계 이상의 의미에 대한 연구는 제한적이었다.

방법: 본 연구에서는 50명의 급성골수성백혈병 환자로부터 진단 시 분리된 골수 내 백혈병세포를 대상으로 유동세포분석법 및 반정량적 역전사-중합연속효소반응법을 이용하여 세포 내 Notch1의 표현 정도를 확인하고 Notch1과 surviving 및 p27Kip1 표현과의 연관성을 확인하였다.

결과: 총 50명의 급성골수성백혈병 환자 중 40명 (80%)에서 Notch1이 표현되었으며, Notch1이 발현된 세포에서 그렇지 않은 세포에 비해 G_0/G_1 주기에 해당하는 세포의 비율이 의미 있게 높았다(86.1 ±5.4% vs. 74.2±5.9%, $P<0.05$). Survivin mRNA는 38명(76.0%)에서 관찰되었으며, Notch1의 표현과 의미 있는 연관성을 보였다(r=0.7170, $P<0.001$). p27Kip1 mRNA 역시 총 40명(80.0%)의 환자에서 관찰되었으며, survivin과 마찬가지로 Notch1 표현과 의미 있게 연관되어 있었다(r=0.8770, $P<0.001$). Notch1이 발현되지 않는 환자들 중 1명을 제외한 모든 환자에서 survivin과 p27kip1이 동시에 발현되지 않았다. 하지만 Notch1 발현 유무에 따라서 관해유도율이나 생존율 등 임상결과의 의미 있는 차이는 관찰되지 않았다.

결론: 다른 종양에서 보고된 바와 유사하게 급성 골수성백혈병에서도 Notch1의 발현이 빈번히 관찰되었으며 Notch1의 발현이 세주주기의 변화에 관여함을 확인하였다. 또한 급성골수성백혈병세포에서 Notch1의 발현은 survivin과 p27Kip1의 발현과 의미 있는 상관관계를 보여 이들 물질들과의 기능적 연관성이 의심되었다. 빈번한 Notch1 발현의 백혈병 발생과정에서의 임상적 의의를 밝히고 survivin

및 p27Kip1 발현의 비정상적인 조절과의 기능적 연관성에 확인하기 위하여 추가 연구가 필요할 것으로 생각한다.

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