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Review

NOTCH Signaling Roles in Acute Myeloid Leukemia Cell Growth and Interaction with other Stemness-related Signals

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Abstract. *NOTCH activation plays oncogenic roles in acute T-lymphoblastic leukaemia (T-ALL). However, whether NOTCH is oncogenic or tumor-suppressive in acute myeloid leukaemia (AML) is still controversial. Herein, the roles of NOTCH in AML are reviewed. AML cells express NOTCH and NOTCH ligands; however, cell-autonomous activation is not observed. Activating NOTCH1 mutations are rare in AML, unlike in T-ALL. NOTCH ligand stimulation generally suppresses the in vitro growth of AML cells but promotes transient growth of some samples. Conversely, knockdown of NOTCH1 and NOTCH2 does not affect the growth of AML cells, whereas it suppresses the growth of T-ALL cells. These findings suggest that NOTCH is dispensable or suppressive for AML cell growth. However, the effects of NOTCH differ depending on cell conditions, and various stemness-related signals modify these effects; hence, forced NOTCH activation in vitro may not exhibit effects in bone marrow. Thus, further understanding is required for the development of AML therapies targeting NOTCH signalling.*

NOTCH signalling plays crucial roles in cell fate decisions during development, stem cell self-renewal, and differentiation in various systems such as neurogenesis and haematopoiesis (1). Dysregulation of NOTCH signaling has been reported in human haematological malignancies and various solid tumors (2). NOTCH activation plays oncogenic roles in many cancers, while it acts as a tumor suppressor in certain malignancies. Therefore, the NOTCH pathway is a potential, context-dependent therapeutic target (3). Notably, more than half patients with acute T-lymphoblastic leukaemia (T-ALL) have activating *NOTCH1* mutations (4). However, the roles of NOTCH signalling in acute myeloid leukaemia (AML) are still controversial. We first reported that NOTCH1 and JAGGED1 proteins are expressed in AML cell lines and primary AML cells (5). Thus far, we have investigated the roles of NOTCH signaling in AML cells compared with T-ALL cells. We have also investigated the crosstalk between NOTCH and other stemness-related signalling pathways such as HEDGEHOG and wingless-type mouse mammary tumor virus integration site family (WNT).

NOTCH is a crucial regulator of normal stem cells and, therefore, may play important roles in leukaemia stem cells. To develop NOTCH-targeting therapeutics for leukaemia stem cells, the mechanisms of NOTCH regulation must be clearly understood in a context-dependent manner. In this review, present our findings in addition to outstanding work from other investigators, focusing on the role of NOTCH signalling in AML cells.

NOTCH Signalling Pathway

NOTCH is a transmembrane receptor that forms a heterodimer consisting of extracellular (N^{EC}) and transmembrane (N^{TM}) subunits (Figure 1). NOTCH is expressed on both haematopoietic cells and leukaemia cells. NOTCH ligands are also transmembrane proteins expressed on bone marrow stromal cells. Mammalian cells express four kinds of NOTCH receptors, NOTCH1-4, and five NOTCH ligands, delta-like protein (DLL) 1, DLL3, DLL4, JAGGED1, and JAGGED2. Ligand binding to NOTCH receptors causes cleavage of N^{TM} by γ-secretase and releases an intracellular fragment of NOTCH (ICN). ICN is translocated to the nucleus, where it induces expression of its target genes such as hairy and enhancer of split 1 (*HES1*) and v-Myc avian myelocytomatosis viral oncogene homolog (*MYC*). Although NOTCH activation generally leads to maintenance of stem cells, its roles differ in cell type- and

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context-dependent manners. In lymphopoiesis, NOTCH1 and NOTCH2 affect T-cell differentiation and marginal zone B-cell differentiation, respectively, while their roles in myeloid lineages are not fully clarified.

NOTCH Expression and Mutation in AML Cells

Activating *NOTCH1* mutations are involved in the pathogenesis of T-ALL. Weng *et al*. reported that 56% of patients with T-ALL have mutations in the heterodimerization domain that cause ligand-independent ICN cleavage and/or mutations in polypeptide enriched in proline, glutamine, serine, and threonine (PEST) domain that prolong the half-life of ICN (4). Previously, we demonstrated the presence of NOTCH1 and JAGGED1 proteins in AML cells by immunoblotting (5). Among 23 cell lines and patient samples, NOTCH1 or JAGGED1 were expressed in 16 samples. Eight samples expressed both NOTCH1 and JAGGED1, which has the potential to cause autonomous activation of NOTCH. Flow cytometric analysis also showed that NOTCH1 or JAGGED1 were expressed in more than half of the AML patient samples and all AML cell lines examined. NOTCH1 expression on AML cell lines was more intense than on Jurkat, a T-ALL cell line (6).

Next, we searched for *NOTCH1* mutations in 20 AML cell lines and patient samples. We detected a missense mutation in the PEST domain in an AML sample (FAB M4) expressing CD2 and CD4 (7). Wouters *et al*. reported that *NOTCH1* mutations were detected in acute myeloid/T-lymphoid leukaemia cases (8). Our case, which was negative for CD7, did not fit this category. We also reported that *NOTCH1* mutations were not detected in 20 cases of myelodysplastic syndrome (9), which is in agreement with the report by Palomero *et al*., which stated that an activating *NOTCH1* mutation was detected in the heterodimerization domain in only one AML case out of 121 patients with AML or myelodysplastic syndrome (10). Thus, *NOTCH1* mutations are very rare in AML, unlike in T-ALL.

Effects of NOTCH Activation in AML Cells

To examine the function of NOTCH in AML cells, we tested the effects of recombinant NOTCH ligands on the *in vitro* growth of AML cell lines. We established a novel cell line (TMD7) from blast cells of a patient with AML with trilineage myelodysplasia, for which NOTCH ligand stimulation caused a growth response (11). Stimulation with DLL1 and DLL4 bound on culture plates promoted short-term growth, while it suppressed long-term growth. The number of clonogenic cells recovered after suspension culture (CCR) in the presence of the ligands, which reflects the self-renewal capacity of leukaemia stem cells, was also decreased in comparison with CCR without the ligands. We also found that soluble ligands in the culture medium did not affect growth; the ligands must

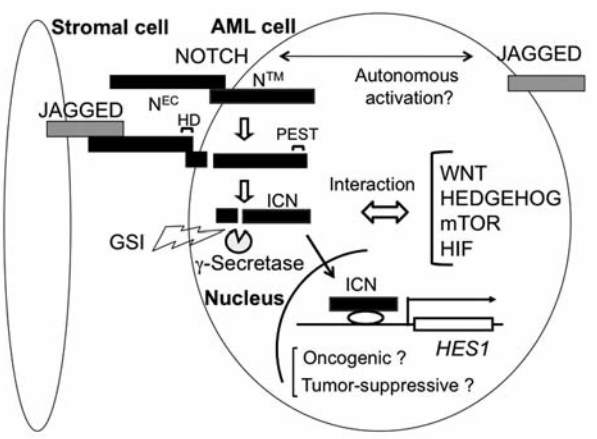

Figure 1. *Schematic representation of NOTCH signaling, as described in this review. N^{EC}, NOTCH extracellular subunit; N^{TM}, NOTCH transmembrane subunit; HD, heterodimerization domain; PEST, polypeptide enriched in proline, glutamine, serine, and threonine; ICN, intracellular fragment of NOTCH; GSI, γ-secretase inhibitor; WNT, wingless-type mouse mammary tumor virus integration site family; mTOR, mechanistic target of rapamycin; HIF, hypoxia-inducible factor; HES1, hairy and enhancer of split 1.*

be immobilized on culture plates to affect the cells (12). For a monoblastic leukaemia cell line (THP-1), JAGGED1, DLL1, and DLL4 suppressed both short- and long-term growth and induced differentiation into macrophage-like cells (13).

Next, we examined the effects on the *in vitro* growth of cells obtained from 12 AML patient samples. Ligand stimulation caused three types of short-term growth responses: growth promotion, growth suppression, or no significant effect. Ligand stimulation suppressed CCR and induced differentiation in the cells of some samples; promotion of CCR was not observed in the cells of any of the samples examined (14). Thus, we found that NOTCH activation acts as a tumor suppressor in AML cells. Recently, two studies supported this view by demonstrating that induction of constitutively active NOTCH in AML cells inhibited growth (15, 16). In contrast, Kode *et al*. reported that activating mutations of β-catenin in osteoblasts stimulates JAGGED1 expression, which subsequently activates NOTCH in haematopoietic stem cells and causes AML (17). This indicates that NOTCH activation plays oncogenic roles in the pathogenesis of AML.

Acute promyelocytic leukaemia (APL) is a sub-type of AML characterized by expression of the promyelocytic leukemia (*PML*)-retinoic acid receptor alpha (*RARA*) fusion gene. Treatment with all-*trans* retinoic acid (ATRA) differentiates APL cells into neutrophil-like cells and induces apoptosis. We found that treatment with ATRA plus DLL1 differentiated the APL-derived cell line NB4 and primary APL cells into monocyte-like cells and reduced apoptosis compared

with ATRA-alone, although DLL1 stimulation itself did not affect growth (18). Thus, NOTCH signalling affects the direction of differentiation. Recently, Grieselhuber *et al*. reported that primary APL samples overexpress *JAGGED1* mRNA in comparison with other AML subtypes. Induction of PML–RARA expression in a cell line increased JAGGED1 protein levels and cleaved the NOTCH1 fragment. NOTCH inhibition reduced growth of APL cells (19). These findings indicate that NOTCH activation plays oncogenic roles in APL.

In a monocytic leukaemia cell line (U937), we showed that DLL1 stimulation reduced tumor necrosis factor-α-induced apoptosis by decreasing activation of caspases, although DLL1 stimulation itself did not affect growth (20). Thus, NOTCH signalling suppresses the apoptosis induced by stimulation in a subset of myeloid leukaemia cells.

Recently, Adamia *et al*. reported that the majority of AML cases express aberrantly spliced *NOTCH2* mRNA and that the cases expressing splice variant *NOTCH2* showed lower expression of NOTCH target genes such as *HES1*. This suggests that splice variant *NOTCH2* might act as dominant-negative. AML cases expressing splice variant *NOTCH2* had a poor prognosis (21).

Downstream Molecules in AML Cells Stimulated with NOTCH Ligands

As mentioned above, DLL1 stimulation promoted short-term growth of TMD7 cells while it suppressed that of THP-1 cells. To clarify the difference in mechanisms, downstream molecules of NOTCH were examined. When stimulated with DLL1, ICN bands emerged in immunoblots for both cell lines, and *HES1* expression was up-regulated, as determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). In THP-1 cells, DLL1 stimulation down-regulated *MYC* expression, while it up-regulated expression of FBJ murine osteosarcoma viral oncogene homolog (*FOS*), cyclin D1 (*CCND1*), and cyclin-dependent kinase inhibitor-1A (*CDKN1A*). Comparatively, DLL1 stimulation in TMD7 cells down-regulated expression of FOS, while it did not significantly affect *MYC, CCND1*, or *CDKN1A* expression levels (22). Microarray analysis also showed that gene expression profiles accompanying NOTCH activation differ between THP-1 and TMD7 cells (23). Additionally, DLL1 stimulation activated the nuclear factor-kappa B (NF-κB) pathway in THP-1 cells by increasing the expression of certain components of the pathway and inducing phosphorylation of NF-κB inhibitor (IκB), IκB kinase (IKK), and v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA). In contrast, DLL1 stimulation did not affect NF-κB activity in TMD7 cells (23). Although these results cannot clearly account for the differences in growth, it is apparent that the effects of NOTCH activation on the downstream molecules depend on the cell type and cause different cellular responses.

Effects of NOTCH Inhibition in AML Cells

NOTCH activity can be inhibited by γ-secretase inhibitors (GSI) or antibodies to NOTCH receptors or NOTCH ligands (3). NOTCH inhibition is a promising therapy for various types of cancer such as T-ALL in which NOTCH plays oncogenic roles. Although GSI treatment suppresses *in vitro* growth and induces apoptosis of T-ALL cell lines (4, 24), successful clinical trials of GSI for T-ALL have not been reported.

We examined the effects of GSI on the *in vitro* growth of AML cell lines. GSI-XXI (compound E) did not significantly suppress the growth of AML cells, while it slightly promoted the growth of THP-1 cells. GSI-XII (Z-IL-CHO) suppressed the growth of AML cell lines examined; however, this effect might be due to off-target effects of GSI because GSI inhibits various substrates such as cadherin, CD44, and v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (ERBB4) (25). We also found that GSI treatment induces erythroid differentiation of erythroblastic leukaemia cell lines (26). To our knowledge, this is the only report to show differentiation-induction of leukaemia cells by GSI.

To examine the effects of NOTCH inhibition excluding off-target effects, we used small-interfering RNA (siRNA) to knock-down *NOTCH1* and *NOTCH2* expression. Neither *NOTCH1* nor *NOTCH2* knockdown affected the growth of TMD7 and THP-1 cells, while both suppressed the growth of T-ALL cell lines (27). This suggests that AML cells, or at least the two AML cell lines examined, do not require NOTCH signalling for their growth. In these experiments, we found that *NOTCH2* knockdown increased the level of the cleaved NOTCH1 fragment without increasing NOTCH1 expression in TMD7 and THP-1 cells. The mechanism and biological meaning behind this interesting phenomenon remain to be determined.

Interaction Between NOTCH and WNT or Hedgehog Signaling

Haematopoietic stem cells (HSCs) are regulated by various stemness-related signaling pathways such as NOTCH, WNT, and HEDGEHOG. Therefore, we experimentally examined the crosstalk between NOTCH and WNT or HEDGEHOG signalling in AML and T-ALL cells by administering their ligands and inhibitors.

WNT signaling regulates self-renewal of HSCs (28). We examined the effects of recombinant WNT3a protein on the *in vitro* growth of AML and T-ALL cell lines. WNT3a treatment did not affect the short-term growth, while it slightly promoted CCR for some AML and T-ALL cell lines, which suggests the promotion of self-renewal capacity. WNT3a treatment did not affect expression and activity of NOTCH (29). Treatment with a WNT inhibitor, quercetin, suppressed the growth of AML and T-ALL cell lines and reduced NOTCH1 expression in a

T-ALL cell line, DND-41 (30). The combinatorial administration of quercetin and GSI synergistically suppressed the growth of DND-41 cells (31). Treatment with a combination of these inhibitors might overcome the failure of GSI-alone in treating T-ALL.

Hedgehog signalling also regulates HSC self-renewal (32). Treatment with recombinant sonic Hedgehog protein did not affect short-term growth, while it slightly promoted CCR of some AML cell lines, but no effect on NOTCH expression was observed (33). Treatment with a Hedgehog inhibitor, cyclopamine, suppressed the growth of some AML and T-ALL cell lines (30, 31). Cyclopamine treatment reduced NOTCH1 expression in DND-41 cells, and combinatorial administration of cyclopamine and GSI additively suppressed the growth of DND-41 cells (31). Thus, WNT and Hedgehog signalling might be related to the NOTCH pathway.

Interaction Between NOTCH and Mechanistic Target of Rapamycin (mTOR), Hypoxia-inducible Factor (HIF), or Bone Morphogenetic Protein (BMP) Signalling

Abnormal activation of mTOR signalling is also involved in the growth of leukaemia cells (34). NOTCH activation is known to activate mTOR signaling by down-regulating the transcription of phosphatase and tensin homolog (*PTEN*), a suppressor of mTOR signaling, through induction of HES1 expression (35). We, therefore, examined the effects of *NOTCH* knockdown by siRNA on mTOR signaling. *NOTCH1* and *NOTCH2* knockdown reduced the level of mTOR protein in THP-1 cells. Contrastingly, NOTCH activation by stimulation with NOTCH ligands increased the expression of mTOR in THP-1 cells (27). Thus, we identified a novel link from NOTCH to mTOR signalling in THP-1 cells. Moreover, we examined the effects of an mTOR inhibitor (PP242) on NOTCH signaling in AML and T-ALL cell lines. PP242 treatment up-regulated NOTCH1 and cleaved NOTCH1 in Jurkat cells but suppressed expression and cleavage in DND-41 cells (36). Thus, mTOR activity differentially affect NOTCH signaling in a cell-dependent manner.

HIF-mediated signaling also plays roles in maintenance of both HSCs and leukaemia stem cells under hypoxic environments such as bone marrow niches (37). Two patterns of interaction between HIF and NOTCH signaling were previously reported: i) HIF1α binds to cleaved NOTCH1, which results in its stabilization and the activation of NOTCH signalling (38); and ii) HIF1α represses a negative feedback loop of the NOTCH1–HES1 system by inhibiting HES1 binding to the *HES1* promoter, resulting in enhancement of NOTCH signalling (37). We found that treatment with an HIF1 inhibitor, echinomycin, suppressed *in vitro* growth and induced apoptosis of AML and T-ALL cell lines. Interestingly, echinomycin treatment reduced levels of NOTCH1 and cleaved NOTCH1 in Jurkat and NB4 cells (39). Thus, we found a novel interaction wherein HIF1 inhibition suppresses NOTCH1 expression and activation.

BMP4 signalling also regulates HSCs (40). We showed that stimulation with recombinant BMP4 protein had diverse effects on the growth of AML and T-ALL cell lines but did not affect NOTCH activity (41).

Why Is NOTCH Signaling Not Autonomously Activated in AML Cells?

Our *in vitro* experiments showed that half of all AML samples expressed NOTCH protein or NOTCH ligand protein. Cells expressing both NOTCH and NOTCH ligands are assumed to be autonomously activated by their own NOTCH ligands; however, cleaved NOTCH1 was not detected by immunoblot analysis in most AML samples. *HES1* mRNA expression levels in AML cells are much lower than those in T-ALL cells, as measured by qRT-PCR (42). Moreover, *NOTCH* knockdown by siRNA did not affect the growth of AML cells (27). It seems that NOTCH signalling is dispensable for the growth of AML cells, at least *in vitro*, or that any effects must be weak. Potential explanations for lack of autonomous activation are as follows: i) NOTCH ligands expressed on AML cells might be of insufficient quantity or under inappropriate conditions for NOTCH activation; ii) NOTCH and its ligands within the same cells might experience cis-inhibitory interactions (43); iii) there might be defects in NOTCH receptor glycosylation, which is essential for ligand binding (44); and iv) NOTCH-suppressive molecules such as NUMB, F-box and WD repeat domain containing 7 (FBXW7), or some microRNAs might be overexpressed. These possibilities must be examined in future studies.

NOTCH works through a non-canonical pathway in addition to the canonical pathway. The non-canonical signalling pathway antagonizes WNT/β-catenin signalling and is independent of NOTCH ligand-induced cleavage and transcription (45). Although cleaved NOTCH1 and *HES1* mRNA are not observed, it is possible that non-canonical NOTCH signalling plays an important role in AML cells.

Is NOTCH Activation Oncogenic or Tumor-Suppressive in AML Cells?

The next step is to determine the roles of NOTCH signalling in AML cells, especially *in vivo*. As AML cells express considerably high levels of NOTCH, it is probable that NOTCH signalling plays some role, especially in bone marrow, where stromal cells express NOTCH ligands. Whether NOTCH plays an oncogenic or tumour-suppressive role in AML is still controversial, as mentioned above. The diverse views arise, in part, from the varied roles of NOTCH that depend on cell type, condition, and the environment.

Notably, the observed effects can be different due to the conditions used in each experiment, especially when comparing *in vitro* and *in vivo* studies.

Our *in vitro* experiments showed that exogenous stimulation with recombinant NOTCH ligands suppresses the long-term growth of AML cells despite transiently promoting the short-term growth of a subset of AML samples. Because NOTCH ligands are expressed on bone marrow stromal cells, NOTCH in AML cells can be activated *in vivo*. If NOTCH activation suppresses the growth and self-renewal of leukaemia stem cells, cells expressing NOTCH will disappear. So, why do AML cells express NOTCH? Possible explanations for these apparently contradictory observations are as follows: i) although NOTCH activation itself suppresses growth, its effects on other stemness-related signalling might potently stimulate growth and self-renewal *in vivo* where various signalling pathways and cytokines interact, and ii) the *in vitro* effects of NOTCH activation might differ from *in vivo* behaviour because the ligand density on culture plates is different from that expressed on stromal cells. Supporting the latter model, NOTCH ligand density has been reported to affect the cell fate decision (46), and supraphysiological NOTCH signals have deleterious effects on self-renewal of HSC (47).

The *in vivo* experiments showed that induction of active NOTCH expression in AML cells causes growth arrest and apoptosis in mice (15), which demonstrates that NOTCH activation acts as tumor suppressor. However, genetically-forced NOTCH activation does not necessarily correspond to the true effects of NOTCH activation in bone marrow of patients with AML. It is certain that NOTCH works as a tumor suppressor in chronic myelomonocytic leukaemia because inactivating NOTCH pathway mutations are found in cells from patients with such disease (48). However, such inactivating mutations have not been found in AML cells.

Conclusion

The present review presented various findings regarding the roles of NOTCH signaling in leukaemia cells, especially in AML cells. Although NOTCH is expressed in AML cells, the role of NOTCH, including whether it is oncogenic or tumor suppressive, has not been elucidated. The problems and potential reasons for the diverse interpretations of the role of NOTCH signaling were also presented. Evidence strongly suggests that the roles are diverse in cell type- and case-dependent manners. To develop NOTCH-targeting therapies for AML, further understanding of the roles of NOTCH is required.

References

1 Bigas A and Espinosa L: Hematopoietic stem cells: to be or Notch to be. Blood *119*: 3226-3235, 2012.

2 Ntziachristos P, Lim JS, Sage J and Aifantis I: From fly wings to targeted cancer therapies: a centennial for notch signaling. Cancer Cell *25*: 318-334, 2014.

3 Espinoza I and Miele L: Notch inhibitors for cancer treatment. Pharmacol Ther *139*: 95-110, 2013.

4 Weng AP, Ferrando AA, Lee W, Morris IV JP, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT and Aster JC: Activating mutations of *NOTCH1* in human T-cell acute lymphoblastic leukemia. Science *306*: 269-271, 2004.

5 Tohda S and Nara N: Expression of Notch1 and Jagged1 proteins in acute myeloid leukemia cells. Leuk Lymphoma *42*: 467-472, 2001.

6 Kanamori E, Itoh M, Tojo N, Koyama T, Nara N and Tohda S: Flow cytometric analysis of Notch1 and Jagged1 expression in normal blood cells and leukemia cells. Exp Ther Med *4*: 397-400, 2012.

7 Fu L, Kogoshi H, Nara N and Tohda S: *NOTCH1* mutations are rare in acute myeloid leukemia. Leuk Lymphoma *47*: 2400-2403, 2006.

8 Wouters BJ, Jordà MA, Keeshan K, Louwers I, Erpelinck-Verschueren CA, Tielemans D, Langerak AW, He Y, Yashiro-Ohtani Y, Zhang P, Hetherington CJ, Verhaak RG, Valk PJ, Löwenberg B, Tenen DG, Pear WS and Delwel R: Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in *NOTCH1*. Blood *110*: 3706-3714, 2007.

9 Fu L, Nara N and Tohda S: Involvement of Notch signaling in myelodysplastic syndrome. Leuk Res *31*: 1160-1161, 2007.

10 Palomero T, McKenna K, O-Neil J, Galinsky I, Stone R, Suzukawa K, Stiakaki E, Kalmanti M, Fox EA, Caligiuri MA, Aster JC, Look AT and Ferrando AA: Activating mutations in *NOTCH1* in acute myeloid leukemia and lineage switch leukemias. Leukemia *20*: 1963-1966, 2006.

11 Tohda S, Sakano S, Ohsawa M, Murakami N and Nara N: A novel cell line derived from *de novo* acute myeloblastic leukemia with trilineage myelodysplasia which proliferates in response to a NOTCH ligand, Delta-1 protein. Br J Haematol *117*: 373-378, 2002.

12 Tohda S, Murata-Ohsawa M, Sakano S and Nara N: Notch ligands, Delta-1 and Delta-4 suppress the self-renewal capacity and long-term growth of two myeloblastic leukemia cell lines. Int J Oncol *22*: 1073-1079, 2003.

13 Murata-Ohsawa M, Tohda S and Nara N: Cellular analysis of growth suppression induced by the Notch ligands, Delta-1 and Jagged-1 in two myeloid leukemia cell lines. Int J Mol Med *14*: 223-226, 2004.

14 Tohda S, Kogoshi H, Murakami N, Sakano S and Nara N: Diverse effects of the Notch ligands Jagged1 and Delta1 on the growth and differentiation of primary acute myeloblastic leukemia cells. Exp Hematol *33*: 558-563, 2005.

15 Lobry C, Ntziachristos P, Ndiaye-Lobry D, Oh P, Cimmino L, Zhu N, Araldi E, Hu W, Freund J, Abdel-Wahab O, Ibrahim S, Skokos D, Armstrong SA, Levine RL, Park CY and Aifantis I: Notch pathway activation targets AML-initiating cell homeostasis and differentiation. J Exp Med *210*: 301-319, 2013.

16 Kannan S, Sutphin RM, Hall MG, Golfman LS, Fang W, Nolo RM, Akers LJ, Hammitt RA, McMurray JS, Kornblau SM, Melnick AM, Figueroa ME and Zweidler-McKay PA: Notch activation inhibits AML growth and survival: a potential therapeutic approach. J Exp Med *210*: 321-337, 2013.

17 Kode A, Manavalan JS, Mosialou I, Bhagat G, Rathinam CV, Luo N, Khiabanian H, Lee A, Murty VV, Friedman R, Brum A, Park D, Galili N, Mukherjee S, Teruya-Feldstein J, Raza A, Rabadan R, Berman E and Kousteni S: Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts. Nature *506*: 240-244, 2014.

18 Murata-Ohsawa M, Tohda S, Kogoshi H, Sakano S and Nara N: The Notch ligand, Delta-1, alters retinoic acid (RA)-induced neutrophilic differentiation into monocytic and reduces RA-induced apoptosis in NB4 cells. Leuk Res *29*: 197-203, 2005.

19 Grieselhuber NR, Klco JM, Verdoni AM, Lamprecht T, Sarkaria SM, Wartman LD and Ley TJ: Notch signaling in acute promyelocytic leukemia. Leukemia *27*: 1548-1557, 2013.

20 Murata-Ohsawa M, Tohda S, Kogoshi H and Nara N: The Notch ligand, Delta-1, reduces TNF-alpha-induced growth suppression and apoptosis by decreasing activation of caspases in U937 cells. Int J Mol Med *14*: 861-866, 2004.

21 Adamia S, Bar-Natan M, Haibe-Kains B, Pilarski PM, Bach C, Pevzner S, Calimeri T, Avet-Loiseau H, Lode L, Verselis S, Fox EA, Galinsky I, Mathews S, Dagogo-Jack I, Wadleigh M, Steensma DP, Motyckova G, Deangelo DJ, Quackenbush J, Tenen DG,Stone RM and Griffin JD: NOTCH2 and FLT3 gene mis-splicings are common events in patients with acute myeloid leukemia (AML): new potential targets in AML. Blood *123*: 2816-2825, 2014.

22 Fu L, Katsube K and Tohda S: Transition of cleaved Notch1 and gene expression changes in myeloblastic leukemia cells stimulated with notch ligands. Anticancer Res *29*: 3967-3970, 2009.

23 Itoh M, Fu L and Tohda S: NF-κB activation induced by Notch ligand stimulation in acute myeloid leukemia cells. Oncol Rep *22*: 631-634, 2009.

24 Okuhashi Y, Nara N and Tohda S: Effects of γ-secretase inhibitors on the growth of leukemia cells. Anticancer Res *30*: 495-498, 2010.

25 Kopan R and Ilagan MXG: γ-Secretase: proteasome of the membrane? Nat Rev Mol Cell Biol *5*: 499-504, 2004.

26 Okuhashi Y, Itoh M, Arai A, Nara N and Tohda S: γ-Secretase inhibitors induce erythroid differentiation in erythroid leukemia cell lines. Anticancer Res *30*: 4071-4074, 2010.

27 Okuhashi Y, Itoh M, Nara N and Tohda S: *NOTCH* knockdown affects the proliferation and mTOR signaling of leukemia cells. Anticancer Res *33*: 4293-4298, 2013.

28 Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R and Weissman IL: A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature *423*: 409-414, 2003.

29 Kawaguchi-Ihara N, Murohashi I, Nara N and Tohda S: Promotion of the self-renewal capacity of human acute leukemia cells by WNT3A. Anticancer Res *28*: 2701-2704, 2008.

30 Kawahara T, Kawaguchi-Ihara N, Okuhashi Y, Itoh M, Nara N and Tohda S: Cyclopamine and quercetin suppress the growth of leukemia and lymphoma cells. Anticancer Res *29*: 4629-4632, 2009.

31 Okuhashi Y, Itoh M, Nara N and Tohda S: Effects of combination of notch inhibitor plus hedgehog inhibitor or Wnt inhibitor on growth of leukemia cells. Anticancer Res *31*: 893-896, 2011.

32 Bhardwaj G, Murdoch B, Wu D, Baker DP, Williams KP, Chadwick K, Ling LE, Karanu FN and Bhatia M: Sonic hedgehog induces the proliferation of primitive human hematopoietic cells *via* BMP regulation. Nat Immunol *2*: 172-180, 2001.

33 Kawaguchi-Ihara N, Okuhashi Y, Itoh M, Murohashi I, Nara N and Tohda S: Promotion of the self-renewal capacity of human leukemia cells by sonic hedgehog protein. Anticancer Res *31*: 781-784, 2011.

34 Chapuis N, Tamburini J, Green AS, Willems L, Bardet V, Park S, Lacombe C, Mayeux P and Bouscary D: Perspectives on inhibiting mTOR as a future treatment strategy for hematological malignancies. Leukemia *24*: 1686-1699, 2010.

35 Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, Caparros E,Buteau J, Brown K, Perkins SL, Bhagat G, Agarwal AM, Basso G, Castillo M, Nagase S, Cordon-Cardo C, Parsons R, Zúñiga-Pflücker JC, Dominguez M and Ferrando AA: Mutational loss of *PTEN* induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med *13*: 1203-1210, 2007.

36 Ono A, Oike R, Okuhashi Y, Takahashi Y, Itoh M, Nara N and Tohda S: Comparative effects of PP242 and rapamycin on mTOR signalling and NOTCH signalling in leukemia cells. Anticancer Res *33*: 809-813, 2013.

37 Wang Y, Liu Y, Malek SN, Zheng P and Liu Y: Targeting HIF1α eliminates cancer stem cells in hematological malignancies. Cell Stem Cell *8*: 399-411, 2011.

38 Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, Ruas JL, Poellinger L, Lendahl U and Bondesson M: Hypoxia requires notch signaling to maintain the undifferentiated cell state. Dev Cell *9*: 617-628, 2005.

39 Yonekura S, Itoh M, Okuhashi Y, Takahashi Y, Ono A, Nara N and Tohda S: Effects of the HIF1 inhibitor, echinomycin, on growth and NOTCH signalling in leukaemia cells. Anticancer Res *33*: 3099-3103, 2013.

40 Goldman DC, Bailey AS, Pfaffle DL, Al Masri A, Christian JL and Fleming WH: BMP4 regulates the hematopoietic stem cell niche. Blood *114*: 4393-4401, 2009.

41 Takahashi Y, Ishigaki T, Okuhashi Y, Ono A, Itoh M, Nara N and Tohda S: Effect of BMP4 on the growth and clonogenicity of human leukemia and lymphoma cells. Anticancer Res *32*: 2813-2817, 2012.

42 Kogoshi H, Sato T, Koyama T, Nara N and Tohda S: γ-Secretase inhibitors suppress the growth of leukemia and lymphoma cells. Oncol Rep *18*: 77-80, 2007.

43 del Álamo D, Rouault H and Schweisguth F: Mechanism and significance of cis-inhibition in Notch signalling. Curr Biol *21*: R40-47, 2011.

44 Takeuchi H and Haltiwanger RS: Role of glycosylation of Notch in development. Semin Cell Dev Biol 21: 638-645, 2010.

45 Andersen P, Uosaki H, Shenje LT and Kwon C: Non-canonical Notch signaling: emerging role and mechanism. Trends Cell Biol *22*: 257-265, 2012.

46 Dallas MH, Varnum-Finney B, Delaney C, Kato K and Bernstein ID: Density of the Notch ligand Delta1 determines generation of B- and T-cell precursors from hematopoietic stem cells. J Exp Med *201*: 1361-1366, 2005.

47 Chiang MY, Shestova O, Xu L, Aster JC and Pear WS: Divergent effects of supraphysiologic Notch signals on leukemia stem cells and hematopoietic stem cells. Blood *121*: 905-917, 2013.

48 Klinakis A, Lobry C, Abdel-Wahab O, Oh P, Haeno H, Buonamici S, van De Walle I, Cathelin S, Trimarchi T, Araldi E, Liu C, Ibrahim S, Beran M, Zavadil J, Efstratiadis A, Taghon T, Michor F, Levine RL and Aifantis I: A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. Nature *473*: 230-233, 2011.

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