NOTCH activation promotes glycosyltransferase expression in human myeloid leukemia cells

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Abstract

NOTCH signaling diversely regulates the growth of acute myeloid leukemia (AML) cells. It is known that glycosylation of NOTCH receptors modulates NOTCH activation. However, little is known about glycosylation of NOTCH in AML cells. We examined the effects of ligand-induced NOTCH activation on the expression of NOTCH-modifying glycosyltransferases in two AML cell lines, THP-1 and TMD7. The cells were stimulated with recombinant NOTCH ligands JAGGED1 and DELTA1, and subjected to immunoblot analysis to evaluate the expression levels of glycosyltransferases. Ligand stimulation promoted the expression of POFUT1, LFNG, MFNG, RFNG, GXYLT1, GXYLT2, and XXYLT1 in THP-1 cells, and that of RFNG and GXYLT1 in TMD7 cells. We found that NOTCH activation promoted the expression of several glycosyltransferases in AML cells. This suggests that NOTCH activation modulates its sensitivity to NOTCH ligands by increased glycosylation of NOTCH receptors in AML cells. Further investigation is needed to elucidate its biological significance.

Introduction

NOTCH signaling plays crucial roles in the regulation of hematopoietic stem cells, and *NOTCH* mutations give rise to cancers such as leukemia.[1] Indeed, *NOTCH1*-activating mutations are detected in more than half of the acute T-lymphoblastic leukemia (T-ALL) patients.[2]

To date, the role of NOTCH in acute myeloid leukemia (AML) has yet to be clarified.[1,3] We previously reported that NOTCH activation by stimulation with NOTCH ligands JAGGED1 and DELTA1 changed the growth of primary AML cells and AML cell lines such as TMD7 and THP-1.[3-8] In brief, DELTA1 stimulation promoted the short-term growth of TMD7 cells while it suppressed the long-term growth.[5] JAGGED1 stimulation did not significantly affected the growth.[5] The growth of THP-1 cells was suppressed by stimulation with these ligands.[6] NOTCH signaling is activated upon binding of the NOTCH extracellular domain (NECD) to JAGGED and/or DELTA ligands on adjacent cells. The addition of *O*-fucose moieties to epidermal growth factor-like (EGF) repeats within the NECD (Figure 1) by *O*-fucosyltransferase-1 (POFUT1) is essential for NOTCH activation. Subsequently, Fringe (FNG) glycosyltransferases mediate the addition of N-acetylglucosamine (GlcNAc) to these *O*-fucose moieties. Mammals encode three distinct Fringe homologues: lunatic Fringe (LFNG), manic Fringe (MFNG), and radical Fringe (RFNG). While this addition of GlcNAc was reported to promote NOTCH–DELTA binding and reduces NOTCH–JAGGED binding,[9-11] the precise roles of GlcNAc have yet to be determined.[12,13]

The addition of *O*-glucose moieties to the EGF repeats by *O*-glucosyltransferase (POGLUT1) is also essential for NOTCH activation. Conversely, the addition of two xylose residues to these *O*-glucose molecules by glucoside α1–3 xylosyltransferases 1 and 2 (GXYLT1 /2) and xyloside α1-3 xylosyltransferase 1 (XXYLT1) was reported to inhibit NOTCH activity in *Drosophila*.[14] The NOTCH-modifying glycosyltransferase enzymes exhibit aberrant expression in various cancers.[13] However, little is known about glycosylation of NOTCH in AML cells. To elucidate the relationship between NOTCH and its glycosylation, we examined the effects of ligand-mediated NOTCH activation on the expression of glycosyltransferases in AML cell lines. Given the technical difficulties associated with measuring glycosylation levels of NOTCH protein, we chose to evaluate glycosyltransferase mRNA and protein expression instead.

Materials and Methods

Cell lines

Two AML cell lines, THP-1 and TMD7 were used. THP-1 monocytic leukemia cell line was obtained from the Health Science Research Resources Bank (Osaka, Japan). TMD7 cell line was established from blast cells of a patient with AML with myelodysplasia-related changes in our laboratory.[4]

NOTCH ligand stimulation

Recombinant human JAGGED1 and DELTA1 were synthesized as extracellular domains fused to the human immunoglobulin gamma-1 heavy chain, constant region (IgG1-Fc) by Dr. S. Sakano (Asahi Kasei Corporation, Fuji, Japan).[4] The NOTCH ligands (5 μg/well) were immobilized in 24-well culture plates. Meanwhile, immobilized human IgG-Fc (5 μg/well: Athens Research & Technology, Athens, GA, USA) was used as a control for the influence of IgG. Cells were cultured in ligand-coated wells using MEM Alpha (GIBCO Invitrogen, Waltham, MA, USA) supplemented with 10% fetal bovine serum. After 24 h, cells were harvested for protein and total RNA extraction.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

RNA samples were reverse-transcribed into first-strand cDNA. Quantitative PCR was performed using a Faster DNA Master SYBR Green I kit, QuantiTect primers (Qiagen, Venlo, Netherlands) for *POFUT1, LFNG, MFNG, RFNG, POGLUT1, GXYLT1, GXYLT2, and XXYLT1*, and LightCycler primer sets (Roche Diagnostics, Basel, Switzerland) for *NOTCH1* and β-actin (*ACTB*). The expression level of each gene was normalized to that of *ACTB*, which was measured simultaneously. Each experiment was repeated at least three times to ensure reproducibility.

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Immunoblot analysis

Cell lysates were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. Membranes were then blocked and probed with primary antibodies specific to POFUT1, LFNG, MFNG, RFNG, POGLUT1, GXYLT1, GXYLT2 (Abcam, Cambridge, MA, USA), and XXYLT1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Antibodies specific to α-tubulin (Abcam) were used a loading control.

Results

Effects of ligand stimulation on mRNA expression of glycosyltransferase genes

We examined the effects of stimulation with JAGGED1 and DELTA1 on the mRNA expression levels of the glycosyltransferases. Stimulation with both ligands up-regulated expression of *RFNG* and *LFNG* in THP-1 cells while the stimulation had no significant effect on the mRNA expression levels of the glycosyltransferase genes in TMD7 cells (Figure 2).

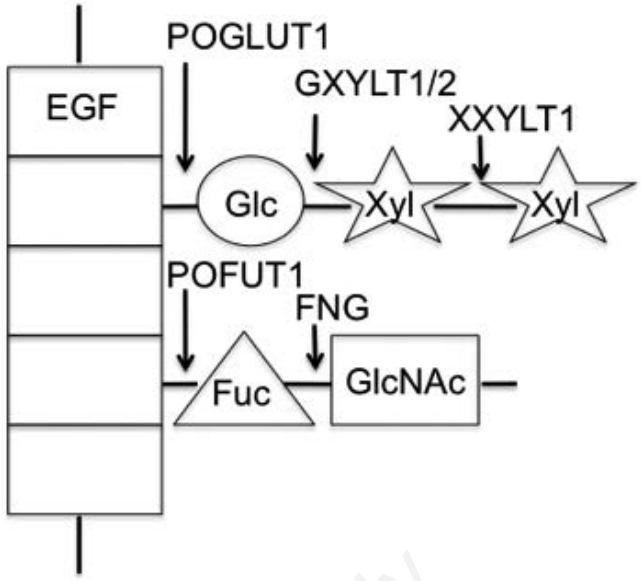

Figure 1. Schematic representation of NOTCH receptor glycosylation. Graphic depiction of the NOTCH extracellular domain, which encodes tandem EGF-like repeats containing the consensus sequences for modification with *O*-Fuc and *O*-Glc by POFUT1 and POGLUT1, respectively. Subsequently, GlcNAc is added to Fuc by FNG, while two Xyl are added to Glc by GXYLT1 /2 and XXYLT1.

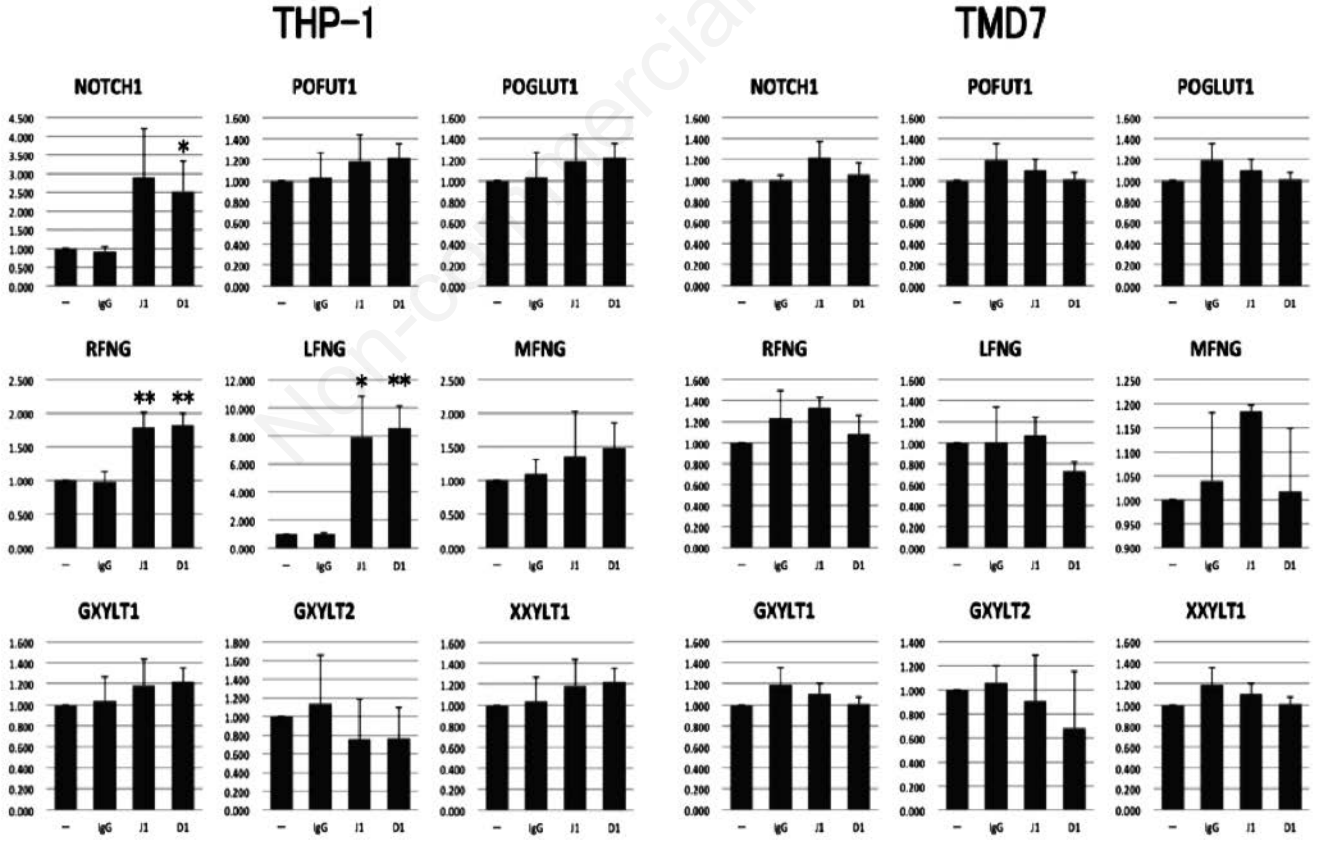

Figure 2. Effects of NOTCH ligand stimulation on the mRNA expression levels of glycosyltransferase genes in THP-1 and TMD7 cells. Cells were cultured in the absence (control marked with -) or presence of human IgG (IgG), JAGGED1 (J1), or DELTA1 (D1). After 24 h, RNA was extracted from the cells and subjected to qRT-PCR analysis. Expression levels were normalized to that of the β-actin gene. Numbers indicate the mean fold changes relative to each control from three independent experiments. *P<0.05, **P<0.01, compared to the IgG.

Effects of ligand stimulation on glycosyltransferase expression

Stimulation with DELTA1 and JAGGED1 resulted in enhanced protein expression of POFUT1, LFNG, MFNG, RFNG, GXYLT1, GXYLT2, and XXYLT1 in THP-1 cells, and that of RFNG and GXYLT1 in TMD7 cells. The enhancing effects of DELTA1 were more potent than those of JAGGED1 in both cell lines (Figure 3).

Discussion and Conclusions

In this study, we found the novel relationship between NOTCH and glycosyltransferase expression in AML cells. Thus far, it was reported that primary AML cells overexpressed POGLUT1,[15] and that RNAi-mediated knockdown of POGLUT1 expression suppressed NOTCH activation and cell proliferation in U937 myeloid leukemia cells.[16]

In our preliminary experiments, we observed expression of the NOTCH-modifying glycosyltransferases POFUT1, LFNG, MFNG, RFNG, POGLUT1, GXYLT1, GXYLT2, and XXYLT1 in seven AML (THP-1, TMD7, and HEL), T-ALL (Jurkat and KOPT-K1), B-lymphoblastic leukemia (NALM-6), and B-lymphoma (TMD8) cell lines (data not shown). There was no remarkable tendency in the levels of expression of these proteins among myeloid cells, T-cells, and B-cells. Because growth of only THP-1 and TMD7 cells were affected by NOTCH ligand stimulation, we used these cell lines in the current study.

As shown here, NOTCH activation can promote protein (and to a lesser extent mRNA) expression of several glycosyltransferases. However, increases in mRNA expression did not necessarily coincide with increases in protein expression. This discrepancy may have been due to the fact that, because mRNA expression levels were only evaluated at 24 hours post-stimulation, precise changes in mRNA may have been missed. Alternatively, the observed increases in protein expression might have been due to increased levels of translation or prolonged protein half-lives. In any case, the mechanisms governing these differences in expression require further clarification.

It was previously shown that NOTCH glycosylation by Fringes promotes DELTA-induced NOTCH activation.[9,10] Inversely, here, we demonstrated that NOTCH activation promotes Fringe expression. These data suggest that NOTCH activation enhances NOTCH activity in a positive feedback manner by promoting the glycosylation of NOTCH receptors. However, this model does not explain the role of JAGGED-induced NOTCH activation. Meanwhile, contrary to the effects of Fringes, GXYLT was reported to negatively regulate NOTCH activation in *Drosophila*.[14] In our study, however, NOTCH activation promoted GXYLT1 expression. The significance of these contradictory phenomena requires further analysis.

In the present study, the response to the ligand stimulation was different between THP-1 and TMD7 cells. The difference can be related to the difference in the changes of gene expression following the ligand stimulation in these two cell lines as shown in our previous paper.[8] We reported that changes of the expression levels of various genes are different between THP-1 and TMD7 cells and that expression of some genes such as *FOS*, *TP53*, and *CDKN2C* changed in opposite directions. This suggests the diversity of the effects of NOTCH signaling in a cell context-dependent manner.

To the best of our knowledge, this is the first report to demonstrate that NOTCH activation promotes the expression of glycosyltransferases in leukemia cells. In the future, clarification of this mechanism and drug-induced modulation of NOTCH glycosylation might lead to novel molecular-targeted therapies for leukemias.

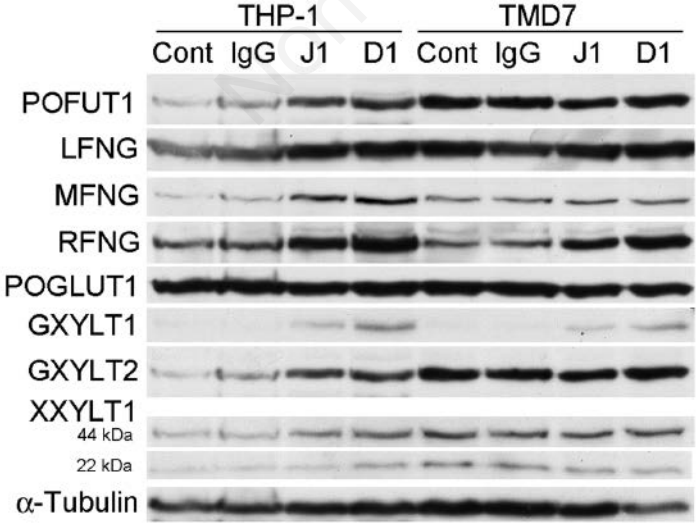

Figure 3. Effects of NOTCH ligand stimulation on glycosyltransferase expression in THP-1 and TMD7 cells. Cells were cultured in the absence (Cont) or presence of human IgG, JAGGED1 (J1), or DELTA1 (D1). After 24 h, cells were harvested, and lysates were subjected to immunoblot analysis using the indicated antibodies.

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