INTRODUCTION

Natural killer (NK) cells are innate lymphoid cells, which act on the early defenses against tumors and virus-infected cells. To induce target cell death, they elicit cytotoxic protein release following calcium-dependent granule exocytosis and produce TNF-α, FasL, and TRAIL. Immature NK cells likely use TRAIL-dependent cytotoxicity rather than FasL- or granule release-dependent cytotoxicity, whereas mature NK cells mainly use the latter two. During the immune response, the expanded activated NK cells come to express c-FLIPL to the death-inducing signaling complex leads to Fas resistance in natural killer-cell lymphoma

Azuchi Masuda, Yasushi Isobe, Koichi Sugimoto, Mayumi Yoshimori, Ayako Arai, Norio Komatsu

Abstract

Activation-induced cell death (AICD) mediated by the Fas/Fas ligand (FasL) system plays a key role in regulating immune response. Although normal natural killer (NK) cells use this system for their homeostasis, malignant NK cells seem to disrupt the process. Extranodal NK/T-cell lymphoma, nasal type (ENKL) is a rare but fatal disease, for which novel therapeutic targets need to be identified. We confirmed that ENKL-derived NK cell lines NK-YS and Hank1, and primary lymphoma cells expressed procaspase-8/FADD-like interleukin-1β-converting enzyme (FLICE) modulator and cellular FLICE-inhibitory protein (c-FLIP), along with Fas and FasL. Compared with Fas-sensitive Jurkat cells, NK-YS and Hank1 showed resistance to Fas-mediated apoptosis in spite of the same expression levels of c-FLIP and the death-inducing signaling complex (DISC) formation. Unexpectedly, the long isoform of c-FLIP (c-FLIPL) was coimmunoprecipitated with Fas predominantly in both ENKL-derived NK cell lines after Fas ligation. Indeed, c-FLIPL was more sufficiently recruited to the DISC in both ENKL-derived NK cell lines than in Jurkat cells after Fas ligation. Knockdown of c-FLIPL per se enhanced autonomous cell death and restored the sensitivity to Fas in both NK-YS and Hank1 cells. Although ENKL cells are primed for AICD, they constitutively express and efficiently utilize c-FLIPL, which prevents their Fas-mediated apoptosis. Our results show that c-FLIPL could be a promising therapeutic target against ENKL.

KEYWORDS
activation-induced cell death, c-FLIPL, extranodal NK/T-cell lymphoma, nasal type, Fas, FasL
Fas and eventually undergo AICD mediated by their own secretion of FasL.\(^4,5\) The AICD signaling involves clustering of Fas on the cell surface and recruitment of the FADD to the trimerized intracellular death domain of Fas.\(^6\) In turn, FADD recruits procaspase-8/FLICE, leading to the formation of the DISC.\(^6\) Once the DISC is completely formed, procaspase-8/FLICE is activated by its autolytic cleavage and forms caspase-8, which initiates the apoptotic signaling pathway.\(^6\)

Extranodal NK/T-cell lymphoma, nasal type (ENKL) is rare in North America and Europe but frequently develops in East Asia.\(^7\) Most ENKL cells are derived from EBV-infected NK cells.\(^7\) Although l-asparaginase-containing chemotherapy has significantly improved the clinical outcome, such therapeutic regimens still fail in approximately 30% of cases, resulting in a fatal course.\(^8\) Therefore, novel therapeutic targets are needed for ENKL. Lymphoma cells abundantly express FasL, which could induce surrounding tissue necrosis and vascular damage.\(^9\) Although they can also express Fas,\(^9,10\) the alteration of the Fas/FasL system in ENKL remains to be fully elucidated.

Cellular FLICE-inhibitory protein (c-FLIP) is known to be a crucial modulator of procaspase-8/FLICE.\(^11\) In particular, the long isoform of c-FLIP (c-FLIP\(_{L}\)) is structurally similar to procaspase-8/FLICE, has the potency to interfere with caspase-8 activation and restores sensitivity to Fas-mediated apoptosis in several cancer cells.\(^12-15\) In ENKL-derived cell lines, cycloheximide treatment was shown to abolish expression of c-FLIP\(_{L}\) and to increase sensitivity to several apoptotic stimuli.\(^16\) However, cycloheximide reduces expression not only of c-FLIP\(_{L}\) but also of the short isoform (c-FLIP\(_{S}\)) and RIP kinases, which are involved in caspase-8-mediated pathways.\(^16-18\) In addition, the protein synthesis inhibitor also induces cell death in a Fas-independent manner.\(^19\) Although c-FLIP\(_{L}\) might function in ENKL, its direct evidence for Fas resistance has not yet been provided. Hence, we investigated the biological behavior of c-FLIP\(_{L}\) in ENKL cells.

## 2 | MATERIALS AND METHODS

### 2.1 | Antibodies

We used the following primary Abs: anti-Fas mouse (Ms) IgM 7C11 (Beckman Coulter) or CH-11 (MBL); anti-Fas Ms IgG1 ZB4 (Beckman Coulter); anti-Fas Ms IgG1 UB2 (Beckman Coulter); anti-Fas Ms IgG1 G-9 (Santa Cruz Biotechnology); anti-Fas rabbit Ab C-20 (Santa Cruz Biotechnology); anti-FasL Ms IgG1 NOK-1 (BD Biosciences); anti-FasL rabbit Ab Q-20 (Santa Cruz Biotechnology); anti-FADD Ms IgG1 FD19 (Santa Cruz Biotechnology); anti-FADD rabbit Ab (Cell Signaling Technology); anti-caspase-8 Ms IgG1 1C12 (Cell Signaling Technology); anti-caspase-8 rabbit IgG (GeneTex); anti-c-FLIP\(_{L}\) Ms IgG3 5D8 (Santa Cruz Biotechnology); anti-c-FLIP\(_{S}\) rabbit Ab (Sigma-Aldrich); anti-c-FLIP rabbit IgG DSJ1E (Cell Signaling Technology); anti-c-FLIP Ms IgG1 NF6 (AdipoGen); anti-RIP Ms IgG2a 3B (BD Biosciences); anti-BCL2 Ms IgG1 C-2 (Santa Cruz Biotechnology); anti-BCL-xL rabbit IgG E18 (Abcam); anti-MCL1 rabbit IgG Y37 (Abcam); anti-caspase-3 Ms IgG1 31A1067 (Santa Cruz Biotechnology); anti-cleaved PARP1 Ms IgG2b 194C1439 (Santa Cruz Biotechnology); and anti-β-actin Ms IgG2a AC74 (Sigma-Aldrich).

### 2.2 | Cell lines

Two EBV-positive ENKL-derived NK cell lines, NK-YS and Hank1, were kindly provided by Dr Junjiro Tsuchiyama (Kawasaki Medical School, Kurashiki, Japan) and Dr Masao Seto (Kurume University, Kurume, Japan), respectively. Human T-cell leukemia line Jurkat purchased from ATCC was used as a positive control for Fas-mediated apoptosis, because this cell line efficiently undergoes apoptosis by Fas ligation with agonistic Ab 7C11 (Beckman Coulter) and CH-11 (MBL). These cell lines were maintained as described previously.\(^19,20\)

### 2.3 | Detection of Fas and FasL by FCM

We used FCM to detect Fas and FasL in each cell line. To confirm cell surface expression of Fas, 1 × 10\(^6\) cells were stained with FITC-conjugated UB2 (Beckman Coulter) for 30 minutes after blocking of Fcγ receptor on NK cell lines with human IgG (Sigma-Aldrich). Intracytoplasmic expression of FasL was evaluated with NOK-1 (BD Bioscience) and FITC-conjugated goat anti-Ms IgG (Beckman Coulter) using the PFA/saponin procedure as described previously.\(^21\)

### 2.4 | Cytokine assays

We further measured cytokine concentration in culture supernatants of Jurkat and Hank1 (3 × 10\(^6\) cells/mL) in a time course (3, 6, and 12 hours) because the doubling time for each cell line was estimated to be 26 to 28 hours. Detection of FasL, TRAIL, and TNF-α was carried out using Quantikine ELISA Kit (R&D Systems) according to the manufacturer’s instructions.

### 2.5 | Immunohistochemistry for Fas, FasL, and c-FLIP on clinical samples

To evaluate the expression of Fas, FasL, and c-FLIP, immunohistochemistry was carried out on formalin-fixed, paraffin-embedded specimens from ENKL patients. After deparaffinization and rehydration, endogenous peroxidase was blocked with hydrogen peroxide (DAKO Japan). Heat-induced antigen retrieval was carried out using 0.05% citraconic anhydride, pH 7.4 (Nissin EM Co., Ltd.). Primary Abs were C-20 (Santa Cruz Biotechnology) for Fas, Q-20 (Santa Cruz Biotechnology) for FasL, and NF6 (Adipogen) for c-FLIP. The Ab signals were enhanced using EnVision (DAKO Japan). Definition of positivity was made when more than 30% of the tumor cells were stained in the CD56-positive
area. The results were determined by outsourcing pathology services (Kotobiken Medical Laboratories). All specimens were obtained at the biopsy undertaken for initial diagnosis of ENKL, according to the WHO classification 2017\(^7\) at Juntendo University Hospital. This study protocol was approved by the Ethics Review Board of Juntendo University.

### 2.6 Western blot analysis

Cells were dissolved on ice in 250 \(\mu\)L ice-cold lysis buffer (Celllytic M; Sigma-Aldrich) containing protease inhibitor (Sigma-Aldrich) as described previously.\(^{19,21}\) Equal amounts of protein (50 \(\mu\)g/well) were separated on a discontinuous SDS-10% polyacrylamide gel and blotted onto a nitrocellulose membrane (Bio-Rad). Western blot analysis was carried out using the following primary Abs: G-9 (Santa Cruz Biotechnology) for Fas; FD19 (Santa Cruz Biotechnology) and anti-FADD rabbit Ab (Cell Signaling Technology) for FADD; 1C12 (Cell Signaling Technology) and anti-caspase-8 rabbit Ab (GeneTex) for procaspase-8/FLICE and caspase-8; 5D8 (Santa Cruz Biotechnology) for c-FLIPL; anti-c-FLIP\(\gamma\) rabbit Ab (Sigma-Aldrich) for c-FLIP\(\gamma\); D5J1E (Cell Signaling Technology) for both forms and the cleaved c-FLIP\(\gamma\); 38 (BD Bioscience) for RIP1; C-2 (Santa Cruz Biotechnology) for BCL2; E18 (Abcam) for BCL-xL; Y37 (Abcam) for MCL1; 31A1067 (Santa Cruz Biotechnology) for procaspase-3 and the cleaved forms; 194C1439 (Santa Cruz Biotechnology) for cleaved PARP1; and AC74 (Sigma-Aldrich) for \(\beta\)-actin. Antibody signals were visualized using Western Blue (Promega). We quantified the expression levels by comparison with each \(\beta\)-actin level using ImageJ version 1.52k (http://imagej.nih.gov/ij/).

### 2.7 Induction of Fas-mediated apoptosis

Each cell line was washed twice with PBS and incubated (5 \(\times\) 10\(^5\) cells/mL) with 2.0 \(\mu\)g/mL Fas agonistic 7C11 (Beckman Coulter) or CH-11 (MBL). Fas antagonistic ZB4 (Beckman Coulter), or control Ms IgM (Beckman Coulter) for 1 hour at 37°C in fresh medium. Cell viability was assessed using the MTT assay (Promega). The viability was calculated as the percentage absorbance of formazan products, that is, (OD 570 CH-11 treated / OD 570 control Ms IgM treated) \(\times\) 100%. We also evaluated the cell death process by FITC-conjugated annexin V-binding and 7-AAD rejection assays (Beckman Coulter). Apoptotic cell change was assessed on a Cell Lab Quanta SC flow cytometer (Beckman Coulter). During our experimental period, 7C11 (Beckman Coulter) became unavailable due to discontinuation. Therefore, the assessment of cleavage of procaspase-3 and PARP1 by western blot analyses was undertaken after Fas ligation with CH-11 (MBL).

### 2.8 Analysis of DISC formation

Briefly, cells were incubated with 2.0 \(\mu\)g/mL 7C11 (Beckman Coulter) or control Ms IgM (Beckman Coulter) for 10 minutes at 37°C. They were washed twice, harvested, and subjected to the analysis. Whole-cell lysate (200 \(\mu\)g total protein) was added to 1.0 \(\mu\)g C-20 (Santa Cruz Biotechnology) or 1C12 (Cell Signaling Technology) and adjusted to 150 \(\mu\)L of volume with lysis buffer (Sigma-Aldrich). Rabbit or mouse serum-saturated protein G-Sepharose beads (20 \(\mu\)L) (GE Healthcare) were added to each lysate, and immunoprecipitation was carried out for 1 hour while rotating at 4°C. After incubation, the beads were washed three times with ice-cold lysis buffer and boiled for 3 minutes with 20 \(\mu\)L loading buffer. The supernatant was electrophoresed and analyzed by western blot analysis.

Confocal fluorescence imaging was also undertaken. After incubation with 7C11 (Beckman Coulter) or control Ms IgM (Beckman Coulter), NK-YS, Hank1, and Jurkat cells were fixed and permeabilized using the PFA/saponin procedure and stained with primary Abs for 1 hour at room temperature. The primary Abs were as follows: C-20 (Santa Cruz Biotechnology) for Fas; 1C12 (Cell Signaling) for procaspase-8/FLICE and caspase-8; and 5D8 (Santa Cruz Biotechnology) for c-FLIP\(_L\). After staining with primary Abs, cells were washed twice with PBS containing 0.03% saponin and 2% goat serum and further incubated with Alexa 594-conjugated goat anti-rabbit Ab (SouthernBiotech) and Alexa 488-conjugated goat anti-Ms Ab (SouthernBiotech). After washing with the saponin-containing PBS, nuclei were stained DAPI (Invitrogen). Confocal microscopic analysis with Cytospin preparations was undertaken on a Leica confocal scanning microscope (Leica Microsystems).

### 2.9 RNA interference for c-FLIP\(_L\)

The siRNA duplexes against c-FLIP\(_L\) (Silencer Select s444339) and control scrambled siRNA (Silencer s4390843) were synthesized by Applied Biosystems. Transfection of siRNA was carried out using Nucleofector I (Lonza) as described previously.\(^{19}\) NK-YS and Hank1 cells (1 \(\times\) 10\(^5\) cells) were resuspended in 100 \(\mu\)L Solution R and T with 200 nmol/L each siRNA, respectively. After 24 hours, each treated cell line (2 \(\times\) 10\(^5\) cells) was subjected to western blot analysis and apoptosis assay, respectively.

### 2.10 Statistical analysis

The appearance of fusion signals between Fas and c-FLIP\(_L\) was counted in each 100 cells on confocal microscopy. Differences among Jurkat, NK-YS, and Hank1 cells were statistically evaluated. Moreover, the proportion of annexin V-positive cells was compared after knockdown of c-FLIP\(_L\) in NK-YS and Hank1 cells. These data were obtained from 3 separate experiments. Differences in the mean values were determined using Student’s t test using SPSS Statistics software (IBM Japan). All P values were 2-sided, and values <.05 were considered to be significant.
FIGURE 1 Extracranial natural killer (NK)/T-cell lymphoma, nasal type (ENKL) expresses cellular Fas-associated death domain-containing protein (FADD)-like interleukin-1β-converting enzyme (FLICE)-inhibitory protein (c-FLIP) along with Fas and Fas ligand (FasL). A, Flow cytometry showing that ENKL-derived NK cell lines, NK-YS and Hank1, clearly expressed cell surface Fas and intracytoplasmic FasL. B, FasL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), and TNF-α levels in culture supernatants of Hank1 and Jurkat. Each cytokine concentration was measured three times and the mean value was represented in the time-course graph. Hank1 secretes FasL and abundant TNF-α. C, Western blot analysis detected Fas, FADD, procaspase-8/FLICE, and long and short forms of c-FLIP (c-FLIPL and c-FLIPS, respectively) at approximately the same levels in NK-YS, Hank1, and Jurkat cells. D, Immunohistochemistry for Fas, FasL, and c-FLIP was carried out using diagnostic specimens of 9 cases of ENKL. Simultaneous expression of Fas, FasL, and c-FLIP was observed in 7 of 9 examined cases (78%). Two representative cases (UPN1 and UPN2) are presented.
3 | RESULTS

3.1 | ENKL cells express c-FLIP along with Fas and FasL

Flow cytometry confirmed that NK-YS and Hank1 cells coexpressed Fas and FasL (Figure 1A). We also detected secreted FasL but not TRAIL in supernatant of Hank1 cell culture (Figure 1B). Western blot analysis showed that they also had the components of the DISC, including Fas, FADD, procaspase-8/FLICE, c-FLIP<sub>L</sub>, and c-FLIP<sub>S</sub> (Figure 1C). The expression levels of these molecules in both ENKL-derived NK cell lines were approximately the same as those in Fas-sensitive Jurkat cells (Figure 1C). Coexpression of Fas and FasL was also confirmed in clinical samples of ENKL (Figure 1D). Immunohistochemistry was carried out in diagnostic specimens from a total of nine cases (Table S1). All nine cases expressed FasL. Eight of them (89%) expressed Fas simultaneously. Furthermore, seven cases (78%) expressed c-FLIP along with Fas and FasL. Although the results indicate that most ENKL cells were ready to undergo AICD, they were indeed surviving and proliferating. This situation raises the possibility that they should have mechanisms to escape AICD.

3.2 | ENKL-derived NK cell lines show resistance to Fas-mediated apoptosis

We next evaluated the susceptibility to Fas-mediated apoptotic stimuli in NK-YS and Hank1 cells. To eliminate the effects of humoral inhibitory factors, we undertook direct Fas ligation with agonistic 7C11 in both NK cell lines. The MTT assay showed that the stimulation with 7C11, but not with control Ms IgM or antagonistic ZB4, decreased cell viability, particularly in Fas-sensitive Jurkat cells. Although the viability of Jurkat cells was decreased to 10% 1 h after Fas ligation with 7C11, those of NK-YS and Hank1 stayed at approximately 50% and 70%, compared with each control, respectively. The effects were statistically significant (Jurkat, *P < .001; NK-YS, **P < .001; Hank1, ***P < .001; Student’s t test). B, Flow cytometry detected that only Fas-sensitive Jurkat cells increased annexin V-positive cell fractions 1 h after Fas ligation with 7C11. C, More than 40% of Jurkat cells showed apoptotic changes, whereas most NK-YS and Hank1 cells failed to increase apoptotic changes. Only events in Jurkat cells reached significance (*P < .001, Student’s t test).
changes even after Fas ligation (Figure 2B,C). Although NK-YS and Hank1 cells might have reduced their ability to proliferate after Fas ligation, they clearly showed resistance to direct Fas-mediated apoptotic stimuli.

### 3.3 DISC formation in NK-YS, Hank1, and Jurkat cells

To determine whether Fas-mediated signaling is functional, we investigated DISC formation in NK-YS and Hank1 cells. Ten minutes after Fas ligation with 7C11, FADD and the cleaved forms of caspase-8 were coimmunoprecipitated with Fas in NK-YS and Hank1 as well as in Jurkat cells (Figure 3A). Colocalization of these molecules was also visualized on confocal microscopy (Figure 3B). Similar to Fas-sensitive Jurkat cells, yellow fusion signals between Fas and caspase-8 were detected in both ENKL-derived NK cell lines 10 minutes after treatment with 7C11 but not with control Ms IgM (Figure 3B). These findings indicate that the DISC was equally generated among the 3 lines after Fas ligation.

### 3.4 c-FLIP\(_{L}\) is efficiently recruited to DISC in ENKL cells

Although the DISC formation was similarly initiated among NK-YS, Hank1, and Jurkat cells, a cleaved form of c-FLIP\(_{L}\) was
coimmunoprecipitated with Fas and caspase-8, predominantly in both ENKL-derived NK cell lines after Fas ligation (Figure 4A). Despite the same expression levels of c-FLIPL, the intracellular behavior of this molecule in these ENKL-derived NK cell lines seems to be different from that in Fas-sensitive Jurkat cells. The colocalization of Fas and c-FLIPL were frequently detected in NK-YS and Hank1, compared with Jurkat cells. (A) Confocal microscopy also confirmed the colocalization of Fas (red) and c-FLIPL (green) predominantly at the cell membrane in both NK-YS and Hank1, compared with Jurkat cells. (B) Yellow fusion signals between Fas and c-FLIPL were counted in each 100 cells, in triplicate. Compared with Jurkat cells, the mean values were significantly high in NK-YS and Hank1 (Jurkat vs NK-YS, *P < .001; Jurkat vs Hank1, **P < .001; Student’s t test). IP, immunoprecipitation; WB, western blot.

These results suggest that efficient recruitment of c-FLIPL to the DISC should affect the proximal Fas-mediated signaling predominantly in NK-YS and HANK1 cells.

3.5 | c-FLIPL plays a critical role in resistance to Fas-mediated apoptosis in ENKL cells

We then evaluated Fas-mediated apoptosis in the presence or absence of c-FLIPL in both ENKL-derived NK cell lines. RNA
interference for c-FLIP<sub>L</sub> effectively knocked down the expression of c-FLIP<sub>L</sub> in NK-YS and Hank1 cells 24 hours after the transfection (Figure 5A). The RNA interference did not alter the expression levels of c-FLIP<sub>S</sub>, RIP1, or antiapoptotic molecules including BCL2, BCL-X<sub>L</sub>, and MCL1 (Figure 5A). Although the cells transfected with control siRNA hardly showed apoptotic changes even after Fas ligation, knockdown of c-FLIP<sub>L</sub> per se significantly increased annexin V-positive fraction even after treatment with mouse (Ms) IgM in both ENKL cell lines. Fas ligation obviously enhanced the effect. Flow cytometry detected that more than 50% of both ENKL-derived NK cell lines were positive for annexin V. C. These events were statistically significant (CNT siRNA-7C11 vs c-FLIP<sub>L</sub> siRNA-Ms IgM, *P = .005 in NK-YS and **P = .002 in Hank1; c-FLIP<sub>L</sub> siRNA-Ms IgM vs c-FLIP<sub>L</sub> siRNA-7C11, ***P < .001 in NK-YS and ****P < .001 in Hank1; Student’s t test). D. Although Fas ligation with CH-11 promoted the processing of procaspase-3, knockdown of c-FLIP<sub>L</sub> clearly accelerated this process and increased the cleavage of poly (ADP-ribose) polymerase (PARP)1 in NK-YS and Hank1 cells. Only this experiment was carried out using CH-11 for Fas ligation.
Nevertheless, ENKL-derived NK cell lines NK-YS and Hank1 showed resistance to Fas-mediated apoptosis in spite of the DISC formation. Previous studies indicated that loss-of-function mutations of the FAS gene are considered a major mechanism of resistance to Fas-mediated apoptosis in ENKL. Here we confirmed the direct evidence of c-FLIP L for the resistance to AICD in ENKL and provided another mechanism.

Coexpression of Fas and FasL was also observed in HRS cells. Although the normal counterpart of HRS cells should undergo the cell-death fate in germinal centers, HRS cells can survive partially through the function of c-FLIP L. In activated normal NK cells, the expression of c-FLIP seems to be transiently upregulated but subsequently suppressed. The prolonged expression of c-FLIP L in activated normal NK cells has been shown to have an advantage in survival during the AICD process. The present data and the previous findings suggest that c-FLIP L could be a key regulator of the resistance to AICD in NK cells. Our previous observation had shown that EBV infection of NK cells conferred resistance to cell stress such as DNA damage and starvation. When the stress was induced, only EBV-infected NK cells maintained the expression levels of c-FLIP L, partially through the activation of NF-κB. The presence of EBV could contribute to the prolonged expression of c-FLIP L under conditions of proapoptotic stress in NK cells.

We further found that c-FLIP L was more sufficiently recruited to the DISC in Fas-resistant ENKL-derived NK cell lines than in Fas-sensitive Jurkat in spite of the same expression levels. After Fas ligation, the DISC formation is similarly initiated among NK-YS, Hank1, and Jurkat cells. Nevertheless, both ENKL-derived NK cell lines could have an advantageous system for the recruitment of c-FLIP L into the DISC. Our study clearly showed that disparities in the intracellular trafficking, rather than the expression levels, should determine functional differences of c-FLIP L in the inhibition of Fas-mediated signaling. In Ms T cells, mitogenic stimulation seems to induce the posttranslational modification of procaspase-8/FLICE and alters the intracellular localization. Although posttranslational modifications of c-FLIP L remain unknown, differences in the intracellular localization might be acquired during the tumorigenic process of ENKL.

In conclusion, our findings indicate that ENKL-derived NK cell lines are similar to activated normal NK cells in being primed for AICD. However, they constitutively express and efficiently utilize c-FLIP L, which prevents their Fas-mediated apoptosis (Figure 6).
specific downregulation of c-FLIP<sub>L</sub> accelerates the autonomous cell death in ENKL cells. Therefore, c-FLIP<sub>L</sub> could be a promising therapeutic target against ENKL.

ACKNOWLEDGEMENTS

We thank Dr Junjiro Tsuhiyama (Kawasaki Medical School, Kurashiki, Japan) and Dr Masao Seto (Kurume University, Kurume, Japan) for providing NK-YS and Hank1, respectively. We also thank Ms Asami Yamada and Ms Junko Asano for technical assistance.

DISCLOSURE

All authors declare no conflict of interest.

ORCID

Yasushi Isobe https://orcid.org/0000-0001-8030-6621

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Masuda A, Isobe Y, Sugimoto K, Yoshimori M, Arai A, Komatsu N. Efficient recruitment of c-FLIP<sub>L</sub> to the death-inducing signaling complex leads to Fas resistance in natural killer-cell lymphoma. Cancer Sci. 2020;111:807-816. https://doi.org/10.1111/cas.14296