FEBS Letters 585 (2011) 1835–1840

FEBS Letters
journal homepage: www.FEBSLetters.org

Caspase-8 cleavage of the interleukin-21 (IL-21) receptor is a negative feedback regulator of IL-21 signaling

Tatsuya Akagi [a], Kouhei Shimizu [a], Shoukichi Takahama [b], Takahiro Iwasaki [b], Kazuhiro Sakamaki [c], Yaeta Endo [a,b,d,e,*], Tatsuya Sawasaki [a,b,d,e,*]

[a] Cell-Free Science and Technology Research Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[b] The Venture Business Laboratory, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[c] Department of Animal Development and Physiology, Graduate School of Biostudies, Kyoto University, Kyoto 606-8501, Japan
[d] Proteo-Medicine Research Center, Ehime University, Toon, Ehime 791-0295, Japan
[e] RIKEN Systems and Structural Biology Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan

ARTICLE INFO

Article history:
Received 1 February 2011
Revised 5 April 2011
Accepted 13 April 2011
Available online 20 April 2011

Edited by Vladimir Skulachev

Keywords:
Caspase-8
Cell-free system
Interleukin-21
Cytokines
Apoptosis

ABSTRACT

We screened a library of human single-transmembrane proteins (sTMPs), produced by a cell-free system, using a luminescent assay to identify those that can be cleaved by caspase-8 (CASP8). Of the 407 sTMPs screened, only the interleukin-21 receptor (IL21R), vezatin (VEZT), and carbonic anhydrase XIV were cleaved at Asp344, Asp655 and Asp53, respectively. We confirmed that IL21R and VEZT were also cleaved in apoptotic HeLa cells with the cleavage sites. Interestingly, IL21R was cleaved within 30 min after apoptosis induction. Furthermore the CASP8-cleaved form of IL21R did not induce phosphorylation at Tyr705 of STAT3. Our results suggest that the interleukin-21 signaling cascade is negatively regulated by CASP8.

1. Introduction

Procaspase-8 (proCASP8) is part of the trans-plasma membrane death-inducing signaling complex and is located on the cytosolic side [1]. Binding of extracellular ligands, e.g., the FAS ligand, to the death inducing signaling complex activates proCASP8, which is then released from the complex as caspase-8 (CASP8). Pro-apoptotic proteins, e.g., caspase-3 (CASP3) and the BH3 interacting domain death agonist (Bid), are cleaved by CASP8 as part of the apoptotic pathway. These CASP8 substrates are found in the cytosol. However, there have been reports that CASP8 cleaves membrane-bound v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ERBB2) in apoptotic cells [2] and also interacts with other membrane proteins [3,4]. These reports show that CASP8 can cleave membrane proteins. Identification of CASP8-cleavable membrane proteins would therefore increase our knowledge of their functions.

We have developed a high-throughput, automated protein production method that uses a wheat cell-free system [5,6], and this system has been used to construct an in vitro-expressed proteome composed of 13 364 human proteins [7] and a kinase library for which the kinases are N- and C-terminally tagged (NCtagged) with the Flag sequence and biotin, respectively [8]. The kinase library, in conjunction with a commercially available assay system that measures luminescence (AlphaScreen), was used to identify substrates of CASP3. Interestingly, of the 13 364 proteins found in the proteome, 1320 are single transmembrane proteins (sTMPs), i.e., those with a single transmembrane (sTM) region, such as ERBB2. For the study reported herein, we adapted the assay system for use with a human NCtagged sTMP library generated by our cell-free system to screen for CASP8-cleavable sTMPs. Interleukin-21 receptor (IL21R) was found to be a CASP8 substrate, and its cleavage by

Abbreviations: Bid, BH3 interacting domain death agonist; CA14, carbonic anhydrase XIV; CASP8, caspase-8; ERBB2, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2; IL-21, interleukin-21; IL21R, interleukin-21 receptor; IL2RG, interleukin 2 receptor, gamma; NCtagged, N- and C-terminally tagged; sTMP, single transmembrane protein; sTMG, single transmembrane gene; VEZT, vezatin

* Corresponding authors at: Cell-Free Science and Technology Research Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan. Fax: +81 89 927 9941.

E-mail addresses: yendo@eng.ehime-u.ac.jp (Y. Endo), sawasaki@ehime-u.ac.jp (T. Sawasaki).

0014-5793/$36.00
doi:10.1016/j.febslet.2011.04.031

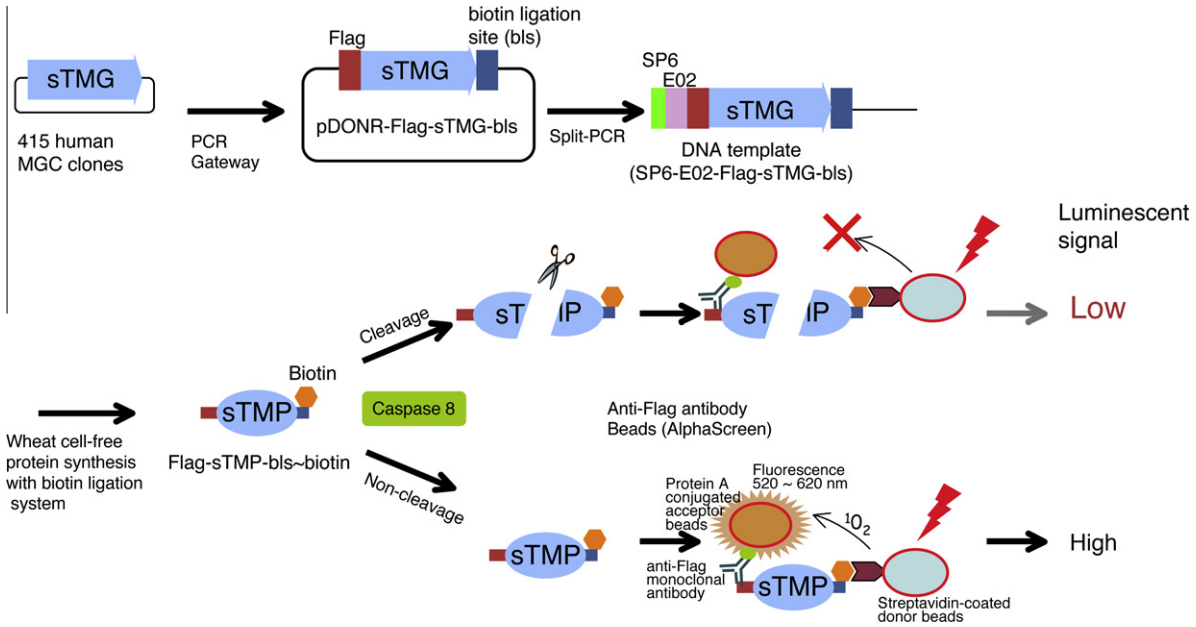

Fig. 1. Strategy for screening sTMPs cleaved by CASP8. Genes having a single transmembrane region (sTM) were selected from the human MGC library.

CASP8 in vivo suggests that CASP8 is a negative feedback regulator of interleukin-21 (IL-21) signaling.

2. Materials and methods

2.1. General

The following procedures have either been described in detail or cited [5,6,8–10]: generation of DNA templates by PCR using split-primers; parallel syntheses of mRNAs and proteins; biotin labeling of the proteins; amino acid sequencing; immunoblotting detection and luminescent detection of caspase cleavage. Anti-Flag and anti-V5 antibodies were purchased from Sigma–Aldrich (St. Louis, MO) and Invitrogen (Carlsbad, CA), respectively. Antibodies specific for endogenous Bid, cleaved PARP, tubulin, STAT3, and STAT3 phosphorylated at Tyr705 were purchased from Cell Signaling Technology (Beverly, MA).

2.2. Construction of DNA templates

The single transmembrane genes (sTMGs) used in this study are listed in Supplementary Table 1. Methods for construction of pDONR-Flag-sTMG-bls plasmids were based on previous report [8]. Genes for expression in the cell were inserted into a pcDNA3.2/V5-DEST by the Gateway method (Invitrogen). The interleukin 2 receptor, gamma (IL2RG) (GenBank Accession No. BC014972) and JAK3 (BC028068) genes from MGC clones were each inserted into a pcDNA3.1 vector to construct pcDNA3.1-IL2RG-Flag and pcDNA3.1-JAK3-myc vectors, respectively. The pcDNA3.2-C8M-IL21R vector was constructed by inserting the IL21R nucleotide sequence corresponding to residues 1–344 into the pcDNA3.2 vector. D → A mutagenesis was carried out using the reagents of a PrimeSTAR Mutagenesis Basal kit (TakaraBio, Otsu, Japan) according to the manufacturer's instructions.

2.3. Cell-free protein synthesis

The sTMPs were produced using the robotic synthesizer GenDecoder 1000 (CellFree Sciences, Yokohama, Japan) and the reagents of ENDEXT kits, i.e., the wheat cell-free system (CellFree Sciences), biotin ligase, and biotin [6,8,9].

2.4. Cleavage assay using the luminescent method and immunoblotting

For each sTMP, 10 μl of the CASP8-cleavage buffer [6.7 mM Tris–HCl, pH 7.5, 167 mM NaCl, 0.8 mM EDTA, 3.3 mM DTT, 3.3% glycerol, 0.03% CHAPS, and 1 U CASP8 (Sigma–Aldrich)] was mixed with 1 μl of the translation mixture that contained a Flag-sTMP-bls~biotin construct, and the mixture was incubated at 30 °C for 2 h in a well of a 384-well Optiplate (Perkin Elmer, Foster City, CA).

2.5. Amino acid sequences of the CASP8 cleavage sites

For IL21R and carbonic anhydrase XIV (CA14), their biotinylated C-terminal fragments produced by CASP8 cleavage were recovered while attached to streptavidin beads and then sequenced directly. For vezatin (VEZT), C-terminally GST fused VEZT was used.

2.6. Cell-based assay

HeLa and Jurkat cells [wild-type (A3, ATCC, Riken Cell Bank, Tsukuba, Japan; CRL-2570) and CASP8-deficient (I9.2, ATCC CRL-2571)] were grown in Dulbecco's modified eagle medium (DMEM) and RPMI medium, respectively, 10% fetal bovine serum (FBS), penicillin (100 mg/ml), and streptomycin (50 μg/ml). Twenty-four hours after transfection, cells were harvested after apoptosis had been induced. For apoptosis induction or inhibition, with 200 ng/ml anti-Fas antibody (Medical and Biological Laboratories Co. Ltd., Nagoya, Japan) in the presence (inhibition) or absence (induction) of 50 μM Z-IETD-FMK (Calbiochem, San Diego, CA) or 50 μM Z-VAD-FMK (Peptide Institute Inc., Osaka, Japan) for 6 h. For analysis of the IL-21 signaling pathway, pcDNA3.2-IL21R or pcDNA3.2-C8M-IL21R vector was cotransfected into HeLa cells with pcDNA3.1-IL2RG-Flag and pcDNA3.2-HA-JAK3 vectors. The cells were treated with 50 ng/μl IL-21 for 30 min, after 24 h of transfection.

3. Results

3.1. Generation of an NCtagged sTMP library for screening of CASP8 substrates

First, we examined the 9362 full-length human gene sequences of the Mammalian Gene Collection (MGC) (http://www.mgc.nci.nih.gov/) to find sTMP sequences using the predictive program TMHMM version 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), and then we classified the sTMG according to the terms used in the Gene Ontology (GO) database (http://www.geneontology.org/). In the end, 574 independent genes having a single transmembrane region were selected and their sequences used to make an NCtagged sTMP library. The NCtagged sTMPs were produced using the wheat cell-free system and an automatic GenDecorder 1000 in the presence of biotin and biotin ligase [8,9] (Fig. 1). Then, 407 human sTMPs, i.e., those with luminescence signals >500 units, were selected for the final sTMP library (Supplementary Table 1). To check the performance of the assay system, we used Bid and ERBB2, which are known as CASP8 substrates [2,11]. Flag-Bid-bls∼biotin and Flag-ERBB2-bls∼biotin were each treated with active CASP8 and their cleavage by CASP8 was first confirmed by immunoblotting with Alexa488-labeled streptavidin (Fig. 2A). Furthermore, the two NCtagged substrates were used for luminescent assay. After CASP8 treatment, the strengths of the luminescent signals of the two protein samples decreased; whereas that of a non-cleavable, control protein (*Escherichia coli* dihydrofolate reductase) did not (Fig. 2B). These results showed that the assay system could be used to detect CASP8-cleavable NCtagged sTMPs in place of or as an adjunct to conventional immunoblotting.

3.2. Screening for CASP8-cleavable sTMPs in the sTMP library

Using the luminescent detection assay, the proteins of the NCtagged sTMP library were screened for CASP8 cleavability (Fig. 2C). The signals for three sTMPs were <65% of their initial signals; whereas, the signals of the other 404 sTMPs were not or were less affected by CASP8 treatment. For the three sTMPs with luminescent signals affected by CASP8, both a general caspase (z-VAD-FMK) and a CASP8-specific (z-IETD-FMK) inhibitor prevented the signal reduction caused by the presence of CASP8 (Fig. 2D). The cleavage of the three sTMPs by CASP8 was confirmed by conventional immunoblotting using anti-Flag antibodies and fluorescent streptavidin (Fig. 3A). The CASP8-cleavage sites in the three proteins were identified by amino acid sequencing of their cleaved C-terminal fragments (Fig. 3B). The cleavage sites of IL21R, VEZT, and CA14 were VESD↓G_{344}, PQAD↓G_{705}, and IQTD↓S_{53}, respectively, which are consensus-type sequences [X-E/Q-X-D↓-G/S (X is any amino acid and ↓ is the cleavage site, http://www.merops.sanger.ac.uk)]. We also found identical or similar sites in orthologous mammalian proteins (Fig. 3B), indicating that these sites may be conserved among mammals.

3.3. Characterization of the CASP8-cleavable sTMPs in HeLa cells made apoptotic by anti-Fas antibodies

We investigated whether the sTMPs found to be cleaved by CASP8 in vitro were also cleaved in HeLa cells made apoptotic by anti-Fas antibodies. Expression of IL21R-V5 and VEZT-V5 was detected in the transfected cells; whereas, CA14-V5 was not expressed. In apoptotic cells, cleavage of the two sTMPs occurred

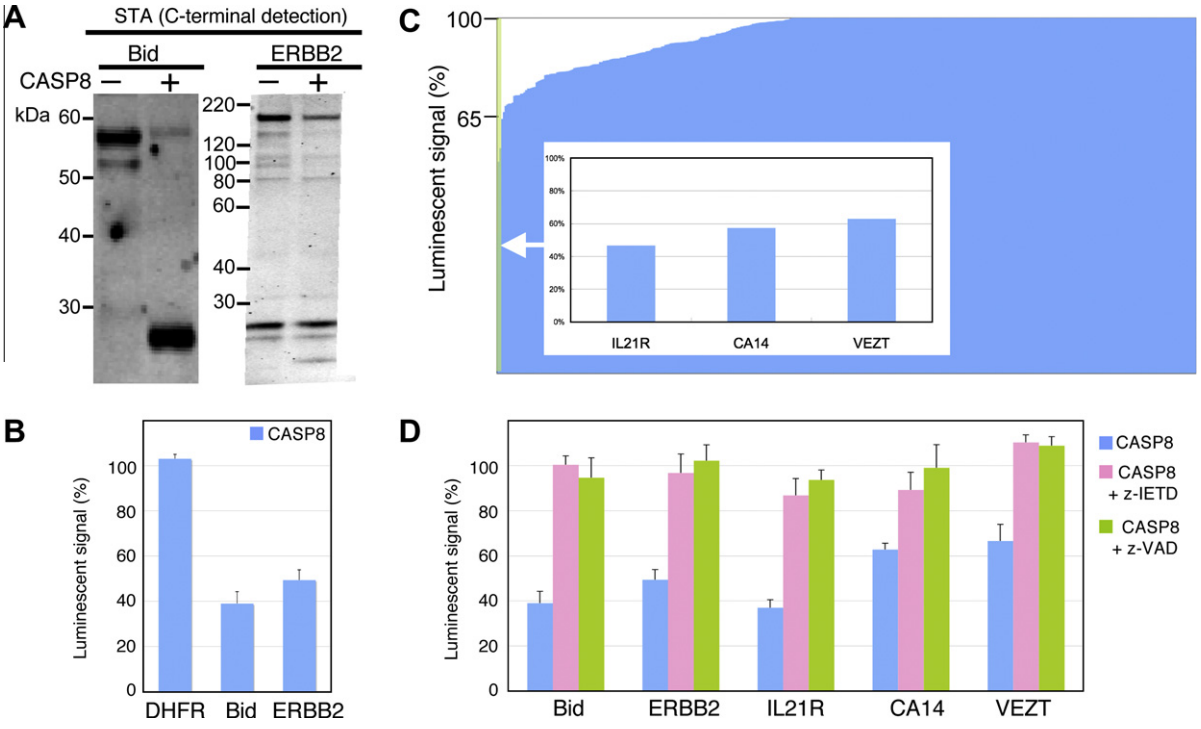

Fig. 2. Screening for CASP8-cleaved sTMP substrates using the NCtagged sTMP library. (A) Immunoblot of NCtagged Bid and ERBB2 that had been incubated in the presence (+) or absence (−) of CASP8. Alexa488-labeled streptavidin (STA) was used for detection. (B) Detection of CASP8-cleaved NCtagged Bid and ERBB2 using the AlphaScreen luminescence system. The luminescence for the controls (no CASP8) was set to 100%. (C) Relative luminescent signals after in vitro CASP8 treatment of NCtagged sTMPs that had been synthesized in the wheat cell-free system. The *x* axis lists the NCtagged sTMPs in ascending order of their luminescent signals after CASP8 treatment. NCtagged sTMPs that returned luminescent signals <65% of the control values (green rectangle) are identified in the inset along with their relative luminescent signals. (D) Inhibition assay using the AlphaScreen.

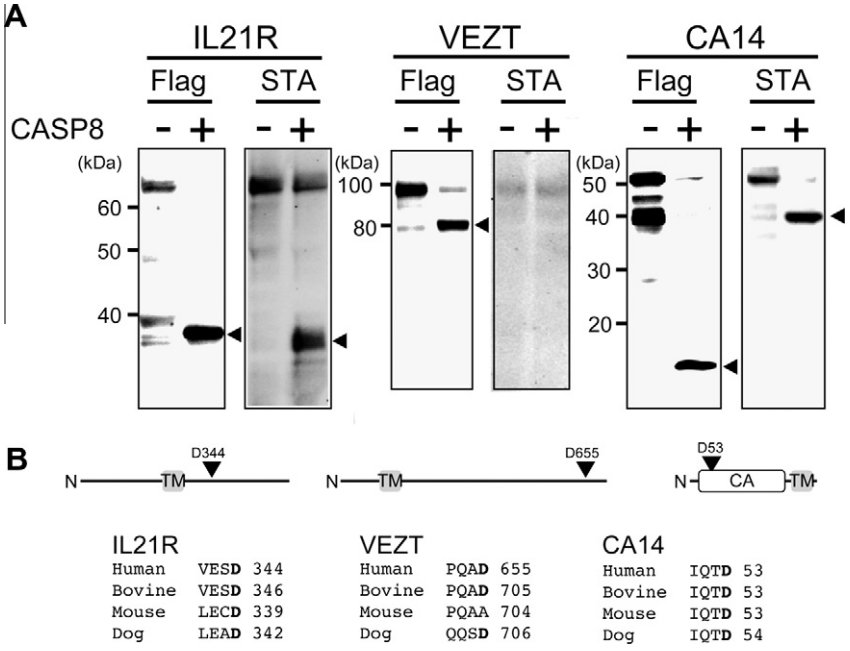

Fig. 3. In vitro cleavage of sTMPs by CASP8. (A) The NCtagged sTMPs were incubated in the presence (+) or absence (−) of CASP8 and their cleavage products (solid arrowheads) were detected using anti-Flag antibodies (Flag) and Alexa488-conjugated streptavidin (STA), which bound to the N- and C-termini of the sTMP constructs, respectively. (B) Sequence alignments of the CASP8-cleavage sites for the three sTMPs are shown at the bottom of the figure.

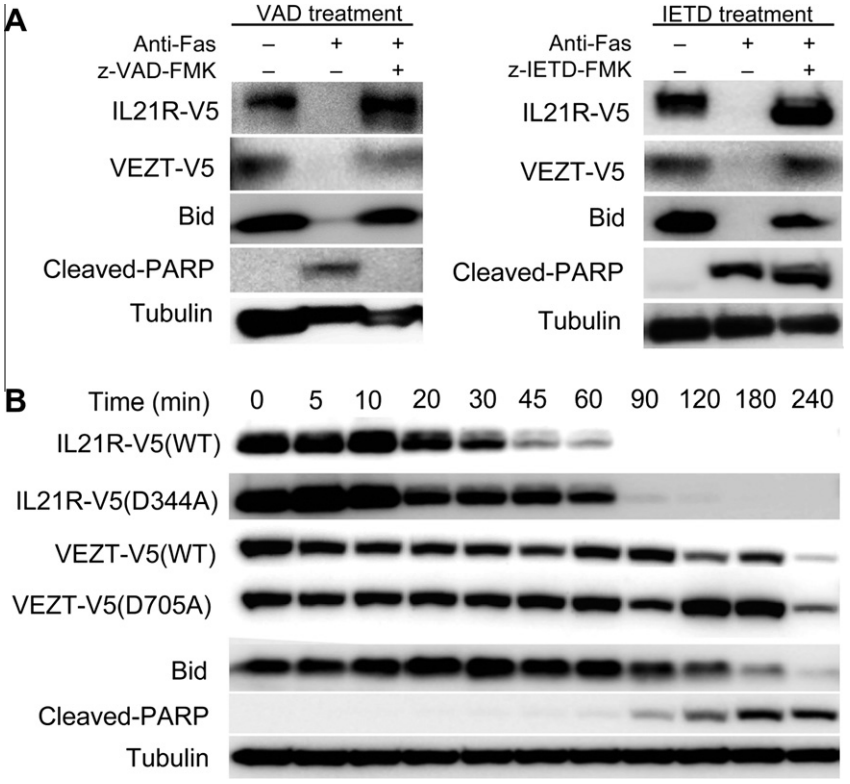

Fig. 4. Cleavage of two of the newly identified sTMP substrates in the apoptotic cells. (A) The cells were treated with DMSO (control), or with anti-Fas antibodies in the presence or absence of z-VAD-FMK or z-IETD-FMK for 6 h and then lysed. (B) Time course for the expression of IL21R and VEZT in apoptotic HeLa cells. IL21R-V5 (wild type, WT), the D344A mutant of IL21R-V5, VEZT-V5 (WT), and the D705A mutant of VEZT-V5 were expressed in apoptotic HeLa cells.

and was completely blocked by z-VAD-FMK and z-IETD-FMK (Fig. 4A). Therefore, our approach, using the sTMP library, identified two new in vivo targets of CASP8.

We investigated some of the events that occurred shortly after IL21R cleavage by CASP8 in apoptotic HeLa cells. Importantly, although IL21R cleavage was detected at 30 min after apoptosis induction using anti-Fas antibodies (and it was almost entirely complete within 90 min) (Fig. 4B), VEZT cleavage was nearly complete only 2 h after apoptotic induction. In vivo cleavage of Bid and PARP (poly (ADP-ribose) polymerase 1) have, respectively, been

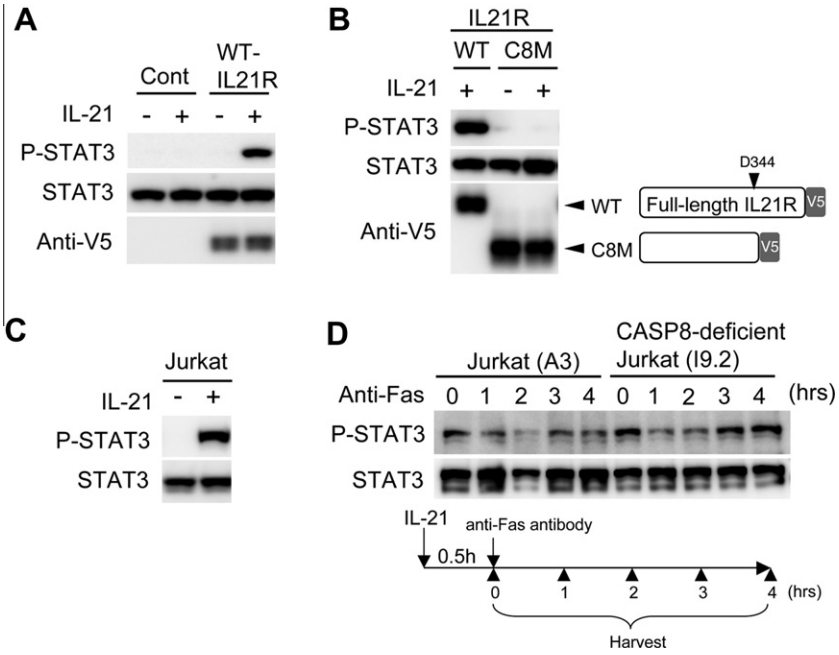

Fig. 5. Characterization of in vivo CASP8-cleaved IL21R (A) HeLa cells Cells were treated with (+) or without (−) IL21 for 1 h after 24 h of transfection and then lysed. Endogenous STAT3 and STAT phosphorylated at Tyr705 (P-STAT3) were detected by antibodies specific for each protein. (B) pcDNA3.1-IL2RG-Flag and pcDNA3.2-HA-JAK3 constructs were expressed with pcDNA3.2-IL21R (WT) or pcDNA3.2-C8M-IL21R (C8M), which is a truncated form that mimics the CASP8-cleaved form. (C) Jurkat cells were treated with (+) or without (−) IL21 for 1 h. (D) Jurkat (A3) and CASP8-deficient Jurkat I9.2 cells were treated with IL21 for 0.5 h and then treated with anti-Fas antibody.

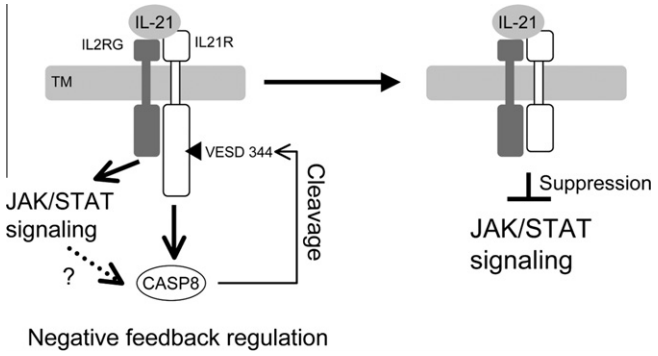

Fig. 6. Model for negative feedback regulation of IL-21 by CASP8 cleavage of IL21R.

used as indicators of CASP8 and caspase-3 activation. Our finding that IL21R is cleaved by CASP8 during initiation phase of apoptosis is consistent with that of previous work, which showed that CASP8 was activated 30 min within the onset of apoptosis [12].

3.4. A biological function for CASP8-cleaved IL21R

IL21 activates the JAK/STAT signaling cascade by forming a complex between IL2RG (IL2Rγ) and IL21R [13,14], and IL-21 also induces apoptosis in B cells [15]. Because STAT3 phosphorylation at Tyr705 is part of the signaling cascade initiated by IL-21 [14], we first attempted to determine the phosphorylation state of Tyr705 in STAT3 when CASP8 was inhibited with a CASP8-specific (z-IETD-FMK) inhibitor in vivo. However, z-IETD-FMK inhibited STAT3 phosphorylation in the absence of CASP8 (data not shown). Therefore, to investigate the biological function of the caspase-cleaved IL21R, we reconstructed the IL-21-dependent JAK/STAT signaling by coexpression of IL21R, JAK3, and IL2RG in HeLa cells. When all three genes were transfected into the cells, STAT3 phosphorylation at Tyr 705 occurred (Fig. 5A). Then, the gene for C8M-IL21R, a truncated form of IL21R (residues 1–344) that mimics the CASP8-cleaved IL21R, was transfected with the genes for JAK3 and IL2RG. However, STAT3 phosphorylation was not observed in the cells although C8M-IL21R was expressed (Fig. 5B), suggesting that CASP8 cleavage of IL21R may block JAK/STAT signaling. Furthermore, because IL-21 induced STAT3 phosphorylation in Jurkat cells (Fig. 5C), normal Jurkat (A3) and Jurkat (I9.2) cells lacking CASP8 activity [4,16] were used to assess IL-21-induced STAT3 signaling after CASP8 activation. After IL-21 treatment for 0.5 h, normal Jurkat and I9.2 cells were made apoptotic by anti-Fas antibodies. In normal Jurkat cells, STAT3 phosphorylation decreased within 4 h. In contrast, in I9.2 cells, STAT3 remained phosphorylated at least up to 4 h after apoptosis was induced (Fig. 5D). Therefore, CASP8 cleavage of IL21R may inhibit IL-21-dependent JAK/STAT signaling.

4. Discussion

The involvement of capsases in interleukin signaling pathway(s) has not been fully delineated. As one intriguing example, caspase-1 processes the proinflammatory cytokine, proIL-1β, into mature IL-1β [17], which implies that at least one caspase is part of an interleukin signaling pathway. In the NCtagged sTMP library, two interleukins (IL-8 and IL-27) and eight interleukin receptors (IL1R2, IL2RG, IL3RA, IL10RB, IL11RA, IL21R, IL22RA1, and IL27RA) were included. However, CASP8 cleaved only IL21R as shown by both the luminescent assay and immunoblotting. Therefore, possibly only IL-21 signaling may be directly related to CASP8 activation. The IL-21 signaling pathway is a modulator of lymphoid proliferation, apoptosis, and differentiation [18]. CASP8 is involved in lymphocyte proliferation and differentiation [19]. Interestingly, in B cells, IL-21 mediated apoptosis involved activation of CASP8 and CASP3, which may be caused by high expression of IL21R via CD40 stimulation [20,21]. In the presence of exogenous IL-21, the CASP8-cleaved form of IL21R did not induce the phosphorylation of Tyr 705 in STAT3 (Fig. 5B), and CASP8 cleavage of IL21R, as part of the apoptosis process, reduced the phosphorylation (Fig. 5D). A

previous study showed that IL-21 induces proliferation of human myeloma cells through tyrosine phosphorylation of STAT3 [22]. Taken together, as shown in Fig. 6, CASP8 appear to act as negative feedback regulators of IL-21 signaling by their cleavage of IL21R.

Acknowledgments

This work was partially supported by the Special Coordination Funds for Promoting Science and Technology by the Ministry of Education, Culture, Sports, Science and Technology, Japan (Nos. 19657041 and 22310127 to T.S.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.04.031.

References

[1] Muzio, M. et al. (1996) FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell 85, 817–827.
[2] Benoit, V., Chariot, A., Delacroix, L., Deregowski, V., Jacobs, N., Merville, M.P. and Bours, V. (2004) Caspase-8-dependent HER-2 cleavage in response to tumor necrosis factor alpha stimulation is counteracted by nuclear factor kappaB through c-FLIP-L expression. Cancer Res. 64, 2684–2691.
[3] Misra, R.S. et al. (2007) Caspase-8 and c-FLIPL associate in lipid rafts with NF-kappaB adaptors during T cell activation. J. Biol. Chem. 282, 19365–19374.
[4] Koenig, A., Russell, J.Q., Rodgers, W.A. and Budd, R.C. (2008) Spatial differences in active caspase-8 defines its role in T-cell activation versus cell death. Cell Death Differ. 15, 1701–1711.
[5] Sawasaki, T., Ogasawara, T., Morishita, R. and Endo, Y. (2002) A cell-free protein synthesis system for high-throughput proteomics. Proc. Natl. Acad. Sci. USA 99, 14652–14657.
[6] Sawasaki, T., Morishita, R., Gouda, M.D. and Endo, Y. (2007) Methods for high-throughput materialization of genetic information based on wheat germ cell-free expression system. Methods Mol. Biol. 375, 95–106.
[7] Goshima, N. et al. (2008) Human protein factory for converting the transcriptome into an in vitro-expressed proteome. Nat. Methods 5, 1011–1017.
[8] Tadokoro, D., Takahama, S., Shimizu, K., Hayashi, S., Endo, Y. and Sawasaki, T. (2010) Characterization of a caspase-3-substrate kinome using an N- and C-terminally tagged protein kinase library produced by a cell-free system. Cell Death Dis. 1, e89.
[9] Sawasaki, T., Kamura, N., Matsunaga, S., Saeki, M., Tsuchimochi, M., Morishita, R. and Endo, Y. (2008) Arabidopsis HY5 protein functions as a DNA-binding tag for purification and functional immobilization of proteins on agarose/DNA microplate. FEBS Lett. 582, 221–228.
[10] Sawasaki, T., Hasegawa, Y., Tsuchimochi, M., Kamura, N., Ogasawara, T., Kuroita, T. and Endo, Y. (2002) A bilayer cell-free protein synthesis system for high-throughput screening of gene products. FEBS Lett. 514, 102–105.
[11] Li, H., Zhu, H., Xu, C.J. and Yuan, J. (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94, 491–501.
[12] Medema, J.P., Scaffidi, C., Kischkel, F.C., Shevchenko, A., Mann, M., Krammer, P.H. and Peter, M.E. (1997) FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J. 16, 2794–2804.
[13] Ozaki, K., Kikly, K., Michalovich, D., Young, P.R. and Leonard, W.J. (2000) Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain. Proc. Natl. Acad. Sci. USA 97, 11439–11444.
[14] Asao, H., Okuyama, C., Kumaki, S., Ishii, N., Tsuchiya, S., Foster, D. and Sugamura, K. (2001) Cutting edge: the common gamma-chain is an indispensable subunit of the IL-21 receptor complex. J. Immunol. 167, 1–5.
[15] Jin, H., Carrio, R., Yu, A. and Malek, T.R. (2004) Distinct activation signals determine whether IL-21 induces B cell costimulation, growth arrest, or Bim-dependent apoptosis. J. Immunol. 173, 657–665.
[16] Juo, P., Kuo, C.J., Yuan, J. and Blenis, J. (1998) Essential requirement for caspase-8/FLICE in the initiation of the Fas-induced apoptotic cascade. Curr. Biol. 8, 1001–1008.
[17] Cerretti, D.P. et al. (1992) Molecular cloning of the interleukin-1 beta converting enzyme. Science 256, 97–100.
[18] Leonard, W.J. and Spolski, R. (2005) Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. Nat. Rev. Immunol. 5, 688–698.
[19] Yi, C.H. and Yuan, J. (2009) The Jekyll and Hyde functions of caspases. Dev. Cell 16, 21–34.
[20] Akamatsu, N. et al. (2007) High IL-21 receptor expression and apoptosis induction by IL-21 in follicular lymphoma. Cancer Lett. 256, 196–206.
[21] de Totero, D. et al. (2006) Interleukin-21 receptor (IL-21R) is up-regulated by CD40 triggering and mediates proapoptotic signals in chronic lymphocytic leukemia B cells. Blood 107, 3708–3715.
[22] Brenne, A.T., Ro, T.B., Waage, A., Sundan, A., Borset, M. and Hjorth-Hansen, H. (2002) Interleukin-21 is a growth and survival factor for human myeloma cells. Blood 99, 3756–3762.