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Biochemical and Biophysical Research Communications 360 (2007) 280–285
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Activation of protein phosphatase causes alternative splicing of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL): Potential effect on immune surveillance

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Received 5 June 2007
Available online 15 June 2007

Abstract

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) belongs to the TNF superfamily of proteins. It is highly expressed on natural killer cells, cytotoxic T lymphocytes, and monocytes after stimulation, and plays a critical role in immune surveillance. Two splice variants of TRAIL were identified recently that show no proapoptotic activity. Phosphorylation level in splicing factors, serine–arginine-rich (SR) and heterogeneous ribonucleoproteins (hnRNPs) govern the mRNA splicing of several apoptosis-related genes. We characterized the apoptotic stimuli-mediated alternative splicing pattern of TRAIL and investigated the possible underlying mechanism of alternative splicing. Etoposide and cycloheximide induced alternative splicing, whereas staurosporine (a broad kinase inhibitor) blocked both constitutive and alternative splicing. *De novo* ceramide synthesis and subsequent protein phosphatase-1 (PP-1) activation enhanced the alternative splicing, as did TNF-α but not interferon alpha (IFN-α) stimulation. We demonstrated that TRAIL alters gene expression through mRNA splicing and may change proapoptotic potential in response to cytokine stimulation.

Keywords: Alternative splicing; TRAIL; Immune surveillance; Signaling pathway; SR proteins; Phosphatase activation; Ceramide synthesis; Apoptosis; Lymphocyte; PBMC; Cytokine

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a rare molecule that kills transformed cells, but largely spares normal cells. It is therefore a promising anti-cancer agent [1]. TRAIL is highly expressed on natural killer cells (NK cells), cytotoxic T lymphocytes (CTLs), and monocytes after interferon alpha (IFN-α) and gamma (IFN-γ) stimulation, and is a key player in tumor surveillance [2–4]. A recent study identified two splice variants of TRAIL (TRAIL-β and TRAIL-γ), although the mechanism responsible for this alternative splicing has not been elucidated [5]. The lack of exon 3 in TRAIL-β and exons 2 and 3 in TRAIL-γ result in massive truncation of the TRAIL extracellular domain and a consequent loss of proapoptotic potential [5].

Post-transcriptional modification of gene expression through alternative splicing is emerging as an important mechanism for introducing significant protein diversity at a low genetic cost [6,7]. Alternative splicing generates functionally distinct products from the same apoptosis-related genes including Bcl-x, caspase-9, caspase-2, Fas, and caspase-8, probably providing distinct regulatory functions

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doi:10.1016/j.bbrc.2007.06.046

for apoptotic fine tuning [8,9]. Pre-mRNA splicing occurs within the spliceosome, a macromolecular complex comprising serine–arginine-rich (SR) and heterogeneous ribonucleoproteins (hnRNPs) [10]. RNA-recognition motifs (RRM) in the N-terminus and a variable-length arginine/serine-rich domain at the C-terminus (the RS domain) characterize SR proteins [10,11]. These molecules are also subjected to post-translational modification during apoptosis [12–14]. The serine residues within the SR domains undergo reversible phosphorylation, leading to modulation of splicing activity and finally to alteration of mRNA splicing. Exon skipping associated with alternative splicing can produce frame shifts, thereby generating a stop codon and splice variants with an opposite/dominant-negative function [15]. Recent studies provided several mechanistic insights into how alternative splicing might modulate the function of apoptosis-related genes. They suggest that *de novo* ceramide generation and subsequent phosphatase activation causes dephosphorylation of SR proteins leading to alteration of mRNA splicing, as observed for Bcl-x in A549 cells, as well as caspase-2 and Fas in U937 cells [16–18].

The present study examined the crosstalk between alternative splicing of TRAIL and intracellular signaling pathways. We examined proapoptotic stimuli-mediated splicing patterns and sought to elucidate the underlying mechanism. The results demonstrated that *de novo* ceramide generation and subsequent phosphatase activation induced alternative splicing of TRAIL mRNA, and that TNF-α stimulation also generated alternative splicing of TRAIL.

Materials and methods

Reagents. Reagents used were as follows: etoposide, staurosporine, cycloheximide, calyculin A, fumonisin B1, D-erythro-C6 ceramide, and PMA (Sigma Chemical Co., St. Louis, MO); human recombinant TNF-α (PeproTec, Rocky Hill, NJ); kinase inhibitors SB203580 and SB202190, and human recombinant IFN-α (Calbiochem, La Jolla, CA).

Cell culture. The local ethics committee approved all experimental protocols and a signed consent was obtained from each healthy volunteer. PBMC from healthy volunteers, as well as U937 and Jurkat cells were grown in 5% CO_2 at 37 °C in RPMI1640 medium (Invitrogen, San Diego, CA), supplemented with 9% heat-inactivated bovine serum (Invitrogen), penicillin, and streptomycin (Invitrogen). Cells were grown and then harvested at midlog phase.

Flow-cytometry analysis for the determination of apoptosis. We analyzed apoptosis by flow cytometry as described previously [18].

RNA isolation and RT-PCR analysis. Total RNA was isolated using the RNeasy Total RNA kit (Qiagen, Hilden, Germany) as described by the manufacturer [18]. One microgram of total RNA was reverse transcribed into cDNA using the Qiagen OneStep RT-PCR kit (Qiagen) and specific primer pairs were as described previously [5,18,19]. The resulting cDNA was used as the template for PCR amplification of TRAIL splice variants (sense 5′-GAA TCC CAT GGC TAT GAT GGA GGT CCA G-3′ and anti-sense 5′-GGA TTC GAG GAC CTC TTT CTC TCA CTA-3′ (GenBank Accession No. U37518)) or caspase-2 splice variant (sense 5′-AAC TGC CCA AGC CTA CAG AA-3′ and anti-sense 5′-GTC AAC CCC ACG ATC AGT CT-3′ (GenBank Accession No. U13021), or GAPDH (sense 5′-GCA GGG GGG AGC CAA AAG GG-3′ and anti-sense 5′-TGC CAG CCC CAG CGT CAA AG-3′ (GenBank Accession No. J04038), The PCR cycling conditions used were as follows: 35 cycles (94 °C, 2 min; 58 °C, 30 s; 72 °C, 1 min) for TRAIL, 35 cycles (94 °C, 30 s; 55 °C, 30 s; 72 °C, 1 min) for caspase-2, and 30 cycles (94 °C, 45 s; 67 °C, 30 s; 72 °C, 1 min) for GAPDH, as described previously [5,18,19]. The resulting fragments were subjected to electrophoresis and analyzed as described previously [18].

Sequencing of RT-PCR products. The RT-PCR products of TRAIL and caspase-2 mRNA were purified and analyzed in both directions using sequencing primers as follows and as described previously [18]: TRAIL forward 5′-TTC ACA GTG CTC CTG CAG TC-3′, reverse 5′-GAG GAC CTC TTT CTC TCA CTA-3′; Caspase-2 forward 5′-AAC TGC CCA AGC CTA CAG AA-3′, reverse 5′-GTC AAC CCC ACG ATC AGT CT-3′.

Results

Characterization of mRNA splicing patterns in response to multiple proapoptotic stimuli

The two primer pairs shown in Fig. 1A were used to distinguish normal transcripts (NT: constitutive splicing) from splice variants (SV: alternative splicing). Induction of alternative splicing in caspase-2 mRNA was carried out as a control experiment [18]. Multiple proapoptotic stimuli generated the larger fragments of caspase-2 and the smaller fragments of TRAIL (Fig. 1B). Sequence analysis confirmed that these fragments were identical in sequence to caspase-2S and TRAIL-β, respectively. Proapoptotic stimuli-mediated alternative splicing patterns were different between caspase-2 and TRAIL mRNA: staurosporine induced a strong alternative splicing of caspase-2 mRNA, but inhibited both constitutive and alternative splicing of TRAIL mRNA in U937 cells and PBMC (Fig. 1B). All treatments induced a loss of mitochondrial membrane potential ($\Delta\Psi_m$) and an increase in hypodiploidism, indicating induction of apoptosis (Fig. 1C).

Dose- and time-dependent induction of alternative splicing of caspase-2 and TRAIL

We chose etoposide for U937 cells and cycloheximide for PBMC because of their potent activity for inducing alternative splicing (Fig. 1B). RT-PCR revealed that alternative splicing of caspase-2 and TRAIL mRNA was dose-dependent (Fig. 2A). The bands of TRAIL-γ mRNA were not detectable (data not shown). Time-course experiments revealed prompt induction of alternative splicing of caspase-2 and TRAIL mRNA 1 h after stimulation (Fig. 2B), which was significantly time-dependent during apoptosis (Fig. 2C). These results indicated that this induction of alternative splicing occurs transcription-independently and is regulated via signaling pathways other than those controlling protein synthesis.

Phosphatase activation and subsequent ceramide synthesis causes alternative splicing of TRAIL mRNA

Ceramide activates serine/threonine protein phosphatases [20]. We next examined whether this mechanism could

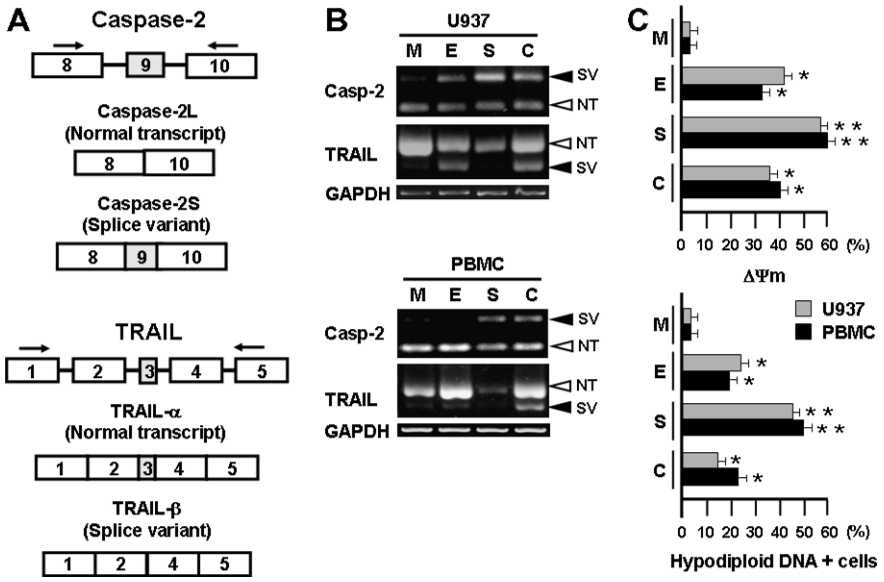

Fig. 1. Characterization of alternative splicing patterns in caspase-2 and TRAIL mRNA. (A) PCR primers were used to distinguish exon-9-containing caspase-2S from exon-9-lacking caspase-2L, and splice variants (TRAILβ: lacks exon-3, and TRAIL-γ: lacks exon-3 and -2) from normal transcripts (TRAIL-α). (B) RT-PCR was used to detect constitutive and alternative splicing of caspase-2 and TRAIL mRNA. Cells were exposed to various proapoptotic stimuli: etoposide (100 μmol/L), staurosporine (1 μmol/L), cycloheximide (100 μg/mL) for 4 h. Total RNA was then extracted and analyzed as described in Materials and Methods. GAPDH is shown as a control. Sample treatment is as follows: mock treatment(M), etoposide(E), staurosporine(S), and cycloheximide(C). (C) FACS was used to evaluate apoptosis, as assayed by the loss of mitochondrial membrane potential ($\Delta\Psi_m$) and DNA fragmentation. Data shown are representative of three independent experiments and expressed as the mean of triplicate samples ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$(Student's *t*-test).

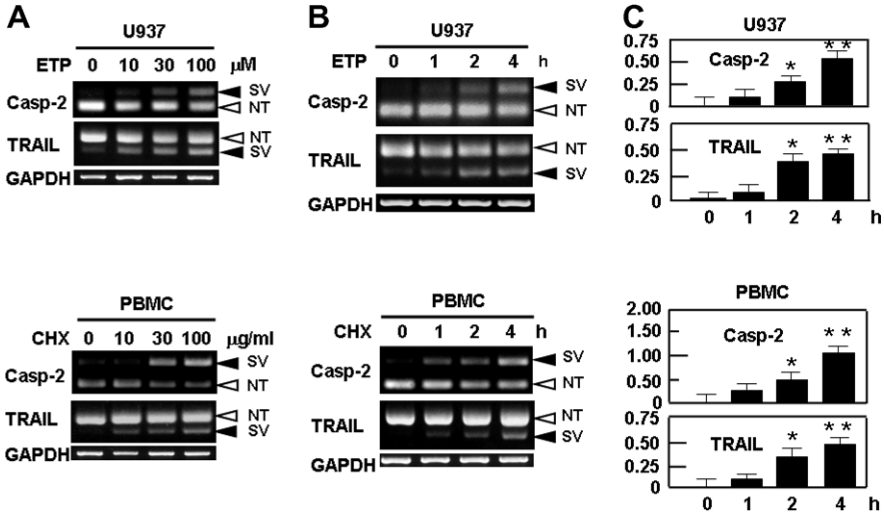

Fig. 2. Dose- and time-dependent induction of alternative splicing. (A) Dose-dependency of induction of caspase-2 and TRAIL alternative splicing. Cells were stimulated with the indicated concentrations for 4 h. (B) Time-dependency of induction of caspase-2 and TRAIL alternative splicing. Cells were stimulated with 100 μmol/L etoposide (U937 cells) or 100 μg/mL cycloheximide (PBMC) for the indicated time periods. (C) Graphic representation of alternative splicing of caspase-2 and TRAIL mRNA. Data shown are representative of three independent experiments and expressed as the mean of triplicate samples ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$(Student's *t*-test).

also induce alternative splicing of TRAIL mRNA. The following chemical inhibitors were used in this series of experiments: calyculin A (a specific inhibitor of serine/threonine protein phosphatase (PP)-1 and PP-2A [21]), okadaic acid (an inhibitor of protein PP-2A [21]), MAP kinase inhibitors SB203580 (a highly specific inhibitor of p38 [22]) and SB202190 (a broad inhibitor of p38 and JNK [23]), and fumonisin B1 (a highly specific inhibitor of *de novo* ceramide synthesis [20]). Calyculin A, but not okadaic acid, inhibited the etoposide-mediated alternative splicing of caspase-2 and TRAIL mRNA in U937 cells (Fig. 3A) and PBMC (Fig. 3E). PP-1-mediated alternative splicing was confirmed by dose-dependent inhibition with calyculin A (Fig. 3B). Neither SB203580 nor SB202190 inhibited alternative splicing of caspase-2 and TRAIL mRNA (data not shown). We next examined whether *de novo* ceramide

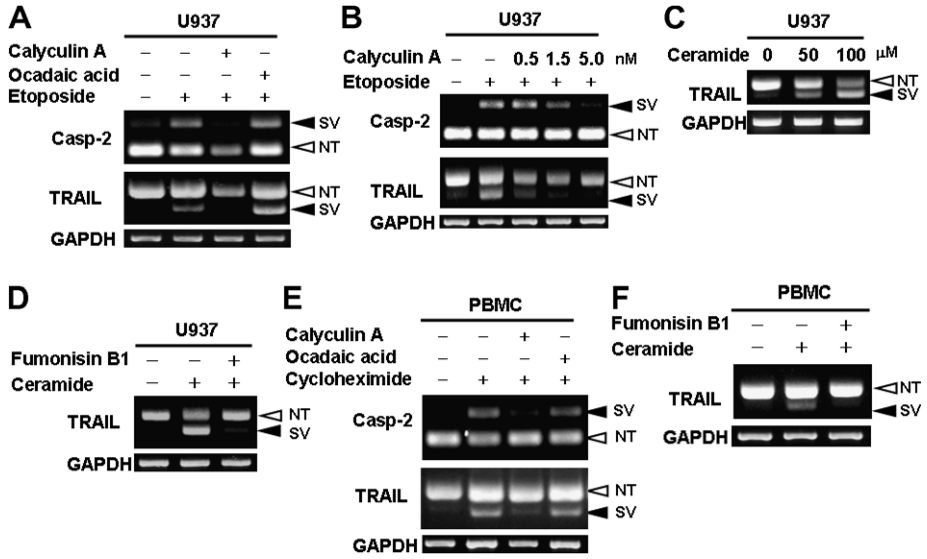

Fig. 3. Chemical inhibition of caspase-2 and TRAIL alternative splicing. (A, E) Cells were pretreated with 5 nmol/L calyculin A or 1 nmol/L okadaic acid for 2 h, and then stimulated with 100 μmol/L etoposide (U937 cells) or 100 μg/mL cycloheximide (PBMC) for 4 h. (B) U937 cells were pretreated with the indicated concentrations of calyculin A for 2 h, and then stimulated with 100 μmol/L etoposide. (C) U937 cells were stimulated with the indicated concentrations of C6-ceramide for 24 h. (D, F) Cells were pretreated with 100 μmol/L fumonisin B1 for 2 h, and then stimulated with 100 μmol/L C6-ceramide for 24 h. GAPDH is shown as a control. Data shown are representative of three independent experiments.

synthesis mediated the alternative splicing of TRAIL mRNA, and found a dose-dependent induction of alternative splicing of TRAIL. In addition, enhancement of alternative splicing decreased the level of constitutive splicing (Fig. 3C). Chemical inhibition with fumonisin B1 blocked alternative splicing of TRAIL mRNA in U937 cells (Fig. 3D) and PBMC (Fig. 3F). Taken together, these results demonstrated that ceramide synthesis and subsequent PP-1 activation governs the alternative splicing of TRAIL mRNA.

TNF-α alters mRNA splicing of TRAIL in U937, Jurkat cells, and PBMC

TNF-α is a proinflammatory cytokine that activates NF-κB pathway and PP-1 [20,24]. Finally, we investigated whether TNF-α alters the mRNA splicing of TRAIL. TNF-α (30 ng/ml) stimulation for 72 h resulted in induction of alternative splicing of TRAIL (Fig. 4), whereas PMA (60 ng/ml) and IFN-α (1000 U/ml) stimulation did not (data not shown). TNF-α stimulation did not induce alternative splicing of caspase-2 mRNA in any cell type (data not shown).

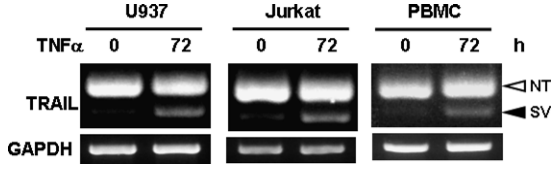

Fig. 4. Induction of alternative splicing by TNF-α stimulation. Cells were stimulated with 30 ng/mL human recombinant TNF-α for 72 h. GAPDH is shown as a control. Data shown are representative of three independent experiments.

Discussion

The present study demonstrated that ceramide synthesis and subsequent PP-1 activation mediates alternative splicing of TRAIL and caspase-2 mRNA in PBMC. Our results are consistent with those reported previously [16–18]. Importantly, our characterization of proapoptotic stimuli-mediated splicing patterns has highlighted two key points: (1) a difference in staurosporine-mediated splicing patterns between caspase-2 and TRAIL (Fig. 1B); and, (2) TNF-α induces alternative splicing of TRAIL mRNA (Fig. 4). The splicing of mRNA involves two major steps: (1) spliceosome assembly, and (2) the removal of intron sequences during a catalytic step [10]. SR proteins participate in RNA-protein and protein-protein interactions during spliceosome assembly, and requiring phosphorylation of SR proteins and other splicing factors during the early stages. Our observations raise the possibility that phosphorylation levels required for spliceosome assembly might be different between caspase-2 and TRAIL and that the difference might affect sequential recognition of splice sites during spliceosome assembly [25]. In this context, the identification of SR protein kinases inhibitors has provided a rationale for controlling or inhibiting splicing and spliceosome assembly [26,27]. Then, phosphatase activation governs the latter catalytic step after assembly [28].

Ceramide generation causes PP-1 activation that is blocked by PP-1 and PP-2A inhibitors, such as calyculin A. Ceramide synthesis occurs through *de novo* or

sphingomyelin hydrolysis pathways in response to extracellular stimuli [29]. The *de novo* pathway is activated in response to TNF-α and chemotherapeutic agents, including etoposide, daunorubicin, hexadecylphosphocholine, and camptothecin derivative CPT-11 [20]. Fumonisin B1 inhibits (dihydro) ceramide synthase, thus effectively inhibiting the *de novo* pathway [20]. This is supported by our results and suggests that chemotherapeutic agents might alter the proapoptotic potential of TRAIL through upregulation of alternative splicing and downregulation of constitutive splicing (Fig. 3C). IFN-α augments TRAIL expression on $CD4^+$ and $CD8^+$ T cells through transcriptional regulation [2]. We demonstrated here that TNF-α alters TRAIL gene expression through post-transcriptional regulation, in this case mRNA splicing. The TRAIL-β splice variant loses proapoptotic potential if massive truncation of the extracellular domain occurs [5]. Taken together, our results imply that variations in the cytokine profile can modulate the proapoptotic potential of TRAIL. In addition, recent studies indicated that TNF-α and IFN-α are antagonistic for the pathogenesis of autoimmune disease [30], further implicating such modifications of TRAIL in autoimmunity. Our results might provide new mechanistic insights into how immune regulation is fine-tuned through mRNA splicing.

In conclusion, the TRAIL gene undergoes alternative splicing through ceramide generation and subsequent phosphatase activation, possibly resulting in the loss of proapoptotic activity.

Acknowledgments

This study was supported in part by a research grant to M.K. from the Ministry of Education, Culture, Sport, Science, and Technology of Japan, and by a Grant-in-Aid for scientific research to M.K. from the Nagasaki Medical Association.

References

[1] A. Ashkenazi, Targeting death and decoy receptors of the tumour-necrosis factor superfamily, Nat. Rev. Cancer 2 (2002) 420–430.
[2] N. Kayagaki, N. Yamaguchi, M. Nakayama, H. Eto, K. Okumura, H. Yagita, Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs, J. Exp. Med. 189 (1999) 1451–1460.
[3] N. Kayagaki, N. Yamaguchi, M. Nakayama, A. Kawasaki, H. Akiba, K. Okumura, H. Yagita, Involvement of TNF-related apoptosis-inducing ligand in human CD4+ T cell-mediated cytotoxicity, J. Immunol. 162 (1999) 2639–2647.
[4] T.S. Griffith, S.R. Wiley, M.Z. Kubin, L.M. Sedger, C.R. Maliszewski, N.A. Fanger, Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL, J. Exp. Med. 189 (1999) 1343–1354.
[5] A. Krieg, T. Krieg, M. Wenzel, M. Schmitt, U. Ramp, B. Fang, H.E. Gabbert, C.D. Gerharz, C. Mahotka, TRAIL-beta and TRAIL-gamma: two novel splice variants of the human TNF-related apoptosis-inducing ligand (TRAIL) without apoptotic potential, Br. J. Cancer 88 (2003) 918–927.
[6] B. Modrek, C. Lee, A genomic view of alternative splicing, Nat. Genet. 30 (2002) 13–19.
[7] L. Cartegni, S.L. Chew, A.R. Krainer, Listening to silence and understanding nonsense: exonic mutations that affect splicing, Nat. Rev. Genet. 3 (2002) 285–298.
[8] C. Shin, J.L. Manley, Cell signalling and the control of pre-mRNA splicing, Nat. Rev. Mol. Cell Biol. 5 (2004) 727–738.
[9] C. Schwerk, K. Schulze-Osthoff, Regulation of apoptosis by alternative pre-mRNA splicing, Mol. Cell. 19 (2005) 1–13.
[10] A. Kramer, The structure and function of proteins involved in mammalian pre-mRNA splicing, Annu. Rev. Biochem. 65 (1996) 367–409.
[11] B.R. Graveley, Sorting out the complexity of SR protein function0073, RNA 6 (2000) 1197–1211.
[12] P.J. Utz, P. Anderson, Posttranslational protein modifications, apoptosis, and the bypass of tolerance to autoantigens, Arthritis. Rheum. 41 (1998) 1152–1160.
[13] M. Kamachi, T.M. Le, S.J. Kim, M.E. Geiger, P. Anderson, P.J. Utz, Human autoimmune sera as molecular probes for the identification of an autoantigen kinase signaling pathway, J. Exp. Med. 196 (2002) 1213–1225.
[14] W.H. Robinson, C. DiGennaro, W. Hueber, B.B. Haab, M. Kamachi, E.J. Dean, S. Fournel, D. Fong, M.C. Genovese, H.E. de Vegvar, K. Skriner, D.L. Hirschberg, R.I. Morris, S. Mulle, G.J. Pruijn, W.J. van Venrooij, J.S. Smolen, P.O. Brown, L. Steinman, P.J. Utz, Autoantigen microarrays for multiplex characterization of autoantibody responses, Nat. Med. 8 (2002) 295–301.
[15] Z.H. Jiang, J.Y. Wu, Alternative splicing and programmed cell death, Proc. Soc. Exp. Biol. Med. 220 (1999) 64–72.
[16] C.E. Chalfant, K. Rathman, R.L. Pinkerman, R.E. Wood, L.M. Obeid, B. Ogretmen, Y.A. Hannun, De novo ceramide regulates the alternative splicing of caspase 9 and Bcl-x in A549 lung adenocarcinoma cells. Dependence on protein phosphatase-1, J. Biol. Chem. 277 (2002) 12587–12595.
[17] N. Iwanaga, M. Kamachi, K. Aratake, Y. Izumi, H. Ida, F. Tanaka, M. Tamai, K. Arima, H. Nakamura, T. Origuchi, A. Kawakami, K. Eguchi, Regulation of alternative splicing of caspase-2 through an intracellular signaling pathway in response to pro-apoptotic stimuli, J. Lab. Clin. Med. 145 (2005) 105–110.
[18] K. Aratake, M. Kamachi, N. Iwanaga, E. Kawasaki, Y. Izumi, H. Ida, F. Tanaka, M. Tamai, K. Arima, H. Nakamura, T. Origuchi, A. Kawakami, K. Eguchi, A cross-talk between RNA splicing and signaling pathway alters Fas gene expression at post-transcriptional level: Alternative splicing of Fas mRNA in the leukemic U937 cells, J. Lab. Clin. Med. 146 (2005) 184–191.
[19] A. Wotawa, S. Solier, E. Logette, E. Solary, L. Corcos, Differential influence of etoposide on two caspase-2 mRNA isoforms in leukemic cells, Cancer Lett. 185 (2002) 181–189.
[20] B.J. Pettus, C.E. Chalfant, Y.A. Hannun, Ceramide in apoptosis: an overview and current perspectives, Biochim. Biophys. Acta 1585 (2002) 114–125.
[21] R.E. Honkanen, T. Golden, Regulators of serine/threonine protein phosphatases at the dawn of a clinical era? Curr. Med. Chem. 9 (2002) 2055–2075.
[22] J.C. Lee, J.T. Laydon, P.C. McDonnell, T.F. Gallagher, S. Kumar, D. Green, D. McNulty, M.J. Blumenthal, J.R. Heys, S.W. Landvatter, et al., A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, Nature 372 (1994) 739–746.
[23] E. Jacinto, G. Werlen, M. Karin, Cooperation between Syk and Rac1 leads to synergistic JNK activation in T lymphocytes, Immunity 8 (1998) 31–41.
[24] H. Wajant, K. Pfizenmaier, P. Scheurich, Tumor necrosis factor signaling, Cell Death Differ. 10 (2003) 45–65.
[25] H. Shen, M.R. Green, A pathway of sequential arginine–serine-rich domain-splicing signal interactions during mammalian spliceosome assembly, Mol Cell. 16 (2004) 363–373.
[26] M. Muraki, B. Ohkawara, T. Hosoya, H. Onogi, J. Koizumi, T. Koizumi, K. Sumi, J. Yomoda, M.V. Murray, H. Kimura,

Furuichi, H. Shibuya, A.R. Krainer, M. Suzuki, M. Hagiwara, Manipulation of alternative splicing by a newly developed inhibitor of Clks, J. Biol. Chem. 279 (2004) 24246–24254.

[27] T. Fukuhara, T. Hosoya, S. Shimizu, K. Sumi, T. Oshiro, Y. Yoshinaka, M. Suzuki, N. Yamamoto, L.A. Herzenberg, L.A. Herzenberg, M. Hagiwara, Utilization of host SR protein kinases and RNA-splicing machinery during viral replication, Proc. Natl. Acad. Sci. USA 103 (2006) 11329–11333.

[28] J.E. Mermoud, P.T. Cohen, A.I. Lamond, Regulation of mammalian spliceosome assembly by a protein phosphorylation mechanism, EMBO J. 13 (1994) 5679–5688.

[29] Y.A. Hannun, C. Luberto, Ceramide in the eukaryotic stress response, Trends Cell Biol. 10 (2000) 73–80.

[30] J. Banchereau, V. Pascual, A.K. Palucka, Autoimmunity through cytokine-induced dendritic cell activation, Immunity 20 (2004) 539–550.