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TWE-PRIL; a fusion protein of TWEAK and APRIL

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Abstract

TWEAK and APRIL are both members of the tumor necrosis factor family, which are involved in respectively angiogensis and immune regulation. While TWEAK is processed at the cell surface, APRIL is processed inside the cell by a furin-convertase and is solely able to perform its function as a soluble factor. Recently, TWE-PRIL has been identified, which is an endogenous hybrid transcript between TWEAK and APRIL. TWE-PRIL is a transmembrane protein that consists of a TWEAK intracellular, transmembrane and stalk region combined with APRIL as its receptor-binding domain. As such TWE-PRIL is expressed at the cell surface. Although TWE-PRIL, like APRIL, can stimulate T and B cell lines, distinct biological functions that may result from its membrane anchoring cannot be excluded. Understanding the function of this newly identified protein will contribute to the elucidation of the complexity of the tumor necrosis factor family.

Keywords: Immune regulation; TNF family; TWEAK; APRIL; Fusion protein

1. Introduction

The balance between life and death is essential in the regulation of the immune system. The discovery of several new members of the tumor necrosis factor (TNF) family has contributed to the elucidation of regulatory mechanisms in this complex system. The TNF family consists of receptors and ligands involved in the regulation of differentiation, survival, proliferation and death pathways [1]. TNF receptor family members are transmembrane type I proteins. Their N-terminal domain contains between one and four cysteine-rich repeats that constitute the ligand-binding site. The C-terminal domain of the receptor is responsible for signaling, which typically occurs *via* adaptor molecules like TRADD [2], FADD [3,4], RAIDD [5] or TRAFs [6]. TRADD can be regarded as an upstream adaptor that creates a platform to which FADD and TRAFs can bind. TRAFs can activate NF-κB and c-*jun* N-terminal kinase, resulting in proliferation and differentiation [7]. In contrast, FADD recruits regulatory domains of the initiator caspases-8 and -10, resulting in cell death. Similarly, RAIDD has been shown to couple to caspase-2 and initiate apoptosis. It is important to note, however, that this separation is not as strict, as FADD, for instance, can recruit RIP kinase and also mediate NF-κB activation [8].

TNF ligands are transmembrane type II proteins that contain a C-terminal domain oriented towards the cytoplasm. The extracellular domain contains a β jellyroll topography [9] and is important for trimerization. The trimerization is, with a possible exception for CD70 important for the function of the ligands. Cleavage in the stalk region between the transmembrane segment- and receptor-binding domains is frequently observed. Processing and subsequent release of the membrane bound ligand occurs either to release a biologically active form (e.g. TNF), or to downregulate transmembrane activity (CD95L). TNF family members can therefore act as soluble or transmembrane ligands depending on the ligand studied. The family of TNF ligands can be divided into

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Abbreviations: TNF, tumor necrosis factor; BCMA, B cell maturation antigen; TACI, transmembrane activator and calcium modulator ligand interactor.

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subfamilies. For instance, one subfamily is made up of all death domain containing receptors. This review will focus on TWE-PRIL, a fusion protein between TWEAK and APRIL, which belong to a subfamily that is made up of BAFF, APRIL, TWEAK and EDA [10].

2. TWEAK

TWEAK (TNFSF12) was first identified as a factor that is able to induce apoptosis in several tissues and cell lines [11–13]. In agreement, a recent paper shows that TWEAK is expressed on human peripheral blood (PB) monocytes upon IFNγ stimulation and seems to be involved in IFNγ stimulated cytotoxicity against TWEAK sensitive tumor cells [14]. In contrast to this cytotoxic effect, it has been reported that TWEAK induces NF-κB activation [11], proliferation of endothelial cells and subsequently angiogenesis [15]. Like several other TNF family members, TWEAK is cleaved at the cell surface by furin, a ubiquitously expressed pro-protein convertase that processes many inactive precursors including hormones, growth factors and receptors [16]. This processing at the furin cleavage site located on the N-terminal side of the TNF homology domain permits the release of a soluble form of TWEAK, which is able to weakly induce apoptosis and play a role in angiogenesis [11,17]. However, TWEAK is active as a membrane-bound form in IFNγ stimulated monocytes as well [14]. This indicates that TWEAK is active in both forms, although a biological difference cannot be excluded (Fig. 1).

3. TWEAK receptors

The group of Marsters *et al*. identified TWEAK as a ligand for the DR3/Apo3/wsl/TRAMP/LARD receptor [12]. The cytoplasmic death domain (DD) of DR3 shows homology with the DD of TNFR-1, Fas, TRAIL-R1 and TRAIL-R2 and DR6, and can induce apoptosis as well as NF-κB activation by recruiting TRADD, TRAF, FADD and caspase-8 [10,18,19]. DR3 is expressed in tissues of the immune system, specifically the spleen, thymus and peripheral blood leukocytes (PBLs) with little or no expression in the other tissues examined [20]. Conflicting data, however, have been reported on this TWEAK–DR3 interaction. Schneider *et al*. show that TWEAK-induced apoptosis is indirect and critically depends on the interaction of endogenous TNFα with TNF-R1 [13]. More importantly, Kym-1 cells do not express DR3, while binding of TWEAK to the cell surface is clearly detected. In addition, Kaptein *et al*. [21] show that TWEAK could not bind to DR3, using an *in vitro* binding study. These groups therefore proposed the existence of an unidentified receptor for TWEAK. Such a receptor was recently cloned from a human umbilical vein endothelial cell (HUVEC) library [22]. The cytoplasmic domain of Fn14 is able to bind to TRAFs 1, 2, and 3. Cross-linking of Fn14 induces HUVEC growth, and its mRNA levels are upregulated *in vitro* by a variety of agents as well as *in vivo* following arterial injury. Soluble Fn14 inhibits endothelial cell migration *in vitro* and corneal angiogenesis *in vivo*. Besides a role in angiogenesis Fn14 also plays a role in apoptosis [23]. Fn14 is expressed on the cell surface of all TWEAK sensitive

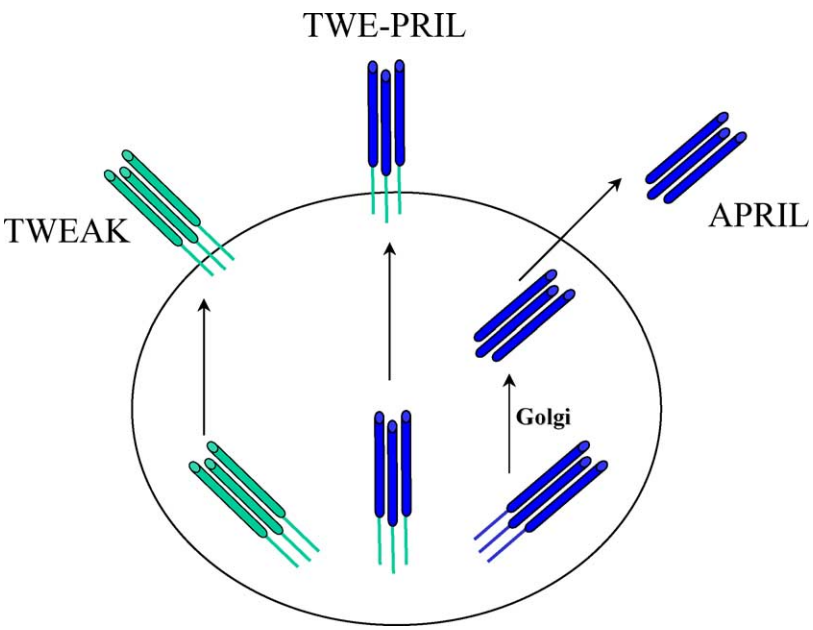

Fig. 1. Generation of APRIL TWE-PRIL and TWEAK by one cell. APRIL (blue) is produced, processed in the Golgi and then secreted as a soluble form that only contains the receptor-binding region. TWEAK (green) is produced as a transmembrane protein that goes to the cell surface. TWE-PRIL is produced as a fusion protein between TWEAK and APRIL (green/blue) and contains the transmembrane intracellular region of TWEAK and the receptor-binding region of APRIL. As such it goes to the cell surface.

tumor cell lines. Moreover, an anti-Fn14 mAb that blocks TWEAK–Fn14 interaction could totally abrogate TWEAK-binding and TWEAK-induced cell death in all these tumor cell lines. Taken together, these results revealed that TWEAK-induced cell death and angiogenesis are solely mediated by Fn14 and that the TWEAK–Fn14 interaction can stimulate the NF-κB transcription factor-signaling pathway.

4. APRIL

APRIL transcript levels were reported to be low in normal tissues, among which the highest levels are found in PBLs [24]. In contrast, higher mRNA levels were detected in leukemia cell lines, and lymphoid tissues [24,25] and endogenous APRIL protein has been detected in human myeloid leukemia cell lines, including U937 and Mono Mac 1 4 [26].

APRIL is found close to the telomeric end of chromosome 17p13.1 and in a similar synthetic position on mouse chromosome 11 [27]. In both the mouse and human genome the APRIL gene is flanked within 1–2 kb by TWEAK and on the other side by Smt3ip1/Senp-3, a member of the sentrin/SUMO specific protease family [28]. APRIL shares 50% similarity with BAFF (also THANK, TALL-1, BlyS and zTNF4), as well as several biological activities [10]. The difference between APRIL and BAFF is that BAFF is released from the cell surface by processing at the cell membrane, while APRIL is processed in the Golgi by a furin convertase, prior to its secretion [29]. APRIL can therefore solely act as a soluble factor, which intracellularly regulated, is a exceptional maturation pathway among the TNF ligand family members.

It has been shown that APRIL is able to promote tumor growth. APRIL-transfected NIH-3T3 cells display an increased growth rate *in vitro*, but more importantly greatly enhance tumor outgrowth *in vivo* as compared to their control-transfected counterparts [24]. In contrast, others have shown that APRIL can display pro-apoptotic activity, similar to CD95L [27]. Furthermore, APRIL can have a protective effect against death-induced apoptosis [30]. This is associated with an upregulation of X-linked inhibitor of apoptosis (XIAP), a member of the inhibitor-of-apoptosis (IAP) family that are thought to block death *via* indirect inhibition of caspases [31].

More recent data show that APRIL can act as a co-stimulator of primary T and B cells [32], suggesting a role for this ligand in immune regulation. To analyse this in further detail, we generated mice expressing APRIL as a transgene in T cells [33]. The transgenic mice appear normal and do not show any B cell hyperplasia. *In vitro*, however, transgenic T cells reveal a greatly enhanced survival, which is detected in activated CD4 T cells *in vivo* as well. This increased survival correlated with elevated expression levels of the anti-apoptotic protein Bcl-2. Besides its effects on T cells, transgenic expression of APRIL affects T cell independent humoral responses. This is likely explained by the more recent observation of Litinskiy *et al.*, which show that dendritic cells upregulate APRIL expression upon exposure to interferon-alpha, interferon-gamma or CD40 ligand and that this induces class-switching in B cells [34]. Taken together, these results suggest that APRIL is able to critically link the innate and adaptive immune responses.

Unfortunately, however, these suggestions could not be corroborated by deletion of the APRIL gene as conflicting results have been reported on the APRIL knockout. One report states that deletion of the APRIL gene led to embryonic lethality, which was likely due to a defect in the development of the heart (reviewed in [35]). This might implicate for an important and unique function for APRIL during development. In contrast, however, the group of Ashkenazi have successfully generated a mouse deficient for APRIL. This strain shows no histological abnormalities and more importantly no defects in T or B cell responses (A. Ashkenazi, personal communication). Although, this would argue for a redundant role of APRIL, it is not yet clear how these apparently conflicting findings can be resolved.

5. APRIL receptors

Two receptors of the TNF family have been identified that can bind to both APRIL and BAFF, called B cell maturation antigen (BCMA) and transmembrane activator and calcium modulator ligand interactor (TACI) [36–40]. More recently, a third receptor has been identified, which can bind only to BAFF, BAFF-R/BR3 [41,42]. BCMA and BAFF-R are expressed on B cells, whereas TACI is also expressed on activated T cells [43]. Studies with TACI- and BCMA-deficient mice revealed that the TACI-deficient mice show B cell expansion rather than death, while the phenotype of the BCMA-deficient mice seems normal [44–47]. This might indicate for a negative role for TACI during the regulation of B cell growth and a possibly redundant role for BCMA. In agreement, a recent paper shows that TACI knockout mice develop fatal lymphoproliferation and autoimmunity [48]. Furthermore, TACI agonistic antibodies inhibit proliferation and a chimeric receptor containing the TACI intracellular domain induces apoptosis, suggesting that TACI plays an important role in B cell homeostasis.

The signal transduction pathways utilized by BCMA, BAFF-R and TACI are only partly characterized. The receptors do not contain death domain motifs, however, can bind to TRAF through TRAF-binding sites present in the intracellular domain of the receptors. Activation of the BCMA receptor results in activation of NF-κB, p38, MAPK, and JNK [49], while activation of TACI results

in activation of the transcription factors NF-κB, AP-1 and NF-AT [50].

The thought of the existence of a third receptor of APRIL first came to the light when several cell lines showed positive FACS staining upon incubation with Flag-APRIL [51], while these cell lines were deficient for TACI and BCMA on Northern blot analysis. In agreement, BAFF did not bind to these cells. Taken together, these results support the existence of a third receptor that is specific for APRIL. In agreement, the results obtained on immune regulation could also not be explained by the expression of TACI or BCMA. BCMA is only expressed on B cells, while TACI is only expressed on activated T cells. T cells will respond to APRIL independently of their activation and therefore it seems that a third receptor must be present, which is responsible for this stimulation. In addition, it has recently been reported that besides acting as homotrimers, BAFF and APRIL together can form heterotrimeric complexes. Such complexes have been found in the serum of patients with systemic immune-based rheumatic diseases [52]. However, their exact function remains to be elucidated.

6. TWE-PRIL

TWE-PRIL was first detected when Northern blot analysis were performed on RNA samples obtained from naïve T cells and T cells activated with a combination of anti-CD3 and anti-CD28 [26]. Besides APRIL mRNA a second larger mRNA appeared that could also be detected in two monocytic cell lines as well as tumor cell lines. This mRNA was identified as being a TWEAK–APRIL hybrid. The human TWEAK gene is situated 878 bp upstream of the APRIL transcription start site. Human TWEAK contains seven exons and six introns, while human APRIL contains six exons and five introns, which are transcribed to mRNA. TWE-PRIL mRNA compasses TWEAK exons 1–6, fused to APRIL exons 2–6 using the splice donor/acceptor sites of TWEAK and APRIL. The TWE-PRIL protein is a membrane-bound protein, consisting of the intracellular, transmembrane and stalk region of TWEAK fused to the APRIL receptor-binding domain. Transfection experiments revealed that TWE-PRIL is expressed at the membrane as measured by immunohistochemistry and FACS analysis. In addition, Western blot analysis showed expression of TWE-PRIL with both anti-TWEAK and anti-APRIL antibodies. More importantly, endogenous protein expression of TWE-PRIL is observed at significant levels in T cells, as well as in a monocytic cell line. Previous studies showed that APRIL induces proliferation in Jurkat cells [24,29]. To test whether TWE-PRIL displayed a similar biological activity, TWE-PRIL transfectants were co-cultured with Jurkat and Ramos cells. This indeed resulted in an increase in cell division, indicating that TWE-PRIL is able to induce cell proliferation.

As APRIL and TWE-PRIL share the same receptor-binding domain, they will probably bind to the same receptors. Ramos cells express both TACI and BCMA [51], while Jurkat is deficient for those receptors, yet is sensitive to APRIL. It therefore likely expresses the unidentified receptor for APRIL. As TWE-PRIL promotes proliferation in both these cell lines, this indicates that TWE-PRIL recognizes the same receptors as APRIL. Whether TWE-PRIL and APRIL recognize the receptors with the same affinity, however, remains to be determined. In this case it is interesting to note that the transmembrane form of TNF is superior to soluble TNF in activating TNFR2 [53], suggesting that differences in affinity for the same receptor can occur.

Intergenic splicing of eukaryotic genes has been reported before [54–59]. This is the first time, however, that the splicing affects two genes of the same family and results in a functional protein. Another member of this subfamily, EDA, also consists of two isoforms, EDA-A1 and EDA-A2, which only differ by the insertion of two amino acids [60]. This insertion functions to determine receptor-binding specificity, such that EDA-A1 binds only the receptor EDAR, whereas EDA-A2 binds only the related, but distinct, X-linked ectodysplasin-A2 receptor (XEDAR). Apparently, this TNF subfamily is characterized by receptor sharing (BAFF, APRIL and TWE-PRIL) as well as ligand splicing (TWEAK/APRIL, EDA).

Although it has been reported that TWE-PRIL, like APRIL, induces proliferation in Jurkat and Ramos cells, a different biological role cannot be excluded. The same phenomenon, for example, has also been described for TNF, which acts differently as a soluble form as compared to the membrane-bound form. Membrane bound TNF supports many features of lymphoid organ structure, while soluble TNF is needed for optimal inflammatory responses [61]. As a membrane-bound form TWE-PRIL will solely act in cell–cell contact, while this is not required for processed APRIL. The possibility that APRIL may act as a soluble and as a membrane-bound factor is an intriguing one that will allow temporal and spatial regulation of an immunoregulatory ligand. Further analysis into the role of the different forms is therefore awaited, but will undoubtedly add to the complexity of the TNF family.

References

[1] Smith CA, Farrah T, Goodwin RG. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. Cell 1994;76:959–62.
[2] Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. Cell 1995;81:495–504.
[3] Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 1995;81:505–12.
[4] Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D. A novel protein that interacts with the death domain of Fas/APO1

contains a sequence motif related to the death domain. J Biol Chem 1995;270:7795–8.
[5] Duan H, Dixit VM. RAIDD is a new 'death' adaptor molecule. Nature 1997;385:86–9.
[6] Rothe M, Pan MG, Henzel WJ, Ayres TM, Goeddel DV. The TNFR2–TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell 1995;83:1243–52.
[7] Song HY, Regnier CH, Kirschning CJ, Goeddel DV, Rothe M. Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-kappaB and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. Proc Natl Acad Sci USA 1997;94:9792–6.
[8] Hu WH, Johnson H, Shu HB. Activation of NF-kappaB by FADD, Casper, and caspase-8. J Biol Chem 2000;275:10838–44.
[9] Fesik SW. Insights into programmed cell death through structural biology. Cell 2000;103:273–82.
[10] Bodmer JL, Schneider P, Tschopp J. The molecular architecture of the TNF superfamily. Trends Biochem Sci 2000;27:19–26.
[11] Chicheportiche Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, Garcia I, Browning JL. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem 1997;272:32401–10.
[12] Marsters SA, Sheridan JP, Pitti RM, Brush J, Goddard A, Ashkenazi A. Identification of a ligand for the death-domain-containing receptor Apo3. Curr Biol 1998;8:525–8.
[13] Schneider P, Schwenzer R, Haas E, Muhlenbeck F, Schubert G, Scheurich P, Tschopp J, Wajant H. TWEAK can induce cell death via endogenous TNF and TNF receptor 1. Eur J Immunol 1999; 29:1785–92.
[14] Nakayama M, Kayagaki N, Yamaguchi N, Okumura K, Yagita H. Involvement of TWEAK in interferon gamma-stimulated monocyte cytotoxicity. J Exp Med 2000;192:1373–80.
[15] Lynch CN, Wang YC, Lund JK, Chen YW, Leal JA, Wiley SR. TWEAK induces angiogenesis and proliferation of endothelial cells. J Biol Chem 1999;274:8455–9.
[16] Molloy SS, Anderson ED, Jean F, Thomas G. Bi-cycling the furin pathway: from TGN localization to pathogen activation and embryogenesis. Trends Cell Biol 1999;9:28–35.
[17] Jakubowski A, Browning B, Lukashev M, Sizing I, Thompson JS, Benjamin CD, Hsu YM, Ambrose C, Zheng TS, Burkly LC. Dual role for TWEAK in angiogenic regulation. J Cell Sci 2002;115:267–74.
[18] Chinnaiyan AM, O'Rourke K, Yu GL, Lyons RH, Garg M, Duan DR, Xing L, Gentz R, Ni J, Dixit VM. Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. Science 1996;274:990–2.
[19] Screaton GR, Xu XN, Olsen AL, Cowper AE, Tan R, McMichael AJ, Bell JI. LARD: a new lymphoid-specific death domain containing receptor regulated by alternative pre-mRNA splicing. Proc Natl Acad Sci USA 1997;94:4615–9.
[20] Kitson J, Raven T, Jiang YP, Goeddel DV, Giles KM, Pun KT, Grinham CJ, Brown R, Farrow SN. A death-domain-containing receptor that mediates apoptosis. Nature 1996;384:372–5.
[21] Kaptein A, Jansen M, Dilaver G, Kitson J, Dash L, Wang E, Owen MJ, Bodmer JL, Tschopp J, Farrow SN. Studies on the interaction between TWEAK and the death receptor WSL-1/TRAMP (DR3). FEBS Lett 2000;485:135–41.
[22] Wiley SR, Cassiano L, Lofton T, Davis-Smith T, Winkles JA, Lindner V, Liu H, Daniel TO, Smith CA, Fanslow WC. A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. Immunity 2001;15:837–46.
[23] Nakayama M, Ishidoh K, Kojima Y, Harada N, Kominami E, Okumura K, Yagita H. Fibroblast growth factor-inducible 14 mediates multiple pathways of TWEAK-induced cell death. J Immunol 2003; 170:341–8.
[24] Hahne M, Kataoka T, Schroter M, Hofmann K, Irmler M, Bodmer JL, Schneider P, Bornand T, Holler N, French LE, Sordat B, Rimoldi D, Tschopp J. APRIL, a new ligand of the tumor necrosis factor family stimulates tumor cell growth. J Exp Med 1998;188:1185–90.
[25] Novak AJ, Bram RJ, Kay NE, Jelinek DF. Aberrant expression of B-lymphocyte stimulator by B chronic lymphocytic leukemia cells: a mechanism for survival. Blood 2002;100:2973–9.
[26] Pradet-Balade B, Medema JP, Lopez-Fraga M, Lozano JC, Kolfschoten GM, Picard A, Martinez AC, Garcia-Sanz JA, Hahne M. An endogenous hybrid mRNA encodes TWE-PRIL a functional cell surface TWEAK–APRIL fusion protein. EMBO J 2002;21:5711–20.
[27] Kelly K, Manos E, Jensen G, Nadauld L, Jones DA. APRIL/TRDL-1, a tumor necrosis factor-like ligand, stimulates cell death. Cancer Res 2000;60:1021–7.
[28] Nishida T, Kaneko F, Kitagawa M, Yasuda H. Characterization of a novel mammalian SUMO-1/Smt3-specific isopeptidase, a homologue of rat axam, which is an axin-binding protein promoting beta-catenin degradation. J Biol Chem 2001;276:39060–6.
[29] Lopez-Fraga M, Fernandez R, Albar JP, Hahne M. Biologically active APRIL is secreted following intracellular processing in the Golgi apparatus by furin convertase. EMBO Rep 2001;2:945–51.
[30] Roth W, Wagenknecht B, Klumpp A, Naumann U, Hahne M, Tschopp J, Weller M. APRIL, a new member of the tumor necrosis factor family, modulates death ligand-induced apoptosis. Cell Death Differ 2001;8:403–10.
[31] Deveraux QL, Takahashi R, Salvesen GS, Reed JC. X-linked IAP is a direct inhibitor of cell-death proteases. Nature 1997;388:300–4.
[32] Yu G, Boone T, Delaney J, Hawkins N, Kelley M, Ramakrishnan M, McCabe S, Qiu WR, Kornuc M, Xia XZ, Guo J, Stolina M, Boyle WJ, Sarosi I, Hsu H, Senaldi G, Theill LE. APRIL and TALL-I and receptors BCMA and TACI: system for regulating humoral immunity. Nat Immunol 2001;1:252–6.
[33] Stein JV, Lopez-Fraga M, Elustondo FA, Carvalho-Pinto CE, Rodriguez D, Gomez-Caro R, De Jong J, Martinez AC, Medema JP, Hahne M. APRIL modulates B and T cell immunity. J Clin Invest 2002; 109:1587–98.
[34] Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, Cerutti A. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 2002;3:822–9.
[35] Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: a tutorial on B cell survival. Annu Rev Immunol 2003;21:231–64.
[36] Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K, Xu W, Parrish-Novak J, Foster D, Lofton-Day C, Moore M, Littau A, Grossman A, Haugen H, Foley K, Blumberg H, Harrison K, Kindsvogel W, Clegg CH. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature 2000;404:995–9.
[37] Marsters SA, Yan M, Pitti RM, Haas PE, Dixit VM, Ashkenazi A. Interaction of the TNF homologues BLyS and APRIL with the TNF receptor homologues BCMA and TACI. Curr Biol 2000;10:785–8.
[38] Thompson JS, Schneider P, Kalled SL, Wang L, Lefevre EA, Cachero TG, MacKay F, Bixler SA, Zafari M, Liu ZY, Woodcock SA, Qian F, Batten M, Madry C, Richard Y, Benjamin CD, Browning JL, Tsapis A, Tschopp J, Ambrose C. BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population. J Exp Med 2000; 192:129–35.
[39] Yan M, Marsters SA, Grewal IS, Wang H, Ashkenazi A, Dixit VM. Identification of a receptor for BLyS demonstrates a crucial role in humoral immunity. Nat Immunol 2000;1:37–41.
[40] Xia XZ, Treanor J, Senaldi G, Khare SD, Boone T, Kelley M, Theill LE, Colombero A, Solovyev I, Lee F, McCabe S, Elliott R, Miner K, Hawkins N, Guo J, Stolina M, Yu G, Wang J, Delaney J, Meng SY, Boyle WJ, Hsu H. TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. J Exp Med 2000;192:137–43.
[41] Thompson JS, Bixler SA, Qian F, Vora K, Scott ML, Cachero TG, Hession C, Schneider P, Sizing ID, Mullen C, Strauch K, Zafari M, Benjamin CD, Tschopp J, Browning JL, Ambrose C. BAFF-R, a newly

identified TNF receptor that specifically interacts with BAFF. Science 2001;293:2108–11.
[42] Yan M, Brady JR, Chan B, Lee WP, Hsu B, Harless S, Cancro M, Grewal IS, Dixit VM. Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. Curr Biol 2001;11:1547–52.
[43] Khare SD, Hsu H. The role of TALL-1 and APRIL in immune regulation. Trends Immunol 2001;22:61–3.
[44] Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, Frew E, Scott ML. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science 2001;293:2111–4.
[45] von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. Immunity 2001;14:573–82.
[46] Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T, Tumas D, Grewal IS, Dixit VM. Activation and accumulation of B cells in TACI-deficient mice. Nat Immunol 2001;2:638–43.
[47] Xu S, Lam KP. B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensable for humoral immune responses. Mol Cell Biol 2001;21:4067–74.
[48] Seshasayee D, Valdez P, Yan M, Dixit VM, Tumas D, Grewal IS. Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. Immunity 2003;18:279–88.
[49] Hatzoglou A, Roussel J, Bourgeade MF, Rogier E, Madry C, Inoue J, Devergne O, Tsapis A. TNF receptor family member BCMA (B cell maturation) associates with TNF receptor-associated factor (TRAF) 1, TRAF2, and TRAF3 and activates NF-kappa B, elk-1, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase. J Immunol 2000;165:1322–30.
[50] von Bulow GU, Bram RJ. NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. Science 1997;278:138–41.
[51] Rennert P, Schneider P, Cachero TG, Thompson J, Trabach L, Hertig S, Holler N, Qian F, Mullen C, Strauch K, Browning JL, Ambrose C, Tschopp J. A soluble form of B cell maturation antigen, a receptor for the tumor necrosis factor family member APRIL, inhibits tumor cell growth. J Exp Med 2000;192:1677–84.
[52] Roschke V, Sosnovtseva S, Ward CD, Hong JS, Smith R, Albert V, Stohl W, Baker KP, Ullrich S, Nardelli B, Hilbert DM, Migone TS. BLyS and APRIL form biologically active heterotrimers that are expressed in patients with systemic immune-based rheumatic diseases. J Immunol 2002;169:4314–21.
[53] Grell M, Douni E, Wajant H, Lohden M, Clauss M, Maxeiner B, Georgopoulos S, Lesslauer W, Kollias G, Pfizenmaier K, et al. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell 1995;83:793–802.
[54] Fears S, Mathieu C, Zeleznik-Le N, Huang S, Rowley JD, Nucifora G. Intergenic splicing of MDS1 and EVI1 occurs in normal tissues as well as in myeloid leukemia and produces a new member of the PR domain family. Proc Natl Acad Sci USA 1996;93:1642–7.
[55] Magrangeas F, Pitiot G, Dubois S, Bragado-Nilsson E, Cherel M, Jobert S, Lebeau B, Boisteau O, Lethe B, Mallet J, Jacques Y, Minvielle S. Cotranscription and intergenic splicing of human galactose-1-phosphate uridylyltransferase and interleukin-11 receptor alpha-chain genes generate a fusion mRNA in normal cells. Implication for the production of multidomain proteins during evolution. J Biol Chem 1998;273:16005–10.
[56] Moore RC, Lee IY, Silverman GL, Harrison PM, Strome R, Heinrich C, Karunaratne A, Pasternak SH, Chishti MA, Liang Y, Mastrangelo P, Wang K, Smit AF, Katamine S, Carlson GA, Cohen FE, Prusiner SB, Melton DW, Tremblay P, Hood LE, Westaway D. Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel. J Mol Biol 1999;292:797–817.
[57] Finta C, Zaphiropoulos PG. The human CYP2C locus: a prototype for intergenic and exon repetition splicing events. Genomics 2000;63:433–8.
[58] Millar JK, Christie S, Semple CA, Porteous DJ. Chromosomal location and genomic structure of the human translin-associated factor X gene (TRAX TSNAX) revealed by intergenic splicing to DISC1, a gene disrupted by a translocation segregating with schizophrenia. Genomics 2000;67:69–77.
[59] Communi D, Suarez-Huerta N, Dussossoy D, Savi P, Boeynaems JM. Cotranscription and intergenic splicing of human P2Y11 and SSF1 genes. J Biol Chem 2001;276:16561–6.
[60] Yan M, Wang LC, Hymowitz SG, Schilbach S, Lee J, Goddard A, de Vos AM, Gao WQ, Dixit VM. Two-amino acid molecular switch in an epithelial morphogen that regulates binding to two distinct receptors. Science 2001;290:523–7.
[61] Ruuls SR, Hoek RM, Ngo VN, McNeil T, Lucian LA, Janatpour MJ, Korner H, Scheerens H, Hessel EM, Cyster JG, McEvoy LM, Sedgwick JD. Membrane-bound TNF supports secondary lymphoid organ structure but is subservient to secreted TNF in driving autoimmune inflammation. Immunity 2001;15:533–43.