Crystal structure of Sema6A-PlxnA2 complex

We next crystallized the Sema6A–PlxnA2 complex by mixing Sema6A and PlxnA2 at an equimolar concentration, obtaining a structure at 3.6Å resolution. The Sema6A and PlxnA2 molecules constitute a 2:2 complex in the crystal, which contained a crystallographic two-fold symmetry (Fig. 3a). The two Sema6A molecules in the complex formed the same face-to-face dimer as was observed in the plexin-free state. On the other hand, the PlxnA2 head-on homodimer was no longer present in the complex, and the two plexin molecules independently docked onto the two Sema6A monomers with their carboxy-terminal PSI (plexin–semaphorin–integrin) domains emanating away diagonally. Despite their participation in different molecular interactions, there were no major changes in the structure of individual Sema6A and PlxnA2 monomers, including the conformation of the loops at the interface, upon the complex formation. At the interface, the Sema6A side showed positively charged surface potentials whereas the PlxnA2 side was negatively charged, indicating that complex formation is driven mainly by electrostatic interactions (Fig. 3b).

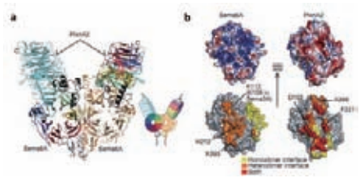

Fig.3 Crystal structure of Sema6A-PlxnA2 2:2 complex. (a) The complex representing post-signalling states. (b) Open-book view of the Sema6A–PlxnA2 interface surface coloured by electrostatic potential (top panel) and by the residue-wise contribution to the interface (bottom panel).

Mutational experiments based on the crystal structure

Cell-based activity measurement using mutant ligands/receptors confirmed that the current complex structure captures genuine receptor-ligand interactions. In addition, functional importance of Sema6A dimer was also indicated (Fig. 4). These mutational experiments based on the crystal structure showed that the hetero-tetrameric configuration of the Sema6A-PlxnA2 complex seen in the crystal structure represents a signalling–competent conformation maintained throughout the signal transduction process.

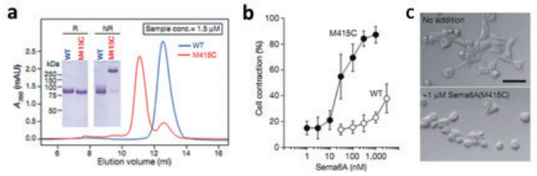

Fig.4 The Sema6A face-to-face homodimer represents a signalling-competent active conformation. (a) Sema6ASP ectodomain fragments (WT or M415C mutant) were subjected to gel filtration chromatography. The peak elution positions for the wild type and M415C mutant corresponded to 106 and 219 kDa, respectively. SDS–PAGE analysis under reducing (R) or non-reducing (NR) conditions (inset) confirmed the 90% formation of a disulphide-linked homodimer in theM415C mutant. (b) Signalling activities of soluble Sema6A proteins. Purified Sema6ASP proteins (WT or M415C) were tested for their ability to induce contraction of HEK293T cells stably expressing PlxnA4. (c) Representative images of cell morphologies both before and after the stimulation are also shown (right). Scale bar, 50 mm.

Conclusion

Plexins on the resting cell surface assume an 'auto-inhibited' state, with their cytoplasmic GAP domain activity suppressed. It is also accepted that ligand engagement at the extracellular side somehow activates GAP. Although it is still unclear how the activity of the GAP domain is structurally regulated (for example, by a monomer/dimer exchange or conformational changes within a single domain), our current structure clearly identifies the structural change that takes place at the extracellular side. In the resting state, plexin assumes an auto-inhibited conformation, possibly by structural constraints stemming from head-on dimerization (Fig. 5, left). Upon semaphorin engagement, the orientation of the two plexin heads becomes more perpendicularly aligned to the membrane (Fig. 5, right).

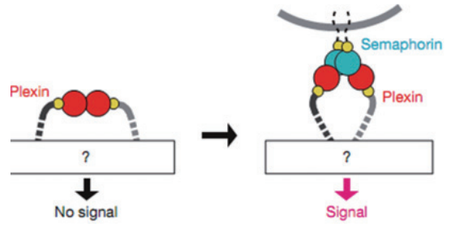

Fig.5 Possible structural mechanism of semaphorin-induced plexin signalling. Transition from the head-on cis homodimer of plexin (left) to the semaphorin-engaged complex (right) changes the relative orientation of the plexin molecular axis. This conformational change is transmitted through the stalk region (thick dotted line) and alters the conformation (for example, dimerization state) of the cytoplasmic GAP domain, resulting in signal initiation. The closer positioning of the two plexin tails in the active conformation is drawn arbitrarily and should be taken as an example, because the association states of the transmembrane and cytoplasmic regions before and after receptor activation remain unknown.

This conformational change is then transmitted, through the long stalk and the transmembrane domain, to the cytoplasmic region, leading to activation of the GAP domain and/or recruitment of Rho family GTPases. Although it remains possible that most of the cell-surface plexins in the resting state do not form a head-on dimer and the inactive phenotype is maintained by another type of mechanism, the structural conservation observed between the two semaphoring-plexin 'terminal' complexes is strongly indicative of the fundamental importance of this conformation in plexin signal transduction. More structural data are needed regarding the rest of the molecule, particularly the stalk region and the GAP domain, under different activation states, in order to understand fully the mechanism underlying semaphorin-induced plexin signal transduction. Such information may lead to the discovery of novel points of semaphorin signal intervention not limited to the receptor–ligand interface.

References

[1] Tamagnone, L. & Comoglio, P.M. Tomove or not tomove? Semaphorin signalling in cell migration. *EMBO Rep*. **5,** 356-361 (2004).
[2]Zhou, Y., et al. Semaphorin signalling: progressmade and promises ahead. *Trends Biochem. Sci*. **33,** 161-170 (2008).
[3]Suzuki, K., Kumanogoh, A. & Kikutani, H. Semaphorins and their receptors in immune cell interactions. *Nature Immunol.* **9,** 17–23 (2008).
[4] Kumanogoh A, Kikutani H.The CD100-CD72 interaction: a novel mechanism of immune regulation. *Trends Immunol.* **22,** 670-676 (2001).
[5] Neufeld, G. & Kessler, O. The semaphorins: versatile regulators of tumour progression and tumour angiogenesis. *Nature Rev. Cancer* **8,** 632–645 (2008).

SHARPIN is a Component of the NF-κB-activating Linear Ubiquitin Chain Assembly Complex
Paper in journals: this is the first page of a paper published in ***Nature***.
[*Nature*] 471, 633-636(2011)

LETTER

doi:10.1038/nature09825

SHARPIN is a component of the NF-κB-activating linear ubiquitin chain assembly complex

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***Cpdm* (chronic proliferative dermatitis) mice develop chronic dermatitis and an immunodeficiency with increased serum IgM[1–3], symptoms that resemble those of patients with X-linked hyper-IgM syndrome and hypohydrotic ectodermal dysplasia (XHM-ED), which is caused by mutations in NEMO (NF-κB essential modulator; also known as IKBKG)[4–6]. Spontaneous null mutations in the *Sharpin* (SHANK-associated RH domain interacting protein in postsynaptic density)[7] gene are responsible for the *cpdm* phenotype in mice[8]. SHARPIN shows significant similarity to HOIL-1L (also known as RBCK1)[9,10], a component of linear ubiquitin chain assembly complex (LUBAC), which induces NF-κB activation through conjugation of linear polyubiquitin chains to NEMO[10–13]. Here, we identify SHARPIN as an additional component of LUBAC. SHARPIN-containing complexes can linearly ubiquitinate NEMO and activated NF-κB. Thus, we re-define LUBAC as a complex containing SHARPIN, HOIL-1L and HOIP (also known as RNF31). Deletion of SHARPIN drastically reduced the amount of LUBAC, which resulted in attenuated TNF-α- and CD40-mediated activation of NF-κB in mouse embryonic fibroblasts (MEFs) or B cells from *cpdm* mice. Considering the pleomorphic phenotype of *cpdm* mice, these results confirm the predicted role of LUBAC-mediated linear polyubiquitination in NF-κB activation induced by various stimuli, and strongly suggest the involvement of LUBAC-induced NF-κB activation in various disorders.**

NF-κB is a transcription factor involved in various functions, including inflammation and cell survival. NF-κB is shown to be activated by various stimuli[14–16]. CD40-mediated degradation of inhibitor of κBα (IκBα, also known as NFKBIA) leads to the activation of NF-κB. This pathway is severely impaired, but not completely abolished, in patients with XHM-ED, a syndrome caused by mutations in NEMO[5,6], a crucial regulator of the canonical NF-κB pathway[17]. CD40-mediated degradation of IκBα was impaired, but not completely abolished in splenic B cells from *cpdm* mice carrying the *Sharpin* null mutation[1–3,8] (Fig. 1a), which indicated that loss of SHARPIN impairs, but does not completely abolish NF-κB activation. The ubiquitin-like (UBL) and NPL4 zinc-finger (NZF) domains of SHARPIN showed significant similarity to those of HOIL-1L, a component of LUBAC that has a crucial role in TNF-α-mediated NF-κB activation[9–12] (Fig. 1b and Supplementary Fig. 1). TNF-α-induced activation of IκB kinase (IKK), phosphorylation and degradation of IκBα, and nuclear localization of the p65 subunit of NF-κB, which are hallmarks of NF-κB activation, were impaired, but not completely abolished, in *cpdm* MEFs (Fig. 1c–e). In addition, expression of NF-κB targets was markedly reduced in *cpdm* MEFs (Supplementary Fig. 2). These results clearly indicated that deletion of *Sharpin* also attenuates TNF-α-induced NF-κB activation.

The UBL domain of HOIL-1L is critical for complex formation with HOIP, the catalytic component of the linear polyubiquitination[10,11]. We proposed that SHARPIN forms a ubiquitin ligase complex with HOIP (SHARPIN–HOIP) and activates NF-κB[10]. Indeed, SHARPIN co-immunoprecipitated with HOIP, but not HOIL-1L (Supplementary Fig. 3). Introduction of SHARPIN–HOIP, but not SHARPIN alone or SHARPIN and HOIL-1L, induced NF-κB transcriptional activity (Supplementary Fig. 4a). Nuclear translocation of p65 was observed in HEK293T cells expressing SHARPIN–HOIP (Supplementary Fig. 4b). Furthermore, SHARPIN–HOIP showed linear polyubiquitination activity in an *in vitro* polyubiquitination assay, and the SHARPIN UBL domain was indispensable for HOIP binding and ubiquitination activity (Supplementary Figs 5 and 6). The NZF domain of SHARPIN seemed to be indispensable for NF-κB activation, but not for linear ubiquitination, and the UBA and RING-IBR-RING regions of HOIP were crucial for NF-κB activation mediated by SHARPIN–HOIP (Supplementary Figs 5d and 6). Given that UBA and RING-IBR-RING domains have crucial roles in HOIP binding to SHARPIN and in linear polyubiquitination, these results indicated that the SHARPIN–HOIP complex can activate NF-κB via linear polyubiquitination. In fact, SHARPIN–HOIP catalysed linear polyubiquitination of NEMO almost as efficiently as HOIL-1L–HOIP when overexpressed in HEK293T cells, as assessed by mass spectrometry and an anti-linear chain specific antibody (Supplementary Fig. 7).

We have noticed that NF-κB was more strongly activated in 293T cells expressing SHARPIN–HOIL-1L–HOIP as compared to cells co-expressing HOIL-1L–HOIP or SHARPIN–HOIP (Supplementary Fig. 4a). Co-immunoprecipitation assays to determine whether SHARPIN formed a ternary complex with HOIL-1L and HOIP showed that SHARPIN co-immunoprecipitated with both HOIP and HOIL-1L when co-expressed with either protein (Supplementary Fig. 3). Moreover, immunoprecipitation of endogenous LUBAC from HEK293T cells revealed that endogenous SHARPIN forms complexes with both HOIP and HOIL-1L (Fig. 2a). In HEK293T and Jurkat cells, SHARPIN co-fractionated primarily with HOIL-1L and HOIP in the relative molecular mass ~600,000 fraction (M_r 600K; Fig. 2b). Sequential precipitation of the ~600K fraction from HEK293 cells that stably expressed Flag–HOIP, HOIL-1L–His_6–haemagglutinin (HA), and Myc–SHARPIN confirmed that HOIL-1L, HOIP and SHARPIN form a ternary complex (Supplementary Fig. 8). These results conclusively indicated that SHARPIN forms a ternary complex with HOIL-1L and HOIP, and we re-defined LUBAC as a 600K complex containing HOIL-1L and/or SHARPIN together with HOIP. Sequential precipitation of HEK293T cells expressing Flag–HOIP, His_6–HOIP and Myc–HOIP showed that there were at least three HOIP molecules per LUBAC complex (Supplementary Fig. 9). SHARPIN–HOIP formed complexes with HOIL-1L–HOIP more efficiently than SHARPIN alone (Supplementary Fig. 10). Thus, LUBAC seems to be composed of a mixture of HOIL-1L–HOIP and SHARPIN–HOIP complexes, although we cannot exclude the possibility that LUBAC may break down into HOIL-1L–HOIP and

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*These authors contributed equally to this work.

The following is a comment on the published paper shown on the preceding page.

SHARPIN is a Component of the NF-κB Activating Linear Ubiquitin Chain Assembly Complex

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(Graduate School of Frontier Biosciences)

Introduction

NF-κB is one of the transcription factors that play a central role in inflammatory responses by inducing the expression of pro-inflammmatory molecules. Besides inflammation, NF-κB is also involved in many biological phenomena including cell survival. Abnormal activation of NF-κB is observed in many pathological conditions such as allergic and auto-inflammatory diseases and malignancies. Therefore, the signal-induced NF-κB activation pathway has been extensively studied[1]. My group have identified that a novel linear polyubiquitin chain and a ligase complex designated as the linear ubiquitin chain assembly complex (LUBAC) that specifically generates the unique polyubiquitin chain play crucial roles in NF-κB activation[2,3]. NF-κB is inactive in resting cells as it resides in the cytoplasm bound to inhibitor proteins called inhibitors of κBs (IκBs). Upon stimuli by inflammatory cytokines, LUBAC recognizes and linearly polyubiquitinates NF-κB essential modulator (NEMO), the regulatory subunit of the IKK (IκB kinase) complex composed of IKKα, IKKβ, and NEMO and induces the phosphorylation of IκBs. Phosphorylated IκBs are degraded in a ubiquitin-dependent manner, which releases NF-κB and allows it to translocate into the nucleus to induce the transcription of target genes[1] (Fig.1). In this manuscript, my group identified SHARPIN as a new subunit of LUBAC. Sharpin was identified as a causative gene of chronic proliferative dermatitis in mice (cpdm), which are characterized by numerous inflammatory symptoms including chronic dermatitis, arthritis and immune disorders[4,5]. Thus, the present results suggested that LUBAC-mediated NF-κB activation plays critical roles in mammals and be involved in various disorders.

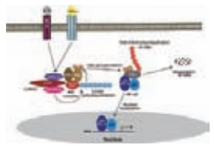

Fig.1 The NF-κB activation pathway. Upon activation by various stimuli, LUBAC recognizes and linearly polyubiquitinates NEMO, and induces the phosphorylation of IκBs. Phosphorylated IκBα is degraded in a ubiquitin-dependent manner, which releases NF-κB and allows it to translocate into the nucleus to induce the transcription of target genes.

Identification of SHARPIN as a new subunit of LUBAC

HOIL-1L and HOIP were first identified as components of LUBAC[2]. HOIL-1L knockout (KO) mice generated in our laboratory revealed that TNF-α-induced NF-κB activation is severely impaired, but not completely abolished in HOIL-1L null cells[3], although knocking-out of molecules essential for NF-κB activation, such as NEMO or IKKβ, is embryonic lethal in mice[6]. Since the expression of HOIP, the catalytic center of LUBAC, is drastically decreased but not completely absent in HOIL-1L KO cells, it has been hypothesized that HOIP may have another binding partner besides HOIL-1L. Then, using database analyses, SHARPIN was identified, as the C-terminus of SHARPIN exhibits significant homology with the N-terminal half of HOIL-1L that is essential for binding to HOIP.

As expected, SHARPIN was co-immunoprecipitated with HOIP, but not HOIL-1L in over expression study. However, SHARPIN could co-immunoprecipitate with HOIL-1L from the lysate of cells in which SHARPIN was co-expressed with not only HOIL-1L but also HOIP (Fig. 2a). Moreover, immunoprecipitation of endogenous LUBAC from HEK293T cells revealed that endogenous SHARPIN forms complexes with both HOIP and HOIL-1L (Fig. 2b). In HEK293T cells, SHARPIN co-fractionated primarily with HOIL-1L and HOIP in the ~600K fraction (Fig. 2c). These results conclusively indicated that SHARPIN forms a ternary complex with HOIL-1L and HOIP, and we re-defined LUBAC as a 600K complex containing HOIL-1L and/or SHARPIN together with HOIP.

Fig.2 SHARPIN forms a tertiary complex with HOIL-1L and HOIP. a. SHARPIN forms a complex with HOIL-1L in the presence but not in the absence of HOIP. Lysates of HEK293T cells transfected as indicated were subjected to immunoprecipitation and then immune complexes were analyzed by immunoblotting. b. Endogenous SHARPIN associates with HOIP and HOIL-1L. c. SHARPIN co-elutes with HOIP and HOIL-1L in fractionated HEK293T cell lysates.

Mechanism underlying NF-κB activation by SHARPIN-containing LUBAC

We have shown that HOIL-1L-HOIP complex can bind to and linearly ubiquitinates NEMO, which induces IKK activation[3]. We then tried to confirm whether it is also the case with SHARPIN-containing LUBAC and found that SHARPIN-containing LUBAC forms a complex with NEMO in a signal-dependent manner, such as upon stimulation with TNF-α, and conjugates linear polyubiquitin to NEMO. Although SHARPIN has been isolated as a SHANK-binding protein[7], SHARPIN has also been identified as a causative gene in the cpdm mouse phenotype[5]. Cpdm mice, which are spontaneous mutant mice lacking SHARPIN, exhibit pleomorphic phenotypes including chronic dermatitis and immune disorders with increased serum IgM. Genetic analysis demonstrated that CD40- or TNF-α-mediated NF-κB activation was impaired, but not completely abolished in splenic B cells or MEFs from cpdm mice, respectively (Fig 3a,b). Also LT-βR- and IL-1β-mediated NF-κB activation is also strongly impaired but not completely abolished in cpdm MEFs. Thus, the current concept for SHARPIN-containing LUBAC-mediated NF-κB activation is as follows: upon stimulation by inflammatory cytokines, such as TNF-α and IL-1β, LUBAC recognizes and linearly polyubiquitinates NEMO, which induces IKK activation and subsequent degradation of IκBα. Free NF-κB translocates into the nucleus and activates the transcription of target genes (Fig. 1).

Fig.3 Ablation of SHARPIN causes impaired NF-κB signaling. a. Impaired CD40-signalling in splenic B cells from cpdm mice. Splenic B cells were treated with an anti-CD40 antibody (1μg/ml).*, non-specific signal. b. Reduced TNF-α–mediated NF-κB activation in cpdm MEFs treated with cycloheximide and TNF-α.

Although SHARPIN is lacking in cpdm mice, the other components of LUBAC, HOIL-1L and HOIP exist in cpdm. Since we have shown that HOIL-1L-HOIP complex can activate NF-κB, mechanism underlying attenuated NF-κB activation in cpdm cells was addressed. Trace amounts of HOIP and HOIL-1L were detected in cpdm MEFs (Fig. 4a). In addition, gel filtration of MEF lysates from cpdm mice revealed the existence of small amounts of HOIL-1L and HOIP in the ~600K fraction, and introduction of Wt SHARPIN increased the amounts of HOIP and HOIL-1L in the ~600K fraction (Fig. 4b). Moreover, TNF-α induced the phosphorylation and degradation of IκBα in MEFs expressing SHARPIN (Fig. 4c). These results clearly indicated that the lack of SHARPIN in cpdm mice reduces the level of LUBAC and attenuates NF-κB activation. In cpdm MEFs transfected with an siRNA specific for HOIL-1L, expression of HOIL-1L and HOIP was severely suppressed and TNF-α–mediated IκBα degradation was almost completely abolished (Fig. 4d). Given that HOIL-1L-HOIP and SHARPIN-HOIP can activate NF-κB, these results strongly indicated that the residual NF-κB activity in cells from cpdm mice, and also perhaps in cells from HOIL-1L KO mice, is due to the presence of residual amounts of HOIL-1L-HOIP or SHARPIN-HOIP, respectively.

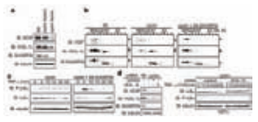

Fig.3 Ablation of SHARPIN causes impaired NF-κB signaling. a. Impaired CD40-signalling in splenic B cells from cpdm mice. Splenic B cells were treated with an anti-CD40 antibody (1μg/ml).*, non-specific signal. b. Reduced TNF-α–mediated NF-κB activation in cpdm MEFs treated with cycloheximide and TNF-α.

Perspectives

In this manuscript, we have clearly shown that LUBAC plays an essential role in canonical pathways of NF-κB activation. We have found TNF-α- and CD40-mediated NF-*k*B activation was attenulated in both cpdm and HOIL-1L KO mice. However, although HOIL-1L KO mice do not display an obvious phenotype, cpdm mice exhibit severe chronic dermatitis and immune deficiency. The phenotypic differences between these mice may result in part from differential effects on IL-1β-induced NF-κB activation and TNF-α–induced caspase 3 activation, both of which were higher in cpdm MEFs than in HOIL-1L$^{-/-}$MEFs. Symptoms of cpdm mice including chronic dermatitis and an immunodeficiency with increased serum IgM, resemble those of patients with X-linked hyper-IgM syndrome and hypohydrotic ectodermal dysplasia (XHM-ED), which is caused by mutations in NEMO[8]. However, the symptoms observed in patients with NEMO mutations are quite diverse. Thus, phenotypic differences between these two mice may be due to differences in NF-κB activation mediated by residual levels of HOIL-1L-HOIP and Sharpin-HOIP. Alternatively, the different phenotypes could be due to differences in the levels of binding proteins, e.g., protein(s) involved in recruitment of LUBAC to the IL-1β receptor, and/or differences in the levels of LUBAC ubiquitination substrates. Further analyses will be needed to clarify mechanisms underlying these phenotypic differences between cpdm and HOIL-1L KO mice.

In addition to activating NF-κB, TNF-α and CD40 also activate the JNK/ERK pathway. TNF-α–induced JNK/ERK activation was slightly higher in cpdm MEFs than Wt MEFs, as observed in HOIL-1L KO MEFs. However, CD40-mediated JNK/ERK activation was mildly suppressed in both cpdm and HOIL-1L$^{-/-}$ B cells. Therefore, our results do not conclusively support a role for LUBAC in JNK/ERK activation. Elucidation of the role of LUBAC in JNK/ERK activation awaits further study.

In summary, we have identified SHARPIN as a novel component of LUBAC. SHARPIN-containing LUBAC assembles linear polyubiquitin chains on NEMO, leading to NF-κB activation. These results do not rule out the presence of other targets of LUBAC-mediated linear polyubiquitination. Given the pleomorphic phenotype of cpdm mice, linear polyubiquitination by LUBAC may be involved in several disorders, including chronic dermatitis and immunodeficiency. Enhanced expression of SHARPIN has been reported in various human cancers. Enhanced expression of LUBAC increases NF-κB activation, suggesting that overexpression of LUBAC may be involved in tumorigenesis through activation of the anti-apoptotic function of NF-κB.

References

[1] Hayden M.S. & Ghosh S. Shared principles in NF-κB signaling. *Cell* **132,** 344-362 (2008).
[2] Kirisako T., Kamei K., Murata S., Kato M., Fukumoto H., et al. A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J* **25,** 4877-4887 (2006).
[3] Tokunaga F., Sakata S., Saeki Y., Satomi Y., Kirisako T., et al. Involvement of linear polyubiquitylation of NEMO in NF-κB activation. *Nat Cell Biol* **11,** 123-132 (2009).
[4] HogenEsch H., Gijbels M.J., Offerman E., van Hooft J., van Bekkum D.W., et al. A spontaneous mutation characterized by chronic proliferative dermatitis in C57BL mice. *Am J Pathol* **143,** 972-982 (1993).
[5] Seymour R.E., Hasham M.G., Cox G.A., Shultz L.D., Hogenesch H., et al. Spontaneous mutations in the mouse Sharpin gene result in multiorgan inflammation, immune system dysregulation and dermatitis. *Genes Immun* **8,** 416-421 (2007).
[6] Gerondakis S., Grumont R., Gugasyan R., Wong L., Isomura I., et al. Unravelling the complexities of the NF-κB signalling pathway using mouse knockout and transgenic models. *Oncogene* **25,** 6781-6799 (2006).
[7] Lim S., Sala C., Yoon J., Park S., Kuroda S., et al. Sharpin, a novel postsynaptic density protein that directly interacts with the shank family of proteins. *Mol Cell Neurosci* **17,** 385-397 (2001).
[8] Jain A., Ma C.A., Liu S., Brown M., Cohen J., et al. Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. *Nat Immunol* **2,** 223-228 (2001).