http://dx.doi.org/10.5012/bkcs.2011.32.11.3893

The Role of Transglutaminase in Double-stranded DNA-Triggered Antiviral Innate Immune Response

Jae-Wook Yoo,[†] Sun Woo Hong,[†,#] Shambhunath Bose,[‡] Hojun Kim,[‡] Soo Youl Kim,[§] Soyoun Kim,[#] and Dong-ki Lee[†,*]

[†]*Global Research Laboratory for RNAi Medicine, Department of Chemistry, Sungkyunkwan University, Suwon 440-746, Korea*
[*]*E-mail: dklee@skku.edu*
[‡]*Institute of Medical Research, College of Medicine, Dongguk University, Seoul 100-715, Korea*
[§]*Cancer Cell and Molecular Biology Branch, Division of Cancer Biology, Research Institute, Gyeonggi-do 410-769, Korea*
[#]*Department of Biomedical Engineering, Dongguk University, Seoul 100-715, Korea*
Received August 26, 2011, Accepted September 2, 2011

Cellular uptake of double-stranded DNA (dsDNA) triggers strong innate immune responses *via* activation of NF-κB transcription factor. However, the detailed mechanism of dsDNA-mediated innate immune response remains yet to be elucidated. Here, we show that the expression of tazarotene-induced gene 3 (TIG3) is dramatically induced by dsDNA stimulation, and the siRNA-mediated down-regulation of TIG3 mRNA results in significant suppression of dsDNA-triggered cytokine expression. Because TIG3 has been previously shown to physically interact with transglutaminase (TG) 1 to activate TG activity, and TG2 has been shown to induce NF-κB activity by inducing IκBα polymerization, we tested whether TG also plays a role in dsDNA-mediated innate immune response. Pre-treatment of TG inhibitors dramatically reduces dsDNA-triggered cytokine induction. We also show that, in HeLa cells, TG2 is the major TG, and TIG3 physically interacts with TG2. Combined together, our results suggest a novel mechanism of dsDNA-triggered innate immune response which is critically dependent on TIG3 and TG2.

Key Words : dsDNA, Innate immunity, TIG3, Transglutaminase

Introduction

Innate immune responses against pathogen associated molecular patterns (PAMPs), such as lipopolysaccharide, triacyl lipopeptide, flagellin, CpG island, double stranded RNA (dsRNA) and double stranded DNA (dsDNA), are triggered by pattern recognition receptors (PRRs) including membrane associated toll-like receptors (TLRs) and cytosolic receptors.[1-4] DsRNA, a gene fragment of RNA viruses, is recognized by retinoic acid inducible gene-I (RIG-I) like helicases (RLH). Mitochondrial interferon-beta promoter stimulator 1 (IPS-1, also called VISA, Cardif and MAVS), act as a adaptor molecule and transfer not only RLH-mediated signals to FADD and RIP1 for activating NF-κB but also TBK1 and IKK-ε for activating IRF3. Subsequently, NF-κB and IRF3, major transcription factors activated during *anti*-viral innate immune responses, induce the expression of *anti*-viral related genes such as pro-inflammatory cytokines, type I interferons and *anti*-viral effectors.[4-6]

Recent studies revealed that DNA-dependent activator of IFN regulatory factors (DAI, also called DLM-1/ZBP1), recognizes cytosolic dsDNA[7,8] and activates IRF3 pathway and NF-κB pathway through inducing TBK1 and IRF3 association and RIP1 recruitment, respectively.[9-11] AIM2 is another cytosolic dsDNA sensor and this protein senses dsDNA *via* its HIN200 domain and binds to ASC, the adaptor molecule, *via* their PYD domains leading to form inflammasome complex which activates pro-caspase-1 to mature caspase-1. Caspase-1 subsequently leads to programmed cell death through activation of both IL-1β and IL-18.[12-15]

Members of transglutaminases (TGs) family are Ca^{2+}-dependent cross-link enzymes which generate ε-(γ-glutamyl)lysine isopeptide bond through an acyl-transfer reaction between the γ-carboxamide group of glutamine residue of one peptide and the ε-amino group of lysine residue of another peptide.[16-18] The products of the cross-link reaction are further polymerized by TGs, which consequently form high-molecular-mass protein aggregates.[19] In mammals, nine separated TG isoenzymes have been reported including type I TG, the keratinocyte TG which activates on terminal differentiation of keratinocyte, and type 2 TG, the ubiquitous tissue TG.[20] The localization, tissue distribution, and physiological features are the criterion of categorizing TG subtypes.[21,22]

Recently, it has been reported that recombinant TG2 inhibitors have *anti*-inflammatory effects of allergic conjunctivitis to ragweed in a guinea pig model.[23] Dysregulation of TG2 associated with abnormal activation of T cell leading to autoimmune inflammatory myopathies and celiac disease.[24,25] The increase of TG2 expression during LPS treatment in BV2 microglia triggered the activation of NF-κB activity through forming high molecular weight IκBα aggregates.[26]

TG1 is an important TG isoenzyme which is activated during keratinocyte terminal differentiation leading to form cornified envelope.[27,28] Recent study showed that TG1 forms a complex with type II tumor suppressor protein Tazarotene-induced gene 3 (TIG3, also called RARRES3 and RIG1) and

the complexation markedly increases TG1 activity which triggers the differentiation of keratinocyte.[29,30] TIG3 is a low molecular-weight protein which has been identified in retinoid-treated cultured epidermal keratinocytes.[31] In epithelial cancer cells, TIG3 acts as a type II tumor suppressor to inhibit cell proliferation *via* suppression of Ras activity which consequently induces cellular apoptosis at the Golgi apparatus.[32,33]

Here, we demonstrate a novel signaling pathway involving both TIG3 and TG2 upon dsDNA-triggered innate immune response. TIG3 expression is induced by dsDNA stimulation and subsequent co-localization with TG2 enhances its enzymatic activity which consequently leads expression of various *anti*-viral cytokines through activation of NF-κB activity.

Experimental

Cell Culture. HeLa, HEK293, T98G and AGS cell lines were purchased from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum and penicillin/streptomycin (100 U/mL penicillin and 100 g/mL streptomycin), and cultured at 37 °C with 5% CO_2. Cells were regularly subcultured to maintain exponential growth.

siRNA Transfection. One day before transfection, cells were plated in 12 well plates for 50% confluency in complete medium without antibiotics. After 24 h, siRNAs were transfected into the cells using Lipofectamine2000 reagent (Invitrogen) according to the manufacturer's protocol. poly-(dA:dT) transfection was performed 48 h after siRNA transfection for indicated time.

Quantitative Real-time PCR. Cells were harvested 48 h after transfection, and total RNA was extracted using Trizol (Ambion) according to the manufacturer's instructions. 500 ng of total RNA was used for cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and an oligo-(dT) 15 primer. Aliquots (1/10) of the cDNA reaction mixture were analyzed by quantitative real-time PCR on a StepOne™ Real-Time PCR Systems (Applied Biosystems) according to the manufacturer's protocol. Primer sequences for the PCRs were described in Supplementary Table 1.

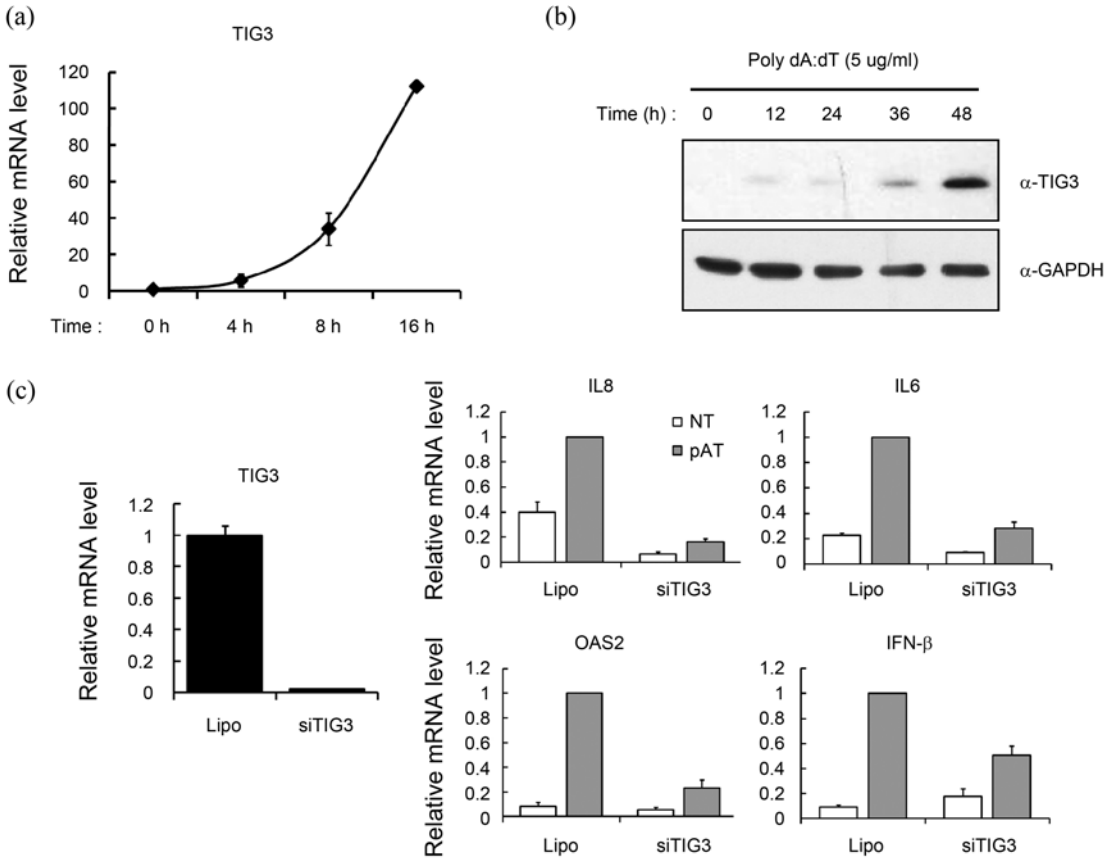

Figure 1. Induction of TIG3 expression and its role upon dsDNA stimulation (a) Endogenous TIG3 mRNA level was measured by quantitative RT-PCR. 5 μg/mL of poly(dA:dT) was transfected into HeLa cell line with indicated time course (x-axis). Values shown on the y-axis are means + standard deviations (SD) from at least three experiments. (b) Endogenous TIG3 protein expression was measured by western blot. 5 μg/mL of poly(dA:dT) was transfected into HeLa cell line with indicated time course. Rabbit *anti*-serum immunized by TIG3 N-term peptide injection was used for primary antibody to detect endogenous TIG3 protein GAPDH western blot was measured for internal control. (c) 10 nM of siRNA targeting TIG3 was transfected into the HeLa cells. After 48 h, the cells were subsequently stimulated by poly(dA:dT). The expression of TIG3 level (black bar) or various cytokines level (white bar: before poly(dA:dT) stimulation, gray bar: after poly(dA:dT) stimulation) were measured. Values shown on the y-axis are means + standard deviations (SD) from at least three experiments.

Co-Immunoprecipitation. One μg of Flag-tagged vector only, wild-type TIG3 or TIG3 1-134 in p3XFlag-CMV (Sigma) was co-transfected with TG1 or TG2 in pcDNA3.0 (Invitrogen) expression vector into 293T cell line which was grown in 60 mm dish. 24 h later, cells were lysed with lysis buffer [50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA] and quantified by BCA method. 500 μg of whole cell lysate was incubated with 1 μg of antibody specific for TG1 (SantaCruz Biotechnology) or TG2 (CUB7402, NeoMarkers) or 20 μL of Flag antibody conjugated agarose beads (Sigma) for 2 h at 4 °C.

Binding partners of either TG1 or TG2 antibody were subsequently precipitated with protein A/G agarose beads (SantaCruz Biotechnology). After washing three times the agarose beads, 1X sample buffer was added to each samples and boiled to elute precipitated proteins. Subsequent western blot was performed.

Western Blot. Cells were washed twice with 1X PBS, lysed with lysis buffer. Protein concentration was measured by BCA method. 30 μg of total lysate or 15 μL of immunoprecipitated product was mixed with 5X sample buffer and loaded to 10% sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE). Samples were electrophoretically transferred onto PVDF membrane (Immune-Blot™ PVDF membrane, BioRad). Proteins of interest were detected with specific antibodies as indicated, and then visualized with an enhanced luminal-based chemiluminescent Western blot detection kit (WESTSAVE™, Abfrontier Korea).

Dual-Reporter Luciferase Assay. One day before transfection, HeLa cells were plated in 24 well plate and grown to 80% confluency in complete medium without antibiotics. Cystamine (50 μM) or MDC (100 μM) was treated and 12 h later, first transfection was performed with NF-κB reporter plasmid (100 ng, pNF-κB-Luc, Stratagene). Renilla luciferase vector (1 ng, pRL-SV40, Promega) was co-transfected for normalization. In case of plasmid transfection, Lipofectamine and Plus reagent (Invitrogen) was used according to manufacturer's instruction. The 24 h later the second transfection of poly(dA:dT) (5 μg/mL, Sigma) was performed for indicated time and luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega). Results were quantified with the 20/20n Luminometer (Turner Biosystems).

Results and Discussion

The Role of TIG3 Upon dsDNA-triggered Innate Immune Responses. We have accidentally found that the

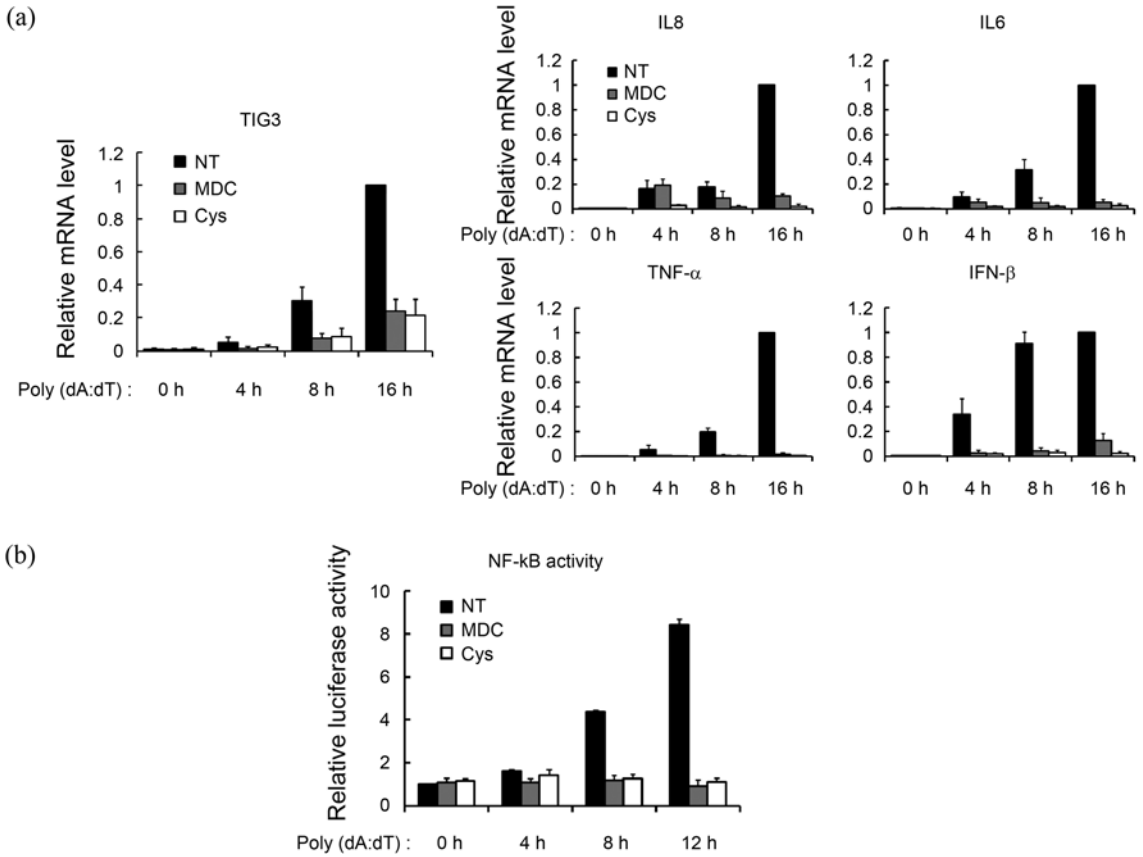

Figure 2. The suppression of poly(dA:dT)-triggered innate immune responses by transglutaiminase inhibitor pre-treatment. (a) Cystamine (50 μM) and MDC (100 μM) were pre-treated before poly(dA:dT) stimulation. TIG3 and various cytokines mRNA level were measured by quantitative RT-PCR. (b) NF-κB activity was detected by dual-luciferase reporter assay. Fire fly luciferase vector was transfected into HeLa cells 24 h before inhibitors treatment. 1/100 of Renila luciferase was used for internal control. Untreated (black bar), MDC treated (dark gray bar) and cystamine treated (white bar) were represented with indicated time-course (x-axis). Values shown on the y-axis are means + standard deviations (SD) from at least three experiments.

expression of TIG3, a H-Rev-107 family of class II tumor suppressor,[31-33] was highly induced upon dsDNA stimulation. The mRNA level of TIG3 started to increase in 4 h after transfection of poly(dA:dT) and continued to increase (Fig. 1(a)). The protein level of TIG3, which was undetectable before dsDNA stimulation, also increased and was visible starting 12 h after poly(dA:dT) transfection (Fig. 1(b)).

We then tested whether other members of H-Rev-107 tumor suppressor family or tazarotene induced gene (TIG) family are similarly induced upon dsDNA stimulation in various cell lines. Among tested genes, only TIG3 showed dramatic induction of gene expression upon dsDNA stimulation in all tested cell lines (Supp. Fig. 1). We also tested whether TIG3 gene induction can be triggered by dsRNA stimulation, such as poly(I:C) transfection. Supp. Figure 1 shows that the TIG3 gene expression was not affected by poly(I:C) stimulation, which led us to hypothesize specific relationship between TIG3 and dsDNA-triggered innate immune response.

To understand the functional role of TIG3 in dsDNA-triggered innate immune stimulation, we suppressed TIG3 expression using siRNA and then measured the induction level of various cytokines upon poly(dA:dT) transfection. The induction of cytokines such as IL8, IL6, OAS2 and IFN-β was significantly inhibited by siRNA-mediated inhibition of TIG3 gene induction, suggesting that the up-regulation of TIG3 gene expression plays an important role in dsDNA-triggered cytokine induction (Fig. 1(c)).

Inhibition of TG Activity Reduces dsDNA-triggered Cytokine Induction *via* Attenuation of NF-κB Activity. Recent studies reported that TIG3 can activate TG1 through physical association, which then subsequently leads to terminal differentiation of keratinocytes.[29,30] In addition, several studies demonstrated the activation of NF-κB activity by the TG activity of TG2 through polymerization of IκBα.[26,34-37] These previous results led us to hypothesize that TIG3 protein induced upon dsDNA stimulation might influence TG activity in HeLa cells, which in turn activate NF-κB activity to up-regulate cytokine level.

In order to test whether TG activity is involved in dsDNA-triggered *anti*-viral innate immune responses, we first treated HeLa cells with MDC or Cystamine, general inhibitors of TG activity. After 16 h incubation with TG inhibitors,

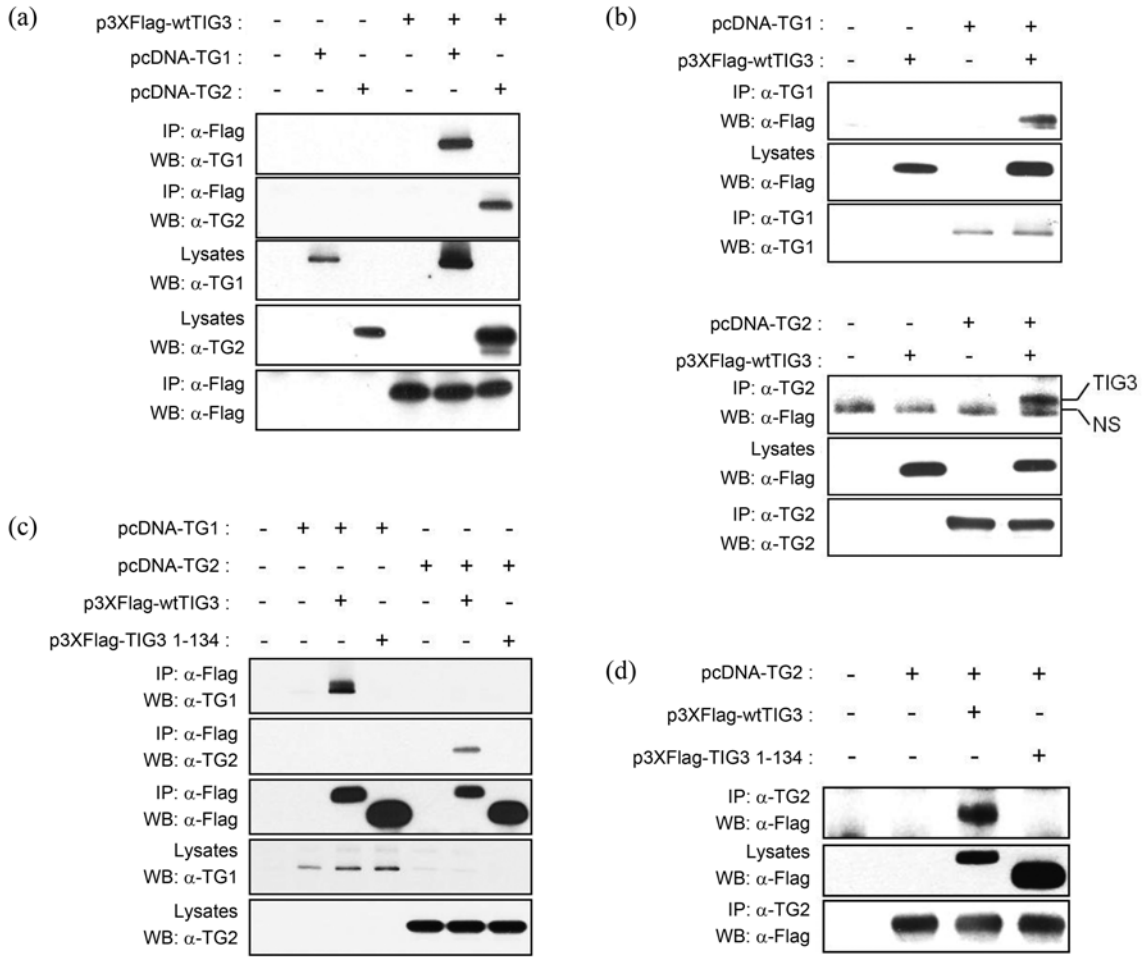

Figure 3. Interaction between TIG3 and TG family proteins. (a-d) TG1 or TG2 constructs were transfected into the 293T cells with Flag-TIG3 (wild-type or C-term truncated 1-134) constructs or an empty vector. Tagged TIG3 (wild-type or 1-134), overexpressed TG1 or TG2 were immunoprecipitated and cell extracts were analysed by western blot. NS indicates non-specific bands. A single result among duplicate experiments was represented.

poly(dA:dT) was transfected into the cells for indicated time. Subsequently, the expression of various cytokines such as IL8, IL6, TNF-α and IFN-β was measured. The expression of innate immune-related cytokines was increased in the cells without TG inhibitor treatment (Fig. 2(a)). In contrast, TG inhibitor pre-treatment significantly reduced cytokine induction, indicating that TG activity is essential for dsDNA-triggered innate immune response (Fig. 2(a)). Interestingly, the induction of TIG3 mRNA level by poly(dA:dT) transfection was also effectively attenuated by MDC or cystamine pre-treatment (Fig. 2(a)).

Next, we tested whether TG inhibitors also suppress NF-κB activity. After pre-treatment of TG inhibitors, we transfected cells with pNF-κB-Luc reporter vector along with poly(dA:dT) and then measured luciferase activity. As shown in Figure 2(b), HeLa cells pre-treated with TG inhibitor almost completely suppressed the NF-κB luciferase activity which is induced upon poly(dA:dT) transfection. These results are consistent with our hypothesis that TG activity regulates the expression of cytokines through NF-κB activation in dsDNA-triggered innate immune response.

TG2 Physically Interacts with TIG3 *via* C-terminal Hydrophobic Region. It has been reported that there are eight proteins in TG protein family.[17] To test which TG subtype is essential for mediating dsDNA-triggered innate immune response in HeLa cells, we measured the expression level of all TG subtypes in HeLa cell line. As shown in Supp. Figure 2(a), TG2 was highly expressed in HeLa cell line, and low level TG1 expression was also detected at mRNA level. TG1 protein expression, however, was not detected (data not shown), whereas strong signal for TG2 protein was detected by Western blotting in HeLa cells (Supp. Fig. 2(b)). On the other hand, we were not able to detect any expression of other TGs, suggesting that TG2 is the major TG in HeLa cell line.

Since TG2 was mainly expressed in HeLa cells, we hypothesized that TG2 might play a major role in dsDNA-triggered innate immune response. Therefore, the increased TIG3 expression level might be associated with the induction of TG2 activity by physical interaction, similar to the published report showing that TIG3 associates with TG1 and increases TG activity.[29,30] To test our hypothesis, we performed co-immunoprecipitation experiment to verify if TG2 physically interacts with TIG3. Flag-tagged wild type TIG3 was co-expressed with either TG1 or TG2 in 293T cell line, and then co-immunoprecipitation was performed either by precipitating TIG3 with Flag-conjugated agarose beads (Fig. 3(a)), or precipitating TG1 or TG2 using *anti*-TG1 or TG2 antibodies, respectively (Fig. 3(b)). In both cases, we found that TIG3 interacts with both TG1 and TG2 (Fig. 3(a) and (b)).

Jans *et al.* have shown that the C-terminal hydrophobic region of TIG3 is critical for the interaction between TIG3 and TG1.[38] Therefore, we further tested whether the C-terminal hydrophobic region of TIG3 is also important for the interaction between TIG3 and TG2. A C-terminal truncated TIG3 variant (TIG3 1-134) with Flag epitope vector were generated and its expression was confirmed by western blot (Fig. 3(c)). As expected, neither TG1 nor TG2 co-precipitated with Flag-tagged TIG3 1-134 (Fig. 3(c) lane 4, 7) whereas full length TIG3 interacted with both TG1 and TG2 (Fig. 3(c) lane 3, 6). Inverse co-immunoprecipitation using TG2 antibody also confirmed that TG2 interacts with full-length TIG3 but not with C-terminal truncated TIG3 1-134 (Fig. 3(d)). Taken together, these results demonstrate that the physical association of TG2 with TIG3 through the C-terminal hydrophobic region of TIG3.

The mechanism of dsDNA-triggered *anti*-viral innate immune responses was reported by several recent studies.[1,8,10,13-15] Also, a number of studies demonstrated the essential role of TG2 in inflammatory responses.[23,26,36,39-41] However, the involvement of TG2 in dsDNA-triggered immune response has not been reported. In this study, we discovered the critical importance of TG activity in dsDNA-triggered immune response based on our serendipitous finding of the role of TIG3 in the same mechanism. Our co-immunoprecipitation experiment demonstrates for the first time that TIG3 interacts not only with TG1, which is mainly expressed keratinocyte for activating terminal differentiation, as previously reported, but also with TG2, which is ubiquitously expressed and known to regulate NF-κB. The interaction between TIG3 and TG2 is mediated by the C-terminal hydrophobic domain of TIG3, similar to the interaction between TIG3 and TG1.

Our findings thus suggest a novel mechanism of dsDNA-triggered innate immune responses mediated by TIG3, TG2 and NF-κB signaling pathway. Further studies should uncover the molecular interaction between TG2 activated by TIG3 and IκBα in detail, to clearly confirm our hypothesis that the activation of NF-κB during dsDNA-triggered immune responses is mediated by IκBα polymerization by TG2 activity rather than the more generally accepted mechanism of IκBα phosphorylation.

Acknowledgments. This work was supported by the basic research program from MEST, Korea (C00541) to D.-k. L.

References

1. Barbalat, R.; Ewald, S. E.; Mouchess, M. L.; Barton, G. M. *Annu. Rev. Immunol.* **2011**, *29*, 185-214.
2. Kawai, T.; Akira, S. *Immunity* **2011**, *34*, 637-650.
3. Kumar, H.; Kawai, T.; Akira, S. *Int. Rev. Immunol.* **2011**, *30*, 16-34.
4. Loo, Y. M.; Gale, M., Jr. *Immunity* **2011**, *34*, 680-692.
5. Onomoto, K.; Onoguchi, K.; Takahasi, K.; Fujita, T. *J. Interferon Cytokine Res.* **2010**, *30*, 875-881.
6. Yoneyama, M.; Fujita, T. *Immunol. Rev.* **2009**, *227*, 54-65.
7. Wang, Z.; Choi, M. K.; Ban, T.; Yanai, H.; Negishi, H.; Lu, Y.; Tamura, T.; Takaoka, A.; Nishikura, K.; Taniguchi, T. *Proc. Natl. Acad Sci. U S A* **2008**, *105*, 5477-5482.
8. Ha, S. C.; Kim, D.; Hwang, H. Y.; Rich, A.; Kim, Y. G.; Kim, K. K. *Proc. Natl. Acad Sci. U S A* **2008**, *105*, 20671-20676.
9. Chi, H.; Flavell, R. A. *Nature* **2007**, *448*, 423-424.
10. Rebsamen, M.; Heinz, L. X.; Meylan, E.; Michallet, M. C.; Schroder, K.; Hofmann, K.; Vazquez, J.; Benedict, C. A.; Tschopp, J. *EMBO Rep.* **2009**, *10*, 916-922.

11. Kaiser, W. J.; Upton, J. W.; Mocarski, E. S. *J. Immunol.* **2008**, *181*, 6427-6434.
12. Burckstummer, T.; Baumann, C.; Bluml, S.; Dixit, E.; Durnberger, G.; Jahn, H.; Planyavsky, M.; Bilban, M.; Colinge, J.; Bennett, K. L.; Superti-Furga, G. *Nat. Immunol.* **2009**, *10*, 266-272.
13. Fernandes-Alnemri, T.; Yu, J. W.; Datta, P.; Wu, J.; Alnemri, E. S. *Nature* **2009**, *458*, 509-513.
14. Hornung, V.; Ablasser, A.; Charrel-Dennis, M.; Bauernfeind, F.; Horvath, G.; Caffrey, D. R.; Latz, E.; Fitzgerald, K. A. *Nature* **2009**, *458*, 514-518.
15. Roberts, T. L.; Idris, A.; Dunn, J. A.; Kelly, G. M.; Burnton, C. M.; Hodgson, S.; Hardy, L. L.; Garceau, V.; Sweet, M. J.; Ross, I. L.; Hume, D. A.; Stacey, K. J. *Science* **2009**, *323*, 1057-1060.
16. Pisano, J. J.; Finlayson, J. S.; Peyton, M. P. *Science* **1968**, *160*, 892-893.
17. Griffin, M.; Casadio, R.; Bergamini, C. M. *Biochem. J.* **2002**, *368*, 377-396.
18. Folk, J. E.; Chung, S. I. *Methods Enzymol*. **1985**, *113*, 358-375.
19. Martinet, N.; Beninati, S.; Nigra, T. P.; Folk, J. E. *Biochem. J.* **1990**, *271*, 305-308.
20. Grenard, P.; Bates, M. K.; Aeschlimann, D. *J. Biol. Chem*. **2001**, *276*, 33066-33078.
21. Lorand, L.; Graham, R. M. *Nat. Rev. Mol. Cell Biol*. **2003**, *4*, 140-156.
22. Chen, J. S.; Mehta, K. *Int. J. Biochem. Cell Biol.* **1999**, *31*, 817-836.
23. Sohn, J.; Kim, T. I.; Yoon, Y. H.; Kim, J. Y.; Kim, S. Y. *J. Clin. Invest.* **2003**, *111*, 121-128.
24. Choi, Y. C.; Park, G. T.; Kim, T. S.; Sunwoo, I. N.; Steinert, P. M.; Kim, S. Y. *J. Biol. Chem.* **2000**, *275*, 8703-8710.
25. Choi, Y. C.; Kim, T. S.; Kim, S. Y. *Eur. Neurol.* **2004**, *51*, 10-14.
26. Lee, J.; Kim, Y. S.; Choi, D. H.; Bang, M. S.; Han, T. R.; Joh, T. H.; Kim, S. Y. *J. Biol. Chem.* **2004**, *279*, 53725-53735.
27. Rice, R. H.; Green, H. *Cell* **1977**, *11*, 417-422.
28. Eckert, R. L. *Physiol. Rev.* **1989**, *69*, 1316-1346.
29. Sturniolo, M. T.; Dashti, S. R.; Deucher, A.; Rorke, E. A.; Broome, A. M.; Chandraratna, R. A.; Keepers, T.; Eckert, R. L. *J. Biol. Chem.* **2003**, *278*, 48066-48073.
30. Sturniolo, M. T.; Chandraratna, R. A.; Eckert, R. L. *Oncogene* **2005**, *24*, 2963-2972.
31. DiSepio, D.; Ghosn, C.; Eckert, R. L.; Deucher, A.; Robinson, N.; Duvic, M.; Chandraratna, R. A.; Nagpal, S. *Proc. Natl. Acad Sci. U S A* **1998**, *95*, 14811-14815.
32. Deucher, A.; Nagpal, S.; Chandraratna, R. A.; Di Sepio, D.; Robinson, N. A.; Dashti, S. R.; Eckert, R. L. *Int. J. Oncol.* **2000**, *17*, 1195-1203.
33. Tsai, F. M.; Shyu, R. Y.; Jiang, S. Y. *Cell Signal* **2007**, *19*, 989-999.
34. Park, S. S.; Kim, J. M.; Kim, D. S.; Kim, I. H.; Kim, S. Y. *J. Biol. Chem.* **2006**, *281*, 34965-34972.
35. Mann, A. P.; Verma, A.; Sethi, G.; Manavathi, B.; Wang, H.; Fok, J. Y.; Kunnumakkara, A. B.; Kumar, R.; Aggarwal, B. B.; Mehta, K. *Cancer. Res.* **2006**, *66*, 8788-8795.
36. Kim, D. S.; Park, S. S.; Nam, B. H.; Kim, I. H.; Kim, S. Y. *Cancer. Res.* **2006**, *66*, 10936-10943.
37. Condello, S.; Caccamo, D.; Curro, M.; Ferlazzo, N.; Parisi, G.; Ientile, R. *Brain. Res.* **2008**, *1207*, 1-8.
38. Jans, R.; Sturniolo, M. T.; Eckert, R. L. *J. Invest. Dermatol.* **2008**, *128*, 517-529.
39. Kim, D. S.; Kim, B.; Tahk, H.; Kim, D. H.; Ahn, E. R.; Choi, C.; Jeon, Y.; Park, S. Y.; Lee, H.; Oh, S. H.; Kim, S. Y. *Biochem. Biophys. Res. Commun.* **2010**, *403*, 479-484.
40. Kim, D. Y.; Park, B. S.; Hong, G. U.; Lee, B. J.; Park, J. W.; Kim, S. Y.; Ro, J. Y. *Br. J. Pharmacol.* **2011**, *162*, 210-225.
41. Kim, J. M.; Voll, R. E.; Ko, C.; Kim, D. S.; Park, K. S.; Kim, S. Y. *J. Mol. Biol.* **2008**, *384*, 756-765.