A Monoclonal Antibody Against the Human SUMO-1 Protein Obtained by Immunization with Recombinant Protein and CpG-DNA-liposome Complex

Dongbum Kim,¹ Joo Young Lee,² Dae-Geun Song,² Sanghoon Kwon,¹ Younghee Lee,³
Cheol-Ho Pan,^{2*} and Hyung-Joo Kwon^{1,4*}

Post-translational modification regulated by conjugation of a small ubiquitin-like modifier (SUMO) is involved in various cellular processes. In this study, we expressed and purified recombinant human SUMO-1 (hSUMO-1). BALB/c mice were immunized with a complex of hSUMO-1 protein and Lipoplex(O) to produce hSUMO-1-specific antibodies. Using conventional hybridoma technology, we obtained four hybridoma clones derived from the mouse with the highest antibody titer against hSUMO-1. Based on Western blot analysis, our hSUMO-1 monoclonal antibody specifically recognizes hSUMO-1, but not other SUMO proteins. These results support that the anti-hSUMO-1 monoclonal antibody produced with the aid of Lipoplex(O) adjuvant is specific and that Lipoplex(O) is useful for development of monoclonal antibodies against recombinant protein. In addition, we analyzed human tissues to examine the distribution of hSUMO-1. Higher expression of hSUMO-1 was detected in normal adrenal gland, esophagus, pancreas, liver, stomach, kidney, and uterus than in corresponding cancer tissues, suggesting a tumor suppressive function of hSUMO-1.

Introduction

SUMOYLATION IS A POST-TRANSLATIONAL MODIFICATION characterized by covalent binding of a SUMO protein to target proteins.⁽¹⁾ Sumoylation regulates maintenance of protein function, including protein stability, protein interaction with other proteins, and modification of transcription factors.^(2,3) SUMO proteins identified in human cells constitute four isoforms—SUMO-1, SUMO-2, SUMO-3, and SUMO-4.^(4,5) Although SUMO proteins are approximately 11 kDa, the exact size of SUMO families is different in various organisms. Normally, SUMO is covalently attached to typical lysine residues within the SUMO modification consensus sequence, ΨKXE, where Ψ is a large hydrophobic residue and X is any amino acid residue in the target protein. SUMO is activated by SUMO-activating enzyme (E1) in an ATP-dependent manner and then transferred to the target protein containing the ΨKXE motif by Ubc9, a SUMO-conjugating enzyme (E2). Finally, SUMO and the target protein complex are linked by several SUMO protein ligases (E3).⁽⁶⁾

SUMO-1 was the first protein identified to be covalently conjugated to GTPase activating protein RanGAP1.^(7,8) SUMO-1-modified RanGAP1 regulates RanBP2 (also known

as Nup358) and Ubc9 complex at the cytoplasmic filaments of the nuclear pore complexes (NPC). SUMO-1 conjugation to IκBα targets the same residue in IκBα used for ubiquitination, thereby inhibiting protein degradation and consequently blocking NFκB-dependent transcriptional activation in mammalian cells.⁽⁹⁾ Interestingly, SUMO-1 shows the opposite role in *Drosophila*: it promotes import of the NF-κB ortholog protein, Dorsal, into the nucleus and enhances transcriptional activity.⁽¹⁰⁾ Recent proteomic analyses in mammalian cells revealed that a number of SUMO substrates and specific modifications by SUMO-1 are involved in essential processes, including chromatin organization, transcription, and RNA metabolism.^(11,12)

CpG-DNA represents synthetic oligonucleotides with immunostimulatory activity mimicking bacterial DNA containing CpG motifs.^(13,14) CpG-DNA has been extensively studied by many research groups as a vaccine adjuvant to prevent malaria, hepatitis B, influenza, and tumors.^(15–20) When patients were administered the CpG-DNA adjuvanted hepatitis B virus antigen, the titers of anti-HBV antibody were significantly higher (more than 150%) than those in patients vaccinated with hepatitis B virus antigen alone.⁽¹⁶⁾ Previously, we isolated natural CpG-DNA from *Mycobacterium bovis*

¹Center for Medical Science Research, ⁴Department of Microbiology, College of Medicine, Hallym University, Chuncheon, Republic of Korea.

²Functional Food Center, Korea Institute of Science and Technology (KIST), Gangneung, Republic of Korea.

³Department of Biochemistry, College of Natural Sciences, Chungbuk National University, Cheongju, Republic of Korea.

*These authors contributed equally to this work.

(specifically, MB-ODN 4531(O)) and confirmed its immunostimulating activity.⁽¹⁷⁾ The activity of MB-ODN 4531(O) was greatly enhanced by encapsulation with a liposome complex composed of phosphatidyl- β -oleoyl- γ -palmitoyl ethanolamine (DOPE) and cholesterol hemisuccinate (CHEMS) (1:1 ratio); we call this CpG-DNA-liposome complex Lipoplex(O).^(18–20) With the aid of Lipoplex(O) as an adjuvant, we successfully produced monoclonal antibodies against transmembrane 4 superfamily member 5 (TM4SF5) and HA protein of the avian influenza virus using B cell epitope peptides as an antigen without a conventional carrier.^(20–22) In this study, we produced an hSUMO-1-specific monoclonal antibody using recombinant hSUMO-1 protein and Lipoplex(O).

Materials and Methods

ODNs and reagents

Natural phosphodiester bond CpG-DNA, MB-ODN 4531(O), was obtained from ST Pharm, Ltd. (Seoul, Korea). MB-ODN 4531(O) consists of 20 bases containing three CpG motifs (underlined): AGCAGCGTTCGTCGCGCCT.⁽¹⁷⁾ Phosphorothioate backbone CpG-DNA, 1826(S), was synthesized by GenoTech (Daejeon, Korea). The CpG-DNA 1826(S) consists of 20 bases containing two CpG-motifs (underlined): TCCAT GACGTTCTGACGTT. The liposomes DOPE and CHEMS were purchased from Sigma-Aldrich (St. Louis, MO).

Recombinant protein expression and purification of hSUMO-1

The human SUMO-1, SUMO-2, SUMO-3, SUMO-4, and AR (aldo-keto reductase family 1 B1; aldose reductase) were expressed as His-tagged proteins. Full-length cDNA of each gene was purchased from Origene (Rockville, MD) and was amplified by PCR reaction using the following primer sets: sense 5'-GAA CAT ATG TCT GAC CAG GAG GCA AAA CC-3' and anti-sense 5'-GAA CTC GAG AAC TGT TGA ATG ACC CCC CG-3' for hSUMO-1; sense 5'-GAA CAT ATG GCC GAC GAA AAG CCC A-3' and anti-sense 5'-GAA CTC GAG GTA GAC ACC TCC CGT CTG C-3' for hSUMO-2; sense 5'-GAA CAT ATG TCC GAG GAG AAG CCC AAG-3' and anti-sense 5'-GAA CTC GAG GAA ACT GTG CCC TGC CAG GC-3' for hSUMO-3; sense 5'-GAA CAT ATG GCC AAC GAA AAG CCC ACA G-3' and anti-sense 5'-GAA CTC GAG GTA GAC ACC TCC CGT AGG CTG-3' for hSUMO-4; sense 5'-GAA GAA CAT ATG GCA AGC CGT CTC CTG CTC-3' and anti-sense 5'-GAA GAA CTC GAG AAA CTC TTC ATG GAA GGG GTA ATC C-3' for AR. The amplified cDNA fragments were then cloned into the expression vector pET-23b (Novagen, Darmstadt, Germany) for the C-terminus His-tag. Expression of the recombinant proteins was induced in *Escherichia coli* BL21(DE3) (Invitrogen, Carlsbad, CA) using 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG, Sigma Aldrich). The bacterial cell pellets were resuspended in a lysis buffer (10 mM PBS containing 1 μ g/mL lysozyme, 5 mM EDTA, 10 μ g/mL leupeptin, 1 mM PMSF, and 1 mM DTT) and disrupted by sonication. After centrifugation, the supernatant was used for Western blot analysis. To purify hSUMO-1, the supernatant of hSUMO-1 was mixed with Ni-NTA beads (Qiagen, Valencia, CA). The bound proteins were eluted with 500 mM imidazole and analyzed by SDS-PAGE.

Preparation of hSUMO-1 and Lipoplex(O) complex

The hSUMO-1 and Lipoplex(O) complex was prepared as reported previously.⁽²⁰⁾ Briefly, DOPE and CHEMS were mixed in ethanol at a molar ratio of 1:1, evaporated with nitrogen gas to make a solvent-free lipid film, and resuspended in a mixture containing equal volumes of water-soluble MB-ODN 4531(O) and hSUMO-1, followed by vigorous stirring at room temperature for 30 min. After adjusting the pH to 7.0, the Lipoplex solution was slightly sonicated for 30 s with a sonicator. The solution was filtered with a 0.22 μ m filter and freeze-thawed three times with liquid nitrogen.

Mice and immunization of human SUMO-1

Mice were maintained under specific-pathogen-free conditions. Four-week-old female BALB/c (H-2b) mice were purchased from Orientbio (Seongnam, Korea). Our animal studies were approved by the Institutional Animal Care and Use Committee of Hallym University (Hallym2012-66). On three occasions at 10-day intervals, the mice were injected intraperitoneally (i.p.) with 50 μ g of hSUMO-1 and 1826(S) or hSUMO-1 and Lipoplex(O) complex, respectively.

Antigen-specific Ig ELISA

Mouse sera were obtained by orbital bleeding from the ophthalmic vein before each injection as well as by sacrifice 10 days after final injection and then stored at -70°C . To measure the antibody titer of total IgG, 96-well immunoplates (Nalge Nunc International, Penfield, NY) were coated with 5 μ g/well of hSUMO-1 protein. The plate was blocked with PBST containing 1% BSA for 1 h. The sera were added to the top row of the plate, and serial 1:3 dilutions in PBST were placed into subsequent rows. The plates were incubated for 2 h at room temperature and washed with PBST. Biotinylated donkey anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) was then added to the wells and incubated for 2 h, followed by the addition of streptavidin conjugated with horseradish peroxidase for 1 h. A colorimetric assay was carried out with a TMB substrate solution (Kirkegaard and Perry Laboratories, Gaithersburg, MD), and the absorbance was measured at 450 nm with a Spectra Max 250 microplate reader (Molecular Devices, Sunnyvale, CA).

Production of mouse anti-hSUMO-1 monoclonal antibody

The spleens from selected mice were used for fusion to generate hybridomas.⁽²³⁾ Fusion was performed by mixing splenocytes with mouse SP2/0 myeloma cells at a 3:1 ratio in a polyethylene glycol solution (PEG, Sigma Aldrich) and cultured in HAT medium (Sigma Aldrich). Hybridoma cells selected by screening supernatants with ELISA were subjected to two rounds of subcloning by a standard limiting dilution protocol to obtain clonal cell population.

Purification of anti-hSUMO-1 monoclonal antibody

Anti-hSUMO-1 monoclonal antibody was purified from the ascitic fluid by protein-A column chromatography. The ascitic fluid was passed through protein-A agarose that was equilibrated with protein-A IgG binding buffer (Pierce, Rockford, IL). After washing, the antibodies were eluted using IgG elution

buffer (Pierce). The eluted fractions were neutralized, pooled, dialyzed against PBS, and concentrated using an Amicon Ultracel 10 K filter (Millipore, Carrigtwohill, Ireland).

Western blot analysis

Recombinant hSUMO-1, 2, 3, 4, and AR were separated by SDS-PAGE. After being transferred to nitrocellulose membranes, proteins were incubated with anti-hSUMO-1 monoclonal antibody for 2 h and washed with PBST. HRP-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories) was then treated as a secondary antibody for 1 h, and immunoreactive bands were detected by addition of an enhanced chemiluminescence solution (Amersham, Piscataway, NJ).

FIG. 1. Preparation of recombinant hSUMO-1 protein and production of hSUMO-1-specific antibody. **(A)** His-tagged human SUMO-1 recombinant protein was expressed in *E. coli* and purified using a Ni-NTA column. Purified protein was analyzed by SDS-PAGE and Western blotting using anti-His antibody. **(B)** Mice were immunized with PBS, 1826(S) plus hSUMO-1, or hSUMO-1 plus Lipoplex(O). Mouse sera were analyzed by ELISA after coating with hSUMO-1.

Tissue microarrays and immunohistochemistry

For analysis of various cancer tissues and corresponding normal tissues, a formalin-fixed, paraffin-embedded Accu-Max tissue array (A301(VI)) was purchased from ISUABXIS

FIG. 2. Isolation of hybridoma clones producing anti-hSUMO-1 antibody. **(A)** Titration curves were obtained by ELISA using the culture supernatant of positive hybridoma clones. **(B)** Monoclonal antibodies were purified from the ascites of mice injected with the hybridoma cell line 1B12-E2 using protein-A agarose column chromatography. The purified antibody was identified by SDS-PAGE and Coomassie staining. **(C)** Titration curve was obtained using normal IgG and purified 1B12-E2 monoclonal antibodies.

(Seoul, Korea) and used with the approval of the Institutional Review Board in Hallym University. The slides were deparaffinized in xylene, rehydrated, and treated with fresh 0.3% hydrogen peroxide in methanol for 15 min. The tissue arrays were stained with the anti-hSUMO-1 monoclonal antibody (10 µg/mL), according to the standard procedures.

Results

Expression and purification of hSUMO-1

Full-length cDNA of hSUMO-1 was cloned into the plasmid vector pET-23b for the expression of hSUMO-1 in *E. coli* BL21(DE3). Upon induction with 0.5 mM IPTG, recombinant hSUMO-1 was produced with a C-terminal poly histidine affinity tag (6x His-tag) for purification by Ni-NTA agarose beads. The fractions obtained during purification steps were analyzed by SDS-PAGE and Coomassie blue staining. Although the expected molecular weight of hSUMO-1 is 11 kDa, recombinant hSUMO-1 was detected with an apparent molecular weight of 17 kDa, which is in accordance with previous reports (Fig. 1A).

Immunization of hSUMO-1 encapsulated in Lipoplex(O)

Many investigators use CpG-DNA with a phosphorothioate-modified backbone as an adjuvant; therefore we used 1826(S) as a control. BALB/c mice were injected with hSUMO-1 and 1826(S) or with the complex of hSUMO-1 and Lipoplex(O). After booster immunization three times at 10-day intervals, mouse sera were collected and checked for antibody titers against hSUMO-1 using ELISA. The complex of hSUMO-1 and Lipoplex(O) induced significantly greater abundance of hSUMO-1-specific IgG than a mixture of hSUMO-1 and 1826(S) (Fig. 1B). Therefore, we concluded that Lipoplex(O) is superior to phosphorothioate-modified CpG-DNA as an adjuvant.

Generation and purification of monoclonal antibody against hSUMO-1

As superior adjuvant activity of Lipoplex(O) was identified (Fig. 1B), the mouse with the highest IgG titer against hSUMO-1 was selected among the mice injected with the complex

of hSUMO-1 and Lipoplex(O) and boosted with the same antigen complex. After 10 days, mouse splenocytes were collected and used for generation of hybridoma cells. The hybridoma cells highly producing hSUMO-1-specific antibodies were screened by indirect ELISA using cell culture supernatants. As a result, four hybridoma cells producing anti-hSUMO-1 antibody were obtained (1B12-B3, 1B12-E2, 1B12-G6, 1B12-H1) and the antibodies showed similar antigen binding affinity (Fig. 2A). Of these clones, the 1B12-E2 clone was selected and injected with pristane-primed mice for generation of ascites. To purify the anti-hSUMO1 monoclonal antibody from the ascites, protein-A agarose column chromatography was performed and the purified antibody was identified by SDS-PAGE and Coomassie staining (Fig. 2B). The monoclonal antibody was analyzed by ELISA for IgG isotyping. Anti-hSUMO-1 MAb isotype was identified as an IgG3.

Western blot analysis with anti-hSUMO-1 monoclonal antibody

A Western blot analysis was performed to evaluate whether the obtained anti-hSUMO-1 monoclonal antibody 1B12-E2 could recognize hSUMO1 protein specifically. The purified recombinant hSUMO-1 protein and other sub-families were separated by SDS-PAGE and subjected to Western blot analysis. The results show that 1B12-E2 can recognize only hSUMO-1 and has no reactivity with other SUMO sub-families (Fig. 3). A commercially available control antibody from Cell Signaling Technology (Danvers, MA) showed the same affinity.

Expression of SUMO-1 in human tissues

In order to investigate expression of hSUMO-1 in various normal and cancer human tissues, we performed an immunohistochemistry analysis on a tissue microarray using anti-hSUMO-1 monoclonal antibody 1B12-E2. As shown in Figure 4, higher expression of hSUMO-1 was detected in normal adrenal gland, esophagus, pancreas, liver, stomach, kidney, and uterus than in corresponding cancer tissues. In the case of skin, expression of SUMO-1 was detected in squamous cell carcinoma but not in corresponding normal tissue. However, prominent expression of hSUMO-1 was not detected in many

FIG. 3. Specificity of anti-hSUMO-1 antibody. Recombinant hSUMO-1, hSUMO2, hSUMO-3, hSUMO4, and AR were separated by SDS-PAGE, and Western blotting was performed using anti-hSUMO-1 antibody prepared from the 1B12-E12 hybridoma clone. A commercially available antibody from Cell Signaling Biotechnology was used as a positive control. A non-specifically bound protein band detected by secondary antibody through overexposure was used as internal control.

FIG. 4. Differential expression of hSUMO-1 in human tissues. Human tissue microarray was analyzed by immunohistochemistry using anti-hSUMO-1 monoclonal antibody (1B12-E12). The human tissue microarray was composed of cancer tissues (lower) and the corresponding normal tissues (upper). SCC, HCC, CA, and RCC represent squamous cell carcinoma, hepatocellular carcinoma, carcinoma, and renal cell carcinoma, respectively. Scale bars, 50 μ m.

other human cancer tissues and corresponding normal tissues (Fig. 5). Therefore, the results suggest that the expression pattern of hSUMO-1 may be a diagnostic marker for some cancers in humans.

Discussion

Selection of an appropriate adjuvant is important for efficacious immunostimulation and production of antibodies *in vivo* after antigen injection.^(15–17) The natural CpG-DNA with a phosphodiester backbone encapsulated with a liposome complex is a promising adjuvant.^(18–20) In this study, we used recombinant hSUMO-1 purified from *E. coli* as an antigen and CpG-DNA-liposome complex (Lipoplex(O)) as an adjuvant for production of a monoclonal antibody specific to hSUMO-1.

Previously, we found that Lipoplex(O) significantly enhances the production of antibodies against B cell epitope peptides derived from tumor antigen and viral surface antigen, and thereby Lipoplex(O) increases the efficacy of pro-

phylactic peptide vaccines in animal studies.^(21,22) We further confirmed that Lipoplex(O) significantly contributes to the production of antibodies against commercially available purified proteins such as ovalbumin and hen egg lysozyme.⁽¹⁸⁾ In this study, we established monoclonal antibodies against hSUMO-1 by immunization with a complex of Lipoplex(O) and recombinant protein expressed in and purified from *E. coli*. Therefore, we can conclude that our immunization protocol using Lipoplex(O) can be widely used for the production of antibodies against peptides, purified natural proteins, and purified recombinant proteins.

We produced four monoclonal antibodies with similar affinity to hSUMO-1. 1B12-E2, one of the four monoclonal antibodies, recognized only hSUMO-1 but not other SUMO families even though we used a whole recombinant hSUMO-1 protein. Compared to the monoclonal antibody purchased from Cell Signaling, 1B12-E2 showed similar activity of antigen binding with hSUMO-1. This may have resulted from the sequence homology feature: hSUMO-1 has relatively lower homology with other hSUMO isoforms (38~43%) while

FIG. 5. Expression of hSUMO-1 in various human tissues. The human tissue microarray was analyzed by immunohistochemistry using anti-hSUMO-1 monoclonal antibody 1B12-E12. The human tissue microarray was composed of cancer tissues (left) and the corresponding normal tissues (right). SCC, HCC, CA, and RCC represent squamous cell carcinoma, hepatocellular carcinoma, carcinoma, and renal cell carcinoma, respectively. Scale bars, 200 μ m.

hSUMO-2, hSUMO-3, and hSUMO-4 have homology of >76%, and hSUMO-2 and hSUMO-3 are highly homologous (87%).⁽¹⁾

Since production of the hSUMO-1 monoclonal antibodies using Lipoplex(O) adjuvant was successful, it will be possible to produce monoclonal antibodies against other SUMO isoforms as well. Because of the high homology between hSUMO-2 and SUMO-3, many of the commercially available

antibodies to hSUMO-2 or hSUMO-3 have cross-reactivity between the two proteins. Therefore, application of a peptide epitope and Lipoplex(O) complex may be more helpful in obtaining antibodies that are specific to only hSUMO-2 or hSUMO-3.

Sumoylation is known to be involved in cancer by stabilizing proteins such as HIF-1 α and Mdm2 through competitive ligation of SUMO proteins to the target residues for

ubiquitination.^(24,25) SUMO-1 is also reported to promote glycolysis in hypoxia.⁽²⁶⁾ Therefore, overexpression or enhanced function of SUMO can be correlated with carcinogenesis and some reports have supported this notion: overexpression of hSUMO-1 and sumoylated Mdm2 in oral squamous cell carcinoma, and association of hSUMO-1 and UBC9 genotypes with tumor response in non-small-cell lung cancer treated with chemotherapy.^(25,27) On the other hand, a tumor suppressive role of sumoylation has been suggested: SUMO-specific protease 1 promotes prostate cancer progression.^(28,29) As the expression levels of hSUMO-1 were higher in normal adrenal gland, esophagus, pancreas, liver, stomach, kidney, and uterus than in corresponding cancer tissues (Fig. 4), our results also support the suppressive effect of hSUMO-1. However, higher expression was found in the skin squamous cell carcinoma. Therefore, the function of hSUMO-1 may vary depending on the tissue types. Further studies are necessary to fully understand the function of hSUMO-1 in the context of cancer progression and to validate the possibility of hSUMO-1 as a diagnostic marker of cancer.

Acknowledgments

This research was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning in the Republic of Korea (2012-R1A2A2A01009887, 20120006130, 20120006695) and supported by the KIST Gangneung Institute intramural grant (2Z03850) program.

Author Disclosure Statement

The authors have no financial interests to disclose.

References

1. Sarge KD, and Park-Sarge OK: Sumoylation and human disease pathogenesis. *Trends Biochem Sci* 2009;34:200–205.
2. Gill G: SUMO and ubiquitin in the nucleus: different functions, similar mechanisms? *Genes Dev* 2004;18:2046–2059.
3. Geiss-Friedlander T, and Melchior F: Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol* 2007;8:947–956.
4. Kim KI, Baek SH, Jeon, YJ, Nishimori S, Suzuki T, Uchida S, Shimbara N, Saitoh N, Tanaka K, and Chung CH: A new SUMO-1-specific protease, SUSP1, that is highly expressed in reproductive organs. *J Biol Chem* 2000;275:14102–14106.
5. Tatham MH, Jaffray E, Vaughan OA, Desterro JM, Botting CH, Naismith JH, and Hay RT: Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. *J Biol Chem* 2001;276:35368–35374.
6. Johnson ES: Protein modification by SUMO. *Annu Rev Biochem* 2004;73:355–382.
7. Matunis MJ, Coutavas E, and Blobel G: A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J Cell Biol* 1996;135:1457–1470.
8. Mahahan R, Delphin C, Guan T, Gerace L, and Melchior F: A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 1997;88:97–107.
9. Desterro JM, Rodriguez MS, and Hay RT: SUMO-1 modification of I κ B α inhibits NF- κ B activation. *Mol Cell* 1998;2:233–239.
10. Bhaskar V, Valentine SA, and Courey AJ: A functional interaction between dorsal and components of the Smt3 conjugation machinery. *J Biol Chem* 2000;275:4033–4040.
11. Li T, Evdokimov E, Shen RF, Chao CC, Tekle E, Wang T, Stadtman ER, Yang DC, and Chock PB: SUMOylation of heterogeneous nuclear ribonucleoproteins, zinc finger proteins, and nuclear pore complex proteins: A proteomic analysis. *Proc Natl Acad Sci USA* 2004;101:8551–8556.
12. Zhao Y, Kwon SW, Anselmo A, Kaur K, and White MA: Broad spectrum identification of cellular small ubiquitin-related modifier (SUMO) substrate proteins. *J Biol Chem* 2004;279:20999–21002.
13. Wagner H: Bacterial CpG DNA activates immune cells to signal infectious danger. *Adv Immunol* 1999;73:329–368.
14. Sparwasser T, Miethke T, Lipford G, Borschert K, Hacker H, Heeq K, and Wagner H: Bacterial DNA causes septic shock. *Nature* 1997;386:336–337.
15. Ellis RD, Martin LB, Shaffer D, Long CA, Miura K, Fay MP, Narum DL, Zhu D, Mullen GE, Mahanty S, Miller LH, and Durbin AP: Phase 1 trial of the Plasmodium falciparum blood stage vaccine MSP1(42)-C1/Aldydogel with and without CPG7909 in malaria naïve adults. *PLoS One* 2010;5:e8787.
16. Cooper CL, Davis HL, Angel JB, Morris ML, Elfer SM, Sequin I, Krieg AM, and Cameron DW: CPG 7909 adjuvant improves hepatitis B virus vaccine seroprotection in anti-retroviral-treated HIV-infected adults. *AIDS* 2005;19:1473–1479.
17. Lee KW, Jung J, Lee Y, Kim TY, Choi SY, Park J, Kim DS, and Kwon HJ: Immunostimulatory oligodeoxynucleotide isolated from genome wide screening of Mycobacterium bovis chromosomal DNA. *Mol Immunol* 2006;43:2107–2118.
18. Kim D, Kwon S, Ahn CS, Lee Y, Choi SY, Park J, Kwon HY, and Kwon HJ: Adjuvant effect of liposome-encapsulated natural phosphodiester CpG-DNA. *BMB Rep* 2011;44:758–763.
19. Kim D, Kwon HJ, and Lee Y: Activation of Toll-like receptor 9 and production of epitope specific antibody by liposome-encapsulated CpG-DNA. *BMB Rep* 2011;44:607–612.
20. Kim D, Kwon S, Rhee JW, Kim KD, Kim YE, Park CS, Choi MJ, Suh JG, Kim DS, Lee Y, and Kwon HJ: Production of antibodies with peptide-CpG-DNA-liposome complex without carriers. *BMC Immunol* 2011;12:29.
21. Rhee JW, Kim D, Park BK, Kwon S, Cho S, Lee I, Park MS, Seo JN, Kim YS, Choi HS, Lee Y, and Kwon HJ: Immunization with a hemagglutinin-driven synthetic peptide formulated with a CpG-DNA-liposome complex induced protection against lethal influenza virus infection in mice. *PLoS One* 2012;7:e48750.
22. Kwon S, Kim D, Park BK, Cho S, Kim KD, Kim YE, Park CS, Ahn HJ, Seo JN, Choi KC, Kim DS, Lee Y, and Kwon HJ: Prevention and therapy of hepatocellular carcinoma by vaccination with TM4SF5 epitope-CpG-DNA-liposome complex without carriers. *PLoS One* 2012;7:e33121.
23. Yokoyama WM, Christensen M, Santos GD, and Miller D: Production of monoclonal antibodies. *Curr Protoc Immunol* 2006, Chapter 2, Unit 2.5.
24. Bae SH, Jeong JW, Park JA, Kim SH, Bae MK, Choi SJ, and Kim KW: Sumoylation increases HIF-1 α stability and its transcriptional activity. *Biochem Biophys Res Commun* 2004;324:394–400.
25. Katayama A, Ogino T, Bandoh N, Takahara M, Kishibe K, Nonaka S, and Harabuchi Y: Overexpression of small ubi-

- quitin-related modifier-1 and sumoylated Mdm2 in oral squamous cell carcinoma: possible involvement in tumor proliferation and prognosis. *Int J Oncol* 2007;31:517–524.
26. Agbor TA, Cheong A, Comerford KM, Scholz CC, Bruning U, Clarke A, Cummins EP, Cagney G, and Taylor CT: Small ubiquitin-related modifier (SUMO)-1 promotes glycolysis in hypoxia. *J Biol Chem* 2011;286:4718–4726.
 27. Han JY, Lee GK, Yoo SY, Yoon SJ, Cho EY, Kim HT, and Lee JS: Association of SUMO1 and UBC9 genotypes with tumor response in non-small-cell lung cancer treated with irinotecan-based chemotherapy. *Pharmacogenomics J* 2010;10:86–93.
 28. Wang Q, Xia N, Li T, Xu Y, Zou Y, Zuo Y, Fan Q, Bawa-Khalfe T, Yeh ET, and Cheng J: SUMO-specific protease 1 promotes prostate cancer progression and metastasis. *Oncogene* 2012;32:2493–2498.
 29. Cheng J, Bawa T, Lee P, Gong L, and Yeh ET: Role of de-sumoylation in the development of prostate cancer. *Neoplasia* 2006;8:667–676.

Address correspondence to:

*Dr. Hyung-Joo Kwon
Department of Microbiology
College of Medicine
Hallym University
Gangwon-do, 200-702
Republic of Korea*

E-mail: hjoonkwon@hallym.ac.kr

Received: June 3, 2013

Accepted: July 17, 2013