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SUMOylated IRF-1 shows oncogenic potential by mimicking IRF-2

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ABSTRACT

Interferon regulatory factor-1 (IRF-1) is an interferon-induced transcriptional activator that suppresses tumors by impeding cell proliferation. Recently, we demonstrated that the level of SUMOylated IRF-1 is elevated in tumor cells, and that SUMOylation of IRF-1 attenuates its tumor-suppressive function. Here we report that SUMOylated IRF-1 mimics IRF-2, an antagonistic repressor, and shows oncogenic potential. To demonstrate the role of SUMOylated IRF-1 in tumorigenesis, we used SUMO-IRF-1 recombinant protein. Stable expression of SUMO-IRF-1 in NIH3T3 cells resulted in focus formation and anchorage-independent growth in soft agar. Inoculation of SUMO-IRF-1-transfected cells into athymic nude mice resulted in tumor formation and infiltration of adipose tissues. Finally, we demonstrated that SUMO-IRF-1 transforms NIH3T3 cells in a dose-dependent manner suggesting that SUMOylated IRF-1 may act as an oncogenic protein in tumor cells.

Introduction

Interferon regulatory factor-1 (IRF-1) was originally identified as a transcriptional activator of the interferon-β gene. Interferon regulatory factor-1 expression is activated upon viral infection and induces the expression of members of the interferon family, including IFNα, IFNβ, IFNγ, and TNFα [1]. Ectopic expression of IRF-1 impedes cell proliferation by transactivating cell cycle-related genes such as 2–5A synthetase and p21 (WAF-1/CIP-1) and also causes apoptosis under certain circumstances by inducing genes such as bak, caspase-8, and caspase-7 [2,3]. For this reason, it was proposed that IRF-1 functions as a tumor suppressor, and many findings have been made that support this hypothesis [4–8]. In tumor cells, IRF-1 is frequently inactivated thus preventing IRF-1-mediated apoptosis and cell cycle arrest via genetic mechanisms such as gene deletion and exon skipping by alternative splicing [9–11].

In contrast, IRF-2, an antagonistic transcriptional repressor of IRF-1, displays oncogenic potential [7,8]. Interferon regulatory factor-2 inhibits IRF-1-mediated apoptosis by repressing IRF-1-dependent transcription, and the overexpression of IRF-2 induces NIH3T3 cell transformation and tumor formation in nude mice [8]. In addition, IRF-2 overexpression enhances the tumorigenicity of B16 mouse melanoma cells, and the level of IRF-2 expression in human esophageal cancer cells is closely correlated with the progression of esophageal cancer cells [12]. Recently, we demonstrated that IRF-2 enhances NF-κB activity by modulating the subcellular localization of NF-κB, and both the repression of IRF-1-dependent transcription and the activation of NF-κB are likely to contribute to its oncogenic potential [13].

SUMOylation is a posttranslational modification in which a SUMO moiety is covalently conjugated with the lysine residues of substrate proteins [14]. When the substrate of SUMOylation is a transcription factor, SUMOylation often modulates its transcriptional activity by regulating the substrate's interactions with transcriptional machinery such as co-activators and co-repressors. Recently, we reported that IRF-1 is aberrantly SUMOylated in tumor cells and that SUMOylation of IRF-1 decreases the degradation of this protein by blocking its ubiquitination [15]. Moreover SUMOylation of IRF-1 attenuates its transcriptional activity and also interferes with IRF-1-mediated apoptosis [15]. Because SUMOylated IRF-1 inhibits IRF-1-dependent transcription, we hypothesized that SUMOylated IRF-1 mimics IRF-2 function and could have some oncogenic potential. In the present report, we showed that the function of SUMOylated IRF-1 is analogous to that

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of IRF-2, and that SUMOylation converted the tumor suppressor IRF-1 into an oncogenic protein.

Materials and methods

Cell culture and transfection. HEK293A and NIH3T3 cells were grown in DME medium supplemented with 10% fetal bovine serum. Transfections of HEK293A and NIH3T3 cells were conducted using Welfect transfection reagent (Welgene, Korea) in accordance with the manufacturer's instructions. For the luciferase assays, cells were seeded in 12-well plates in DMEM 18 h prior to transfection. Total DNA for the transfections was typically 0.5 μg per plate, consisting of 0.1 μg luciferase reporter construct (ISRE-Luc and 3XNF-κB-Luc), 0.1 μg β-galactosidase (β-Gal) internal control vector, and the indicated quantities of expression vector. Human TNFα was purchased from Sigma (St. Louis, MO).

Transformation assay. NIH3T3 cells were transfected with pcDNA3, pcDNA3/IRF-1, pcDNA3/IRF-2, pcDNA3/SUMO-IRF-1, and pcDNA3/SUMO-IRF-1 ΔN, and stable cell lines were obtained after selection with G418 (1 mg/ml) (AG Scientific, San Diego, CA). For the focus forming assay, 1×10^5 NIH3T3 cells were plated onto 35-mm dishes. Cellular foci were observed after 2 weeks by fixing and staining with 0.4% crystal violet. Counts represent the mean values of triplicate experiments. For the soft agarose assay, 1×10^4 NIH3T3 cells were plated in 3 ml 0.35% low-melting agarose (Sigma) in 10% FBS/DME, and were overlaid onto 5 ml of 0.8% agarose in 10% FBS/DMEM. Cellular foci were observed after 2 weeks. For the tumor xenograft experiments, 1×10^6 NIH3T3 cells were injected subcutaneously into the hind limb of a nude mouse that was then was sacrificed 19–21 d post-injection. Tumors were fixed with 4% paraformaldehyde in PBS and stained with hematoxylin and eosin. The animal study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Samsung Biomedical Research Institute (SBRI).

Results

Focus formation in NIH3T3 cells by SUMO-IRF-1

We have recently demonstrated that the level of SUMOylated IRF-1 is elevated in tumor cells and that SUMOylated IRF-1 interferes with IRF-1-mediated apoptosis by repressing IRF-1-dependent transcription [15]. Because the function of SUMOylated IRF-1 is similar to that of IRF-2 in the control of IRF-1-dependent

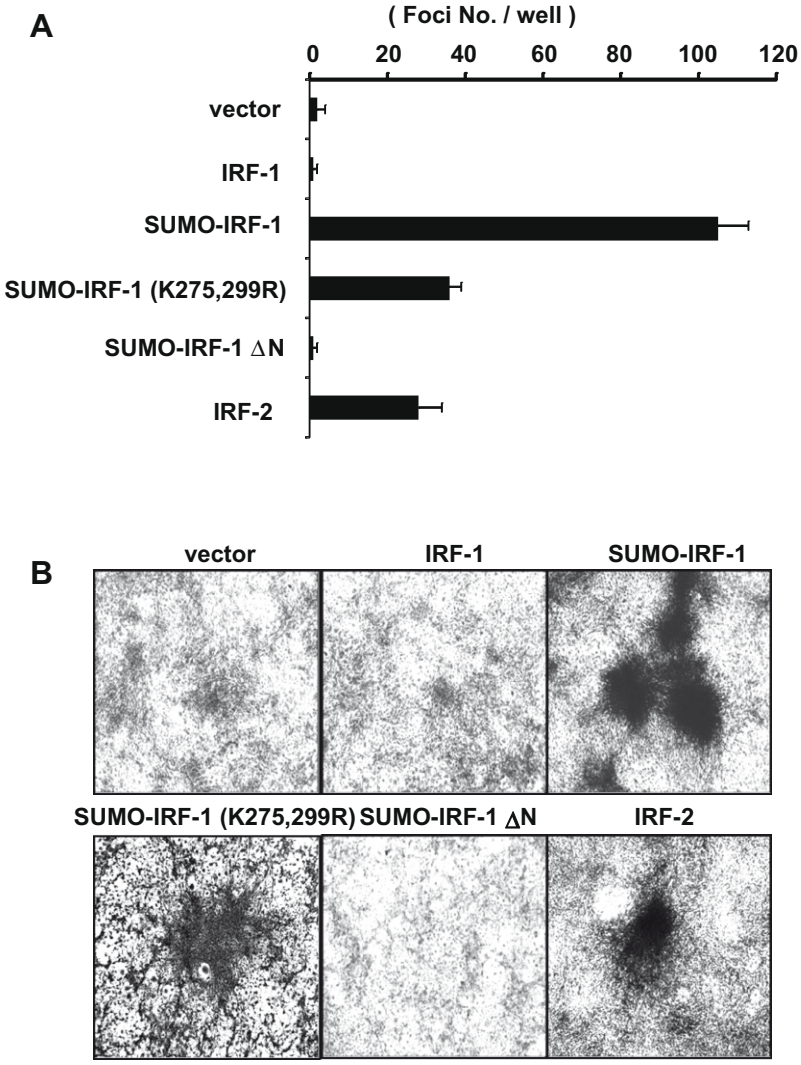

Fig. 1. Focus formation assay in NIH3T3 cells. (A) NIH3T3 cells were transfected with pcDNA3, pcDNA3/IRF-1, pcDNA3/SUMO-IRF-1, pcDNA3/SUMO-IRF-1 (K275,299R), and pcDNA3/SUMO-IRF-1 ΔN. Transfected cells were grown in G418-containing medium, and resistant cells were plated at 10^5 cells per 35-mm culture dish. After 2 weeks of incubation, the colony numbers were counted. The counts represent the means of experiments performed in triplicate. (B) The photographs were taken to confirm colony formation after 2 weeks.

transcription, we hypothesized that SUMOylated IRF-1 acts as an oncogenic protein. To test our hypothesis, we performed a focus formation assay in murine fibroblast NIH3T3 cells. Because it is difficult to exclusively SUMOylate IRF-1 protein, we fused SUMO-1 to the C-terminal end of IRF-1 proteins (SUMO-IRF-1). In this way, we were able to study the function of SUMOylated IRF-1 specifically [16,17]. Cells were transfected with pcDNA3, pcDNA3/IRF-1, pcDNA3/SUMO-IRF-1, pcDNA3/SUMO-IRF-1 (K275,299R), pcDNA3/SUMO-IRF-1 ΔN, and pcDNA3/IRF-2. After selection with G418, the cells were detached and plated in 35-mm plates. Cellular foci were observed after 15 d. While IRF-1 and SUMO-IRF-1 ΔN did not induce focus formation, transfection with either SUMO-IRF-1, pcDNA3/SUMO-IRF-1 (K275,299R), or IRF-2 induced focus formation (Fig. 1A). We observed dense transformed foci in both SUMO-IRF-1- and IRF-2-transfected cells (Fig. 1B). These results suggest that SUMO-IRF-1 has oncogenic potential.

Transforming potential of SUMO-IRF-1

To elucidate the role of SUMO-IRF-1 in transformation, NIH3T3 cells were transfected with the plasmid encoding SUMO-IRF-1, and the stably transfected cell populations were isolated. The polyclonal cells expressing SUMO-IRF-1 were pooled, and the expression levels of a number of cell cycle-related genes were examined by Western blotting. While p53 levels did not change, the level of p21, a cell cycle inhibitor, was downregulated and the level of cyclin D was up-regulated (Fig. 2A). These results suggest that the ectopic expression of SUMO-IRF-1 induces cell proliferation by modulating cell cycle-related genes.

Next, soft agar assays were performed with the cell lines stably expressing SUMO-IRF-1 in order to confirm the transforming potential of SUMO-IRF-1. The ectopic expression of SUMO-IRF-1 exhibited a dramatic increase in colony number, supporting the notion that SUMO-IRF-1 has oncogenic potential (Fig. 2B).

To further explore the oncogenic potential of SUMO-IRF-1, we injected SUMO-IRF-1-expressing NIH3T3 cells subcutaneously into athymic nude mice. The left sides of the mice were injected with vector-transfected cells and the right sides with SUMO-IRF-1. SUMO-IRF-1 induced tumors in all six nude mice within 3 weeks, while no tumor growth was observed in the tissues injected with the vector (Fig. 2C), with infiltration of soft adipose tissue observed in some tumors (Fig. 2D). These results further support the oncogenic potential of SUMO-IRF-1.

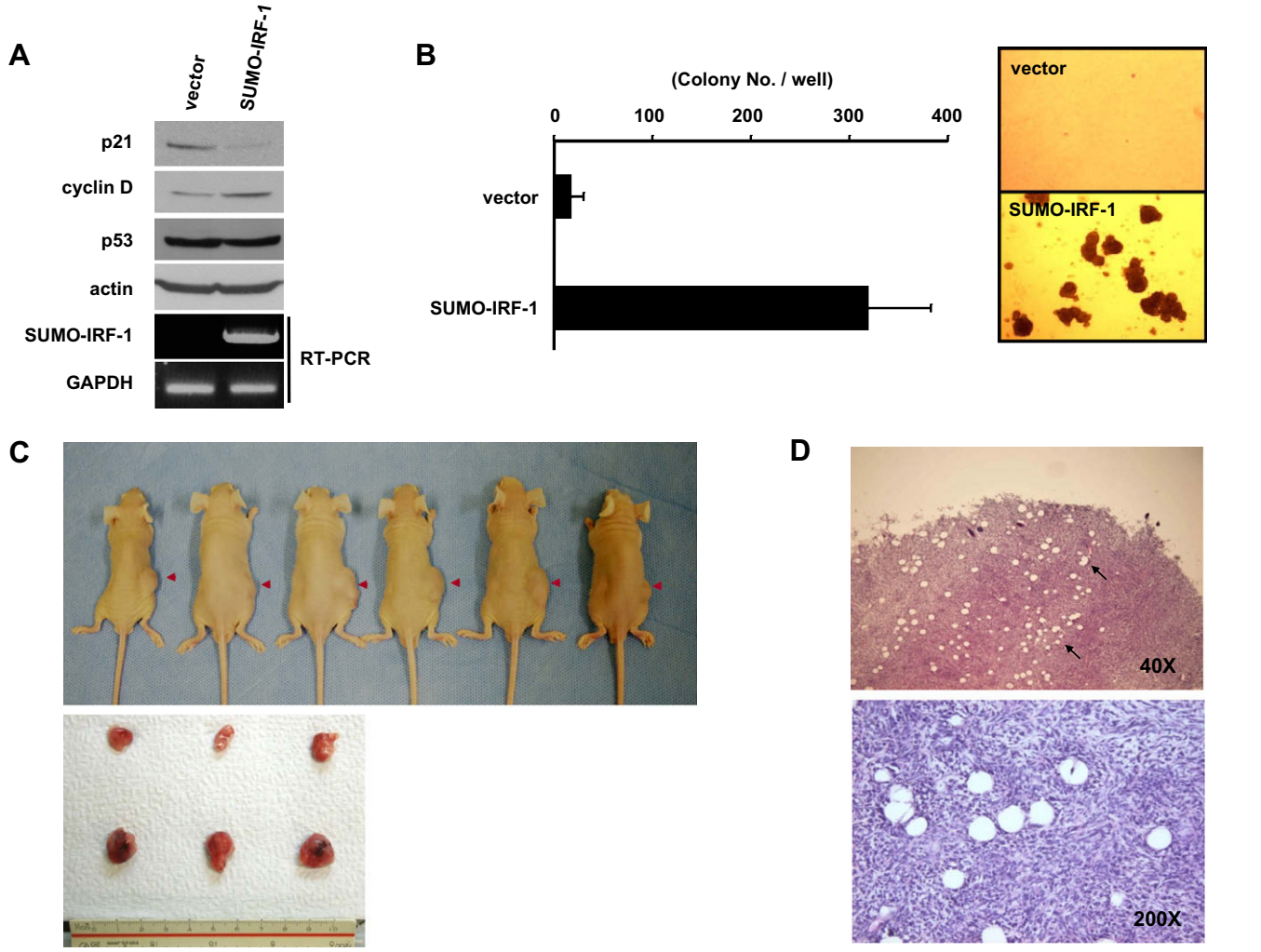

Fig. 2. Oncogenic properties of SUMO-IRF-1. (A) G418-resistant NIH3T3 cells were harvested, and the cell lysates were subjected to immunoblot with anti-p21, anti-cyclin D, anti-p53, and anti-actin antibodies. The expression of SUMO-IRF-1 was confirmed by RT-PCR. (B) SUMO-IRF-1-expressing cells grew as colonies in soft agar. G418-resistant NIH3T3 cells were plated in soft agar, visible colonies were counted in triplicate (left panel), and the colonies were photographed after 2 weeks (right panel). (C) SUMO-IRF-1-expressing NIH3T3 cells (right, arrowhead) grew as tumors in athymic nude mice, in contrast to the vector cells (left) (top panel). Tumors obtained at the end of the experiment are shown (bottom panel). (D) Histologies of the SUMO-IRF-1 NIH3T3-derived tumors stained with hematoxylin and eosin. Invasion of subcutaneous adipose is indicated.

SUMO-IRF-1 induces transformation in a dose-dependent manner

The level of SUMOylated IRF-1 is elevated in tumor cells, and so we examined whether the level of SUMO-IRF-1 correlated with the degree of tumorigenesis. By stable transfection of NIH3T3 cells with either SUMO-IRF-1 or SUMO-IRF-1 (K275,299R), we generated six monoclonal cell lines having either low expression of SUMO-IRF-1, high expression of SUMO-IRF-1, low expression of SUMO-IRF-1 (K275,299R), high expression of SUMO-IRF-1 (K275,299R) or vector-only expression (two cell lines) (Fig. 3A). A focus formation assay with these stable cells showed that higher expression levels of SUMO-IRF-1 or SUMO-IRF-1 (K275,299R) significantly increased the number of foci, as well as the focal size (Fig. 3B). These results indicate a clear correlation between the level of SUMO-IRF-1 expression and the transformation potential, and that SUMO-IRF-1 induces transformation in a dose-dependent manner.

SUMO-IRF-1 mimics IRF-2 function

Because SUMO-IRF-1 shows oncogenic potential in both the focus formation assay and the soft agar assay, we compared the transcriptional regulation by SUMO-IRF-1 with that of IRF-2. Recently we demonstrated that SUMOylated IRF-1 interferes with IRF-1-dependent transcription, and so we added SUMO-IRF-1 (K275,299R) for the analysis of transcription regulation. While SUMO-IRF-1 ΔN did not repress IRF-1-dependent transcription, both SUMO-IRF-1 and SUMO-IRF-1 (K275,299R) downregulated IRF-1-dependent transcription (Fig. 4A). Because IRF-2 protein enhances NF-κB-dependent transcription by modulating the subcellular localization of NF-κB [13], we examined whether SUMO-IRF-1 influences NF-κB-dependent transcription. As shown in Fig. 4B, SUMO-IRF-1 enhanced NF-κB-dependent transcription in a manner similar to IRF-2, and SUMO-IRF-1 ΔN also enhanced NF-κB-dependent transcription (Fig. 4B). In addition, we confirmed the expressions of IRF-1, SUMO-IRF-1, SUMO-IRF-1 (K275,299R) and IRF-2 in the transfected HEK293A cells (Fig. 4C).

Discussion

In this report, SUMO-IRF-1 recombinant protein was used to clarify the role of SUMOylated IRF-1 protein in tumorigenesis,

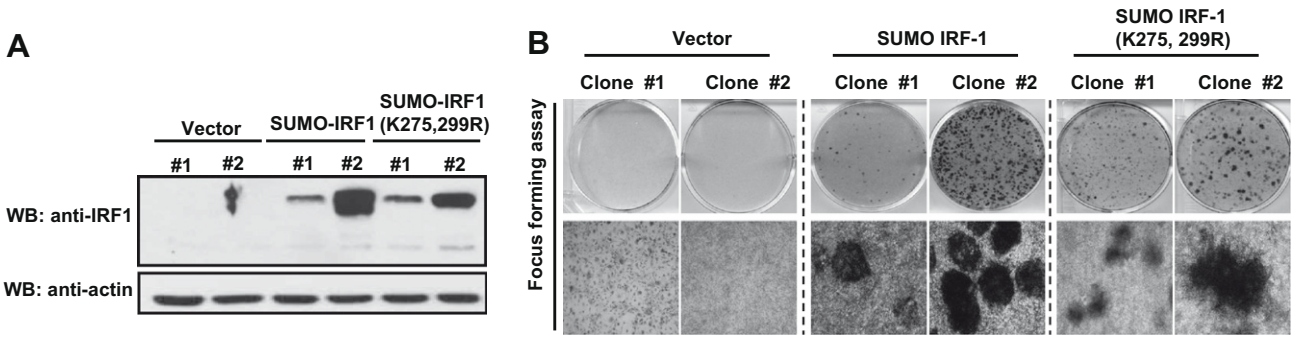

Fig. 3. Dose-dependent effects of the over-expressions of SUMO-IRF-1 and SUMO-IRF-1 (K275,299R) on NIH3T3 transformation. (A) NIH3T3 cells were transfected with either SUMO-IRF-1 or SUMO-IRF-1 (K275,299R), and two stable clones with distinctive expressions were selected for additional experiments. Expression levels of SUMO-IRF-1 were assessed by Western blot with an anti-IRF-1 antibody. (B) Stable clones were plated at 10^5 cells per 35-mm culture dish. After 2 weeks of incubation, cells were stained and photographs were taken.

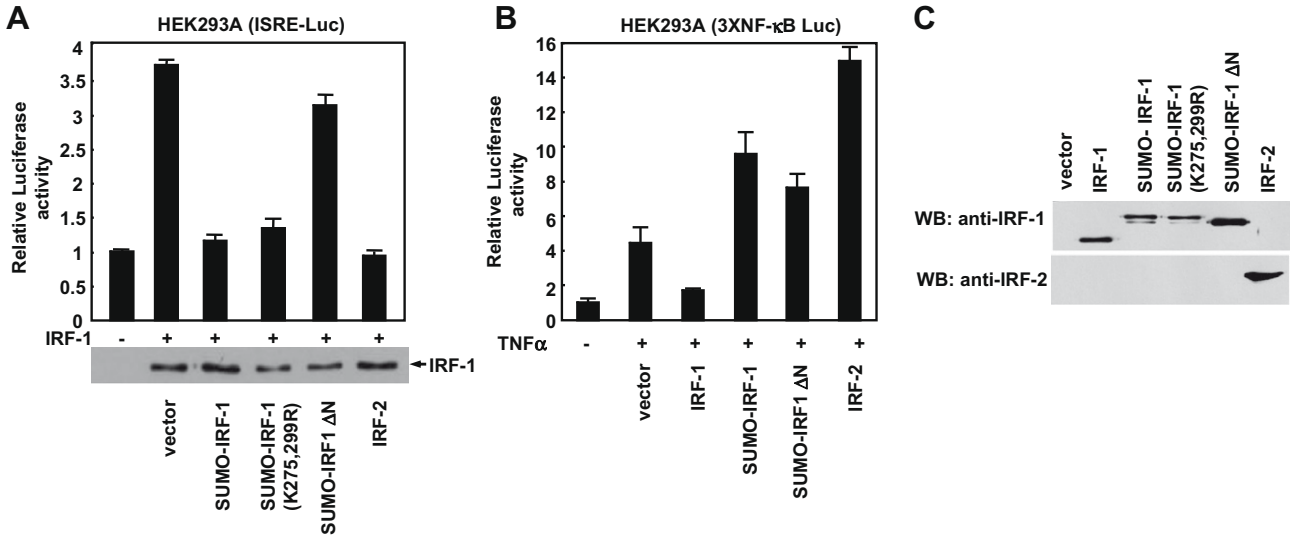

Fig. 4. SUMO-IRF-1 mimics IRF-2 function. (A) SUMO-IRF-1, SUMO-IRF-1 (K275,299R), and IRF-2 downregulate the transcription of IRF-1-dependent transcription. HEK293A cells were transfected with 200 ng ISRE fused to a Luc gene (ISRE-Luc) in the presence or absence of the plasmid encoding IRF-1 (400 ng) together with 500 ng of plasmid encoding either SUMO-IRF-1, SUMO-IRF-1 (K275,299R), SUMO-IRF-1 ΔN, or IRF-2. Expression of IRF-1 was assessed by Western blot with an anti-IRF-1 antibody (bottom panel). (B) SUMO-IRF-1 and IRF-2 enhanced TNFα-induced NF-κB activation. HEK293A cells were transfected with 200 ng 3XNF-κB-Luc together with 500 ng of plasmid encoding either IRF-1, SUMO-IRF-1, SUMO-IRF-1 ΔN, or IRF-2. (C) Transient expressions of IRF-1, SUMO-IRF-1, SUMO-IRF-1 (K275,299R), SUMO-IRF-1 ΔN, and IRF-2 were assessed by Western blot analysis.

and overexpression of SUMO-IRF-1 was found to have oncogenic potential. The stable expression of SUMO-IRF-1 protein in NIH3T3 cells resulted in focus formation and anchorage-independent growth in soft agar. Moreover, the injection of SUMO-IRF-1-transfected cells into athymic nude mice induced tumor formation. To analyze gene regulation by the ectopic expression of SUMO-IRF-1, we examined the expressions of a number of genes and found that the expression levels of p21 and cyclin D were modulated by SUMO-IRF-1.

Recently we demonstrated that SUMOylation of IRF-1 attenuates its tumor-suppressive function as well as its transcriptional activity [15]. In this regard, the function of SUMOylated IRF-1 is quite similar to that of IRF-2, an antagonistic repressor. Interestingly, IRF-2 is an oncogenic protein, and the overexpression of IRF-2 can induce tumor formation *in vitro* and *in vivo* [8]. For this reason, we hypothesized that the SUMOylated IRF-1 may act as an oncogenic protein in tumor cells, since the level of SUMOylated IRF-1 is significantly elevated in tumors. Focus formation assays revealed that SUMO-IRF-1 has an oncogenic potential, and so we examined the expressions of cell cycle-related genes. Western blot analysis revealed that p21 expression was decreased and cyclin D expression was increased by the ectopic expression of SUMO-IRF-1. Previously, it was reported that the transcription of p21, a cell cycle inhibitor, is regulated by IRF-1 and p53 [18]. Because SUMO-IRF-1 can repress IRF-1-dependent transcription, SUMO-IRF-1 could possibly be involved in p21 down-regulation by transcriptional regulation.

In addition, we demonstrated that the expression of cyclin D was increased in SUMO-IRF-1-transfected cells. Because cyclin D expression is up-regulated by the activation of NF-κB [19], SUMO-IRF-1 may cause an elevation in cyclin D expression via stimulation of the NF-κB pathway. However, the disruption of IRF-1-dependent transcription is likely to be more important for transformation by SUMO-IRF-1 than is activation of the NF-κB pathway. This conclusion is supported by our finding that expression of the amino terminal truncated mutant of SUMO-IRF-1 (SUMO-IRF-1 ΔN) enhanced NF-κB-dependent transcription but did not result in focus formation.

To demonstrate the role of SUMOylated IRF-1 in tumor cells, we used the SUMO-IRF-1 recombinant protein. Although this technique is commonly used to demonstrate the role of SUMO [16,17], there might be some functional disparity between the SUMO-recombinant protein and the SUMOylated protein. However, it is difficult to exclusively SUMOylate or deSUMOylate the endogenous IRF-1. Ubiquitin-conjugating enzyme 9 (Ubc9) and SENP can be targeted for down-regulation in order to decrease the SUMOylation of IRF-1, but many other signaling molecules can be influenced by the knockdown of SUMO-related enzymes. Alternatively, the knockdown of IRF-1 can be an effective method for the removal of SUMO-IRF-1; however, the inactivation of IRF-1 could possibly induce tumor formation due to the loss of the protein's tumor-suppressive function. Therefore transformation analysis with SUMOylated protein is quite difficult due to its redundancy, and the recombinant protein is so far the most useful tool for elucidating the role of SUMOylated protein.

Recent reports have demonstrated that the Ubc9 expression level in tumor cells is elevated compared to that of normal cells [15,20–22]. Since Ubc9 is the sole E2-conjugating enzyme in the mediation of SUMOylation, the SUMOylation of many signaling molecules could be altered in these tumors. Interferon regulatory factor-1 is one of the targets regulated by SUMOylation, and the overexpression of Ubc9 appears to be related with IRF-1 SUMOylation. Further research will be required to identify the other SUMOylated proteins whose levels are elevated in tumors.

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References

[1] T. Taniguchi, K. Ogasawara, A. Takaoka, N. Tanaka, IRF family of transcription factors as regulators of host defense, Annu. Rev. Immunol. 19 (2001) 623–655.
[2] E.M. Coccia, N. Del Russo, E. Stellacci, R. Orsatti, E. Benedetti, G. Marziali, J. Hiscott, A. Battistini, Activation and repression of the 2–5A synthetase and p21 gene promoters by IRF-1 and IRF-2, Oncogene 18 (1999) 2129–2137.
[3] P.K. Kim, M. Armstrong, Y. Liu, P. Yan, B. Bucher, B.S. Zuckerbraun, A. Gambotto, T.R. Billiar, J.H. Yim, IRF-1 expression induces apoptosis and inhibits tumor growth in mouse mammary cancer cells in vitro and in vivo, Oncogene 23 (2004) 1125–1135.
[4] Y. Moriyama, S. Nishiguchi, A. Tamori, N. Koh, Y. Yano, S. Kubo, K. Hirohashi, S. Otani, Tumor-suppressor effect of interferon regulatory factor-1 in human hepatocellular carcinoma, Clin. Cancer Res. 7 (2001) 1293–1298.
[5] P. Lengyel, Tumor-suppressor genes: news about the interferon connection, Proc. Natl. Acad. Sci. USA 90 (1993) 5893–5895.
[6] N. Tanaka, M. Ishihara, M. Kitagawa, H. Harada, T. Kimura, T. Matsuyama, M.S. Lamphier, S. Aizawa, T.W. Mak, T. Taniguchi, Cellular commitment to oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF-1, Cell 77 (1994) 829–839.
[7] T. Taniguchi, M.S. Lamphier, N. Tanaka, IRF-1: the transcription factor linking the interferon response and oncogenesis, Biochim. Biophys. Acta 1333 (1997) M9–M17.
[8] H. Harada, M. Kitagawa, N. Tanaka, H. Yamamoto, K. Harada, M. Ishihara, T. Taniguchi, Anti-oncogenic and oncogenic potentials of interferon regulatory factors-1 and -2, Science 259 (1993) 971–974.
[9] C.L. Willman, C.E. Sever, M.G. Pallavicini, H. Harada, N. Tanaka, M.L. Slovak, H. Yamamoto, K. Harada, T.C. Meeker, A.F. List, et al., Deletion of IRF-1, mapping to chromosome 5q31.1, in human leukemia and preleukemic myelodysplasia, Science 259 (1993) 968–971.
[10] H. Harada, T. Kondo, S. Ogawa, T. Tamura, M. Kitagawa, N. Tanaka, M.S. Lamphier, H. Hirai, T. Taniguchi, Accelerated exon skipping of IRF-1 mRNA in human myelodysplasia/leukemia; a possible mechanism of tumor suppressor inactivation, Oncogene 9 (1994) 3313–3320.
[11] E.J. Lee, M. Jo, J. Park, W. Zhang, J.H. Lee, Alternative splicing variants of IRF-1 lacking exons 7, 8, and 9 in cervical cancer, Biochem. Biophys. Res. Commun. 347 (2006) 882–888.
[12] Y. Wang, D.P. Liu, P.P. Chen, H.P. Koeffler, X.J. Tong, D. Xie, Involvement of IFN regulatory factor (IRF)-1 and IRF-2 in the formation and progression of human esophageal cancers, Cancer Res. 67 (2007) 2535–2543.
[13] M. Chae, K. Kim, S.M. Park, I.S. Jang, T. Seo, D.M. Kim, I.C. Kim, J.H. Lee, J. Park, IRF-2 regulates NF-kappaB activity by modulating the subcellular localization of NF-kappaB, Biochem. Biophys. Res. Commun. (2008).
[14] J. Park, T. Seo, H. Kim, J. Choe, Sumoylation of the novel protein hRIP{beta} is involved in replication protein A deposition in PML nuclear bodies, Mol. Cell. Biol. 25 (2005) 8202–8214.
[15] J. Park, K. Kim, E.J. Lee, Y.J. Seo, S.N. Lim, K. Park, S.B. Rho, S.H. Lee, J.H. Lee, Elevated level of SUMOylated IRF-1 in tumor cells interferes with IRF-1-mediated apoptosis, Proc. Natl. Acad. Sci. USA 104 (2007) 17028–17033.
[16] J.S. Steffan, N. Agrawal, J. Pallos, E. Rockabrand, L.C. Trotman, N. Slepko, K. Illes, T. Lukacsovich, Y.Z. Zhu, E. Cattaneo, P.P. Pandolfi, L.M. Thompson, J.L. Marsh, SUMO modification of Huntingtin and Huntington's disease pathology, Science 304 (2004) 100–104.
[17] S. Ross, J.L. Best, L.I. Zon, G. Gill, SUMO-1 modification represses Sp3 transcriptional activation and modulates its subnuclear localization, Mol. Cell 10 (2002) 831–842.
[18] N. Tanaka, M. Ishihara, M.S. Lamphier, H. Nozawa, T. Matsuyama, T.W. Mak, S. Aizawa, T. Tokino, M. Oren, T. Taniguchi, Cooperation of the tumour suppressors IRF-1 and p53 in response to DNA damage, Nature 382 (1996) 816–818.
[19] D. Joyce, C. Albanese, J. Steer, M. Fu, B. Bouzahzah, R.G. Pestell, NF-kappaB and cell-cycle regulation: the cyclin connection, Cytokine Growth Factor Rev. 12 (2001) 73–90.
[20] Y.Y. Mo, Y. Yu, E. Theodosiou, P.L. Rachel Ee, W.T. Beck, A role for Ubc9 in tumorigenesis, Oncogene 24 (2005) 2677–2683.
[21] Y.Y. Mo, S.J. Moschos, Targeting Ubc9 for cancer therapy, Expert Opin. Ther. Targets 9 (2005) 1203–1216.
[22] Z. Lu, H. Wu, Y.Y. Mo, Regulation of bcl-2 expression by Ubc9, Exp. Cell Res. 312 (2006) 1865–1875.