Small Ubiquitin-like Modifier (SUMO) Isoforms and Conjugation-independent Function in DNA Double-strand Break Repair Pathways*

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Background: The SUMO system is involved in double-strand break (DSB) repair.
Results: SUMO2/3 is required for the major NHEJ pathway; SUMO1 stimulates all DSB repair pathways, and a non-conjugatable form of SUMO1 stimulates DSB repair pathways involving DNA end resection.
Conclusion: SUMO1 and SUMO2/3 have distinct activities in DSB repair.
Significance: SUMO1 can stimulate DSB repair as a free protein.

Small ubiquitin-like modifier (SUMO) proteins act in DNA double-strand break (DSB) repair, but the pathway specificity of the three major isoforms has not been defined. In experiments in which we depleted the endogenous SUMO protein by RNAi, we found that SUMO1 functioned in all subpathways of either homologous recombination (HR) or non-homologous end joining (NHEJ), whereas SUMO2/3 was required for the major NHEJ pathway, called conservative NHEJ, but dispensable in other DSB repair pathways. To our surprise, we found that depletion of UBC9, the unique SUMO E2 enzyme, had no effect in HR or alternative NHEJ (Alt-NHEJ) but was required for conservative NHEJ. Consistent with this result, both non-conjugatable mutant and wild-type SUMO1 proteins functioned similarly in HR and Alt-NHEJ. These results detail the functional roles of specific SUMO isoforms in DSB repair in mammalian cells and reveal that SUMO1 functions in HR or Alt-NHEJ as a free protein and not as a protein conjugate.

DNA double-strand breaks (DSB) present a major problem in genome maintenance because the repair machinery must bridge a gap of indeterminate composition. Two mechanistically distinct pathways are present for DSB repair in mammalian cells: homologous recombination (HR) and non-homologous end joining (NHEJ), competing for the repair of DSBs (1–3). There are two major mechanisms present in HR: the error-free homology-directed repair (HDR) pathway and the error-prone single-strand annealing (SSA). HDR and SSA pathways utilize sequence homology and DNA end resection for repair of DSBs (3). Similarly, eukaryotic cells utilize two pathways of NHEJ, the major NHEJ, called conservative NHEJ (C-NHEJ), in which DSB ends are ligated without homology and which protects DSB ends with minimal processing (4, 5), and the alternative NHEJ (Alt-NHEJ), which depends on DNA end resection at the DSB to generate single strands that anneal via microhomology (5–7).

In vertebrates, there are three functional forms of SUMO family proteins: SUMO1, SUMO2, and SUMO3. SUMO2 and SUMO3 share about 95% sequence identity, but are only 45% identical in sequence to SUMO1, thus forming a distinct subfamily as SUMO2/3 (8). The conjugation of SUMO isoforms onto target protein is designated as SUMOylation, an enzymatic cascade triggered by an E1 SUMO-activating enzyme (SAE1/SAE2), followed by a single E2-conjugating enzyme, UBC9, and an E3 SUMO ligase, resulting in a covalent isopeptide bond between the lysine of target protein and glycine-glutamic dipeptide at the carboxyl terminus of the activated SUMO (9, 10).

The SUMO system has been shown to have strong ties to DSB repair. Abolition of activity of SUMO E3 enzymes in human cells impairs DSB repair (11–13). Mutation of the single SUMO E2 enzyme UBC9 in yeast or human cells results in defects in DNA repair, including recombination abnormality and impaired DSB repair (14–16). Furthermore, many DSB repair proteins are modified by SUMO (11, 12, 14, 17–22). Nonetheless, cells expressing individual SUMOylation-defective HR protein mutants often lack notable phenotypes (20, 23). SUMO modification of individual NHEJ proteins, however, regulates their function in DSB repair (14, 21, 22).

SUMO1 has been discovered as a non-covalent binding partner via SUMO-interacting motifs (SIMs) for human HR proteins including RAD51, RAD52, and replication protein A (RPA) (24–28). The SIM-dependent non-covalent binding to SUMO1 is required for loading of the recombinase RAD51 onto resected DSB ends for HR-mediated repair (19, 29).

In this study, we identified the roles for SUMO isoforms in all four DSB repair subpathways. We found that SUMO1 stimulated all four pathways whereas SUMO2/3 was required only in the C-NHEJ pathway. Surprisingly, the single SUMO E2 enzyme UBC9 was dispensable for HR and Alt-NHEJ, and the conjugation-deficient SUMO1 mutant protein was competent for HR and Alt-NHEJ repair. In contrast, UBC9 was required for C-NHEJ and the SUMO1 mutant was defective in this pathway as compared with the wild type. We conclude that although C-NHEJ is SUMOylation-dependent, the HR and Alt-NHEJ pathways are stimulated by non-covalent SUMO1 interactions.
**EXPERIMENTAL PROCEDURES**

Homologous Recombination and Non-homologous End-joining Assays—HDR and SSA assays were performed as described previously in HeLa cells (30, 31). The repair of double-strand break by Alt-NHEJ pathway was based on a vector kindly provided by Jeremy Stark (Beckman Research Institute of the City of Hope) (32) stably integrated into HeLa genome. On day 1, the appropriate cell line was seeded in 15.6-mm-diameter wells. The next day, cells, 50% confluent, were transfected with 30 pmol of each siRNA in the presence of 3 μl of Oligofectamine (Life Technologies). On day 3, cells were transferred to 35-mm-diameter wells. At 48 h after transfection, cells were retransfected with 50 pmol of the same siRNA in the presence of 5 μl of Lipofectamine 2000 (Life Technologies), plus 3 μg of an I-SceI endonuclease expression vector, which causes a DSB cut in the recombination substrate integrated in the genome. In each transfection, the total siRNA amount was adjusted to be the same in each well by adding siControl. On day 7, cells were trypsinized, and 10,000 cells from each well were counted using a FACSCalibur flow cytometer (BD Biosciences) in the Ohio State University Comprehensive Cancer Center Analytical Cytometry shared resource for the percentage of GFP-positive cells.

The C-NHEJ assay utilized quantitative real-time PCR and was carried out as described (33) with the following modifications in 293 cells. Two rounds of transfection procedure were done as above. The genomic DNA isolated 3 days after transfection of the I-SceI plasmid was digested with the restriction enzyme Xhol and purified by Qiagen PCR purification kit before real-time PCR was applied. RPS17 probe (Hs00734303_g1, Applied Biosystems) was used as an internal control, and the quantitative ΔΔC_T method was used to analyze the data.

For plasmid add-back in the rescue assay for all four DSB repair pathways, the transfection procedure was the same except for the amount of reagents used; in the first transfection, 30 pmol of each siRNA in the presence of 3 μl of Oligofectamine (Life Technologies), plus 3 μg of an I-SceI endonuclease expression vector, which causes a DSB cut in the recombination substrate integrated in the genome. In each transfection, the total siRNA amount was adjusted to be the same in each well by adding siControl. On day 7, cells were trypsinized, and 10,000 cells from each well were counted using a FACSCalibur flow cytometer (BD Biosciences) in the Ohio State University Comprehensive Cancer Center Analytical Cytometry shared resource for the percentage of GFP-positive cells.

**RESULTS**

**SUMO1 and SUMO2/3 Function Differently in DSB Repair Pathways**—SUMO proteins have been shown to be involved in DSB repair (11, 12, 14, 19, 21). We used HeLa- or 293-derived cell lines with the specific recombination substrate DNA integrated in the genome to test the specificity of SUMO isoforms in each DSB repair pathway (Fig. 1, A–D, right). siRNA-dependent depletion of each isoform in the appropriate cell line specifically probes the two HR pathways, HDR and SSA, and the two NHEJ pathways, Alt-NHEJ and C-NHEJ (30–33). BRCA1 and Ligase IV, which are known to regulate DSB repair (30, 31, 37, 38), served as positive controls in three functional DSB repair assays: BRCA1 in HDR and SSA (Fig. 1, A and B) and Ligase IV in C-NHEJ (Fig. 1D). Depletion of SUMO1 (Fig. 1E) reduced repair in all four subpathways tested to about 62% HDR, 31% SSA, 41% Alt-NHEJ, and 39% C-NHEJ, respectively, relative to the control siRNA (Fig. 1, A–D), suggesting that the SUMO1 isoform is stimulatory in all mechanisms of DSB repair. On the other hand, depletion of SUMO2/3 had as significant a deficit in the C-NHEJ pathway as did depletion of Ligase IV (Fig. 1D). In the assays for the other three DSB pathways, depletion of SUMO2/3 increased the levels of HDR, SSA and Alt-NHEJ, although these increases were not statistically significant (Fig. 1, A–C). This result reveals that, in contrast to SUMO1, the SUMO2/3 isoforms are required for C-NHEJ, and either these isoforms do not participate in homologous recombination and Alt-NHEJ or these isoforms have a modest inhibitory activity.

Studies have shown that SUMO1 and SUMO2/3 serve distinct functions in mammalian cells as they are conjugated to different target proteins in vivo (8, 39, 40), which is consistent with our result that SUMO1 and SUMO2/3 function differently in the DSB repair pathways. Co-depletion of SUMO1 and SUMO2/3 in C-NHEJ resulted in a similar level of repair as...
single depletion of SUMO2/3, indicating that the SUMO isoforms were participating in the same pathway (Fig. 1D). Co-depletion of SUMO1 and SUMO2/3 reduced HDR to a similar level as SUMO1 depletion alone, and single depletion of SUMO2/3 suggested that these isoforms do not function in HDR (Fig. 1A). By contrast, co-depletion of SUMO2/3 partially rescued the defect caused by SUMO1 depletion in SSA and Alt-NHEJ, consistent with the concept that SUMO2/3 has a modest repressive effect on these pathways of DSB repair (Fig. 1B and C).

Depletions of protein by siRNAs were confirmed by immunoblot (examples shown in Fig. 1E). The above results from functional DSB repair assays were summarized in Fig. 1F. Depletion of SUMO2/3 minimally impacts homologous recombination or Alt-NHEJ, but these isoforms are required in C-NHEJ. By contrast, SUMO1 is stimulatory in all DSB repair subpathways.

Because SUMO isoforms have a major influence on DSB repair pathway choice, it is necessary to rule out the possibility that depletion of SUMO proteins affects cell cycle progression in mammalian cells. We depleted endogenous SUMO1, SUMO2/3, or UBC9, respectively, by siRNA transfection in HeLa cells and assessed whether the population of cells was blocked at a certain cell cycle stage by flow cytometry measurement at time points of 48, 72, and 96 h after transfection (Fig. 2A). Depletion of either protein had no effect on passage through the normal cell cycle, indicating that the DSB repair deficits caused by loss of the endogenous SUMO isoforms or UBC9 protein are not due to cell cycle blockage. Next we tested whether a secondary effect on DSB repair protein stability is present upon depleting SUMO isoforms because SUMOylation occurs commonly as a post-translational modification that might regulate target protein degradation. Following depletion of SUMO isoforms or UBC9 by siRNA transfection in HeLa
cells, we assessed protein abundance of SUMOylation targets in DSB repair: RAD52, MRE11, and Ku70/80 complex, as well as RAD51, which is not a known SUMOylation target. We found that the depletions had no effect on protein abundance of these repair factors as compared with control siRNA (Fig. 2B).

**UBC9 Is Dispensable for HR or Alt-NHEJ—**UBC9 is the only SUMO E2-conjugating enzyme, and it has been implicated in the DNA damage response in animal models and human cells (11, 13, 16, 22, 41, 42). Depletion of UBC9 was included in the experiments in Fig. 1 to test simultaneously for all three SUMO isoforms, and we were surprised to note that UBC9 depletion did not affect three of the four subpathways tested (Fig. 1 and summarized in Fig. 1F). UBC9 was effectively depleted (Fig. 1E), and there was a phenotype due to UBC9 depletion in the C-NHEJ assay, indicating that the observed level of depletion was sufficient to cause a repair defect. UBC9 depletion reduced the C-NHEJ repair efficiency to about 19% as compared with the control siRNA, similar in magnitude to depletion of Ligase IV, suggesting that UBC9 was important for this pathway, consistent with the result that SUMO2/3 was required for C-NHEJ (Fig. 1D).

Because UBC9 was dispensable in three pathways and required in C-NHEJ, we hypothesized that SUMO1 function in HDR, SSA, and Alt-NHEJ was independent of conjugation to another protein. There have been other examples in the published literature of SUMO proteins functioning independent of covalent binding to a target protein. As an example, UBC9 has been shown to have no effect on SUMO1-mediated repression of BRCA1-induced transcriptional activity stimulated by DNA damage (43). Together, our results suggest that the stimulatory effect on homologous recombination or Alt-NHEJ by SUMO1 is not mediated by SUMO conjugation.

**Free, Non-conjugated SUMO1 Stimulates HR and Alt-NHEJ—**Conjugation of SUMO onto substrates requires the covalent interaction between the carboxyl terminus of SUMO and lysine acceptors on target proteins via an isopeptide bond (9, 10, 44, 45). Deletion of the carboxyl-terminal two glycines from the SUMO1 protein renders it incompetent for covalent conjugation to another protein (25, 36, 46). To test whether SUMO1 functions in homologous recombination and Alt-NHEJ without covalent modification, we transfected into cells an siRNA targeting the SUMO1 3′-UTR and a plasmid expressing the SUMOylation-incompetent SUMO1-GG protein, which truncates the critical two carboxyl-terminal glycine residues and which is resistant to the SUMO1-targeting siRNA. Depletion of endogenous SUMO1 and expression of a FLAG-tagged wild-type SUMO1 rescued the DSB repair defect in all four DSB repair assays (Fig. 3, A–D). The exogenous SUMO1 was expressed at slightly lower levels than the endogenous protein (Fig. 3E, upper panel, lanes 1, 3, and 4), and in immunoblots probing for the FLAG epitope, we could detect the major SUMOylation modification of RanGAP1 migrating at a position consistent with a mass of 90 kDa (8, 46) (Fig. 3E, middle panel, lane 3). The SUMO1-GG protein was expressed at similar levels as the wild-type protein and did not result in the SUMO1 conjugation product (Fig. 3E, middle panel, lane 4). Using this protocol to replace the endogenous SUMO1 with either wild-type or conjugation-defective SUMO1, we assayed for the specific DSB repair assays as in Fig. 1. Just as observed in Fig. 1, transfection of this 3′-UTR-specific siRNA (SUMO1-3′) depleted endogenous SUMO1 protein (Fig. 3E, upper panel, lane 2) and reproducibly yielded inhibition of all four DSB repair pathways, as did the siRNA targeting the SUMO1 coding region (Fig. 3, A–D, lane 3). When the 3′-UTR specific siRNA was co-transfected with a plasmid expressing wild-type SUMO1 resistant to this siRNA, repair efficiency in HDR was restored back to 87% of activity relative to the control siRNA (Fig. 3A, lane 4). Transfection of the non-conjugatable SUMO1-GG plasmid rescued DNA repair in the HDR, SSA, and Alt-NHEJ assays to a similar amount as had the wild-type
SUMO1 (Fig. 3, A–C, lane 5). These results unambiguously demonstrate that the stimulatory function of SUMO1 in the HDR, SSA, and Alt-NHEJ pathways was independent of conjugation to a target protein. By contrast, in the C-NHEJ assay, transfection of the wild-type SUMO1-expressing plasmid partially rescued repair, but transfection of the SUMO1/H9004GG-expressing plasmid did not (Fig. 3D). These results together with the data of UBC9 effect on DSB repair pathways (Fig. 1F) further support a SUMOylation-independent mechanism for the action of SUMO1 on homologous recombination and alternative NHEJ, as well as a conjugation-dependent role of SUMO protein, SUMO1, and also SUMO2/3, in conservative-NHEJ.

**DISCUSSION**

Together, our findings identify that SUMO isoforms act differently in double-strand break repair pathways. SUMO1 stimulates all subpathways; SUMO2/3, on the other hand, is required for the C-NHEJ pathway but dispensable for the other pathways. Strikingly, our data reveal a novel role of SUMO1 as
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A free protein, not a protein conjugate in homologous recombination and alternative NHEJ.

A previous observation had shown that overexpression of either wild type or SUMO1-ΔGG mutant inhibited homologous recombination in mammalian cells (25). The present study has different results, perhaps because we did not utilize overexpression, but rather depletion, and clearly indicate that SUMO1 stimulates homologous recombination. Two recent studies have found that non-covalent interaction between yeast SUMO and RAD51 via its SIM has an important role in RAD51 accumulation at DNA damage sites, a crucial step in HR-mediated DSB repair (19, 29). It is then not totally surprising to now discover that SUMO conjugation is dispensable in HR and Alt-NHEJ.

We propose a model in which SUMO1 acts via different mechanisms in response to DSB damage in mammalian cells (Fig. 4). Following DSB, either end resection or end protection occurs depending on the specific DSB repair mechanism utilized to repair the damage. HDR and SSA require more extensive end resection than Alt-NHEJ because microhomology can be revealed by minimal resection near DNA ends. In the HDR pathway, SUMO1 binds non-covalently to an HR factor, such as RAD51, via its SIM (19, 26, 29, 47). Although RAD51 is known not to be SUMOylated, this interaction is crucial for the loading of RAD51 onto the resected DNA ends (19, 29) (Fig. 4A). Similarly, when the DSB is repaired via the SSA or Alt-NHEJ pathway, an interaction between SUMO1 and the SIM of a given repair factor at the resected ends stimulates the efficient repair independent of coherent SUMOylation (Fig. 4, B and C). By contrast, the C-NHEJ pathway, in which DSB ends are ligated without homology and resection, requires conjugation of SUMO1 and SUMO2/3 to the repair protein substrates for DSB repair (Fig. 4D). Therefore, end resection upon DSB might be a common step stimulated by non-conjugatable SUMO1.

The results of this study apply to the cell in which a single DNA break is induced. However, it is possible that covalent conjugation of SUMO1 may be important under other circumstances not tested in this study. For example, conjugation of SUMO1 has been found to be important for the HR-mediated repair of replication forks, elimination of protein conjugates from DNA ends, and removal of interstrand DNA crosslinks, or when the DNA damage load is extensive, such as after exposure of cells to a high radiation dose or a DNA-alkylating agent (19, 47–50).

Notably, cells expressing individual SUMOylation-defective HR protein mutants often exhibit mild phenotypes (20, 23). Consistent with these data, our model suggests that non-covalent SUMO1 interaction mediated by SIM of the substrate may represent a mechanism that could stimulate both homologous recombination and alternative NHEJ in DSB repair.

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REFERENCES
1. Bunting, S. F., Callén, E., Wong, N., Chen, H. T., Polato, F., Gunn, A., Bothmer, A., Feldthahn, N., Fernandez-Capetillo, O., Cao, L., Xu, X., Deng, C. X., Finkiel, T., Nussenzweig, M., Stark, J. M., and Nussenzweig, A. (2010) 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 141, 243–254
2. Daley, J. M., Palmbos, P. L., Wu, D., and Wilson, T. E. (2005) Nonhomologous end joining in yeast. Annu. Rev. Genet. 39, 431–451
3. Páques, F., and Haber, J. E. (1999) Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 63, 349–404
4. Burma, S., Chen, B. P., and Chen, D. J. (2006) Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. DNA Repair 5, 1042–1048
5. Guirouilh-Barbat, J., Huck, S., Bertrand, P., Pirizio, L., Desmaze, C., Sabettier, L., and Lopez, B. S. (2004) Impact of the KU80 pathway on NHEJ-induced genome rearrangements in mammalian cells. Mol. Cell 14, 611–623
6. Corneo, B., Wendland, R. L., Deriano, L., Cui, X., Klein, I. A., Wong, S. Y., Arnal, S., Holub, A. J., Weller, G. R., Pancake, B. A., Shah, S., Brandt, V. L., Meek, K., and Roth, D. B. (2007) Rag mutations reveal robust alternative end joining. Nature 449, 483–486
7. Haber, J. E. (2008) Alternative endings. Proc. Natl. Acad. Sci. U.S.A. 105, 405–406
8. Saitoh, H., and Hinchey, J. (2000) Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. J. Biol. Chem. 275, 6252–6258
9. Bergink, S., and Jentsch, S. (2009) Principles of ubiquitin and SUMO modifications in DNA repair. Nature 458, 461–467
10. Hay, R. T. (2005) SUMO: a history of modification. Mol. Cell 18, 1–12
11. Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K. M., and Jackson, S. P. (2009) Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 462, 935–939
12. Morris, J. R., Boutell, C., Keppler, M., Densham, R., Weekes, D., Alamshah, A., Butler, L., Galanty, Y., Pangon, L., Khichi, T., Ng, T., and Solomon, E. (2009) The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 462, 886–890
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SUMOylation affects the efficiency of DNA repair. Nucleic Acids Res. 38, 4708–4721

Dou, H., Huang, C., Singh, M., Carpenter, P. B., and Yeh, E. T. (2010) Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex. Mol. Cell. 39, 333–345

Padhye, L. and Jentsch, S. (2012) Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. Cell 151, 807–820

Sacher, M., Pfander, B., Hoege, C., and Jentsch, S. (2006) Control of Rad52 recombination activity by double-strand break-induced SUMO modification. Nature Cell Biol. 8, 1284–1290

Yurchenko, V., Xue, Z., and Sadowsky, M. J. (2006) SUMO modification of human XRCC4 regulates its localization and function in DNA double-strand break repair. Mol. Cell. Biol. 26, 1786–1794

Zhao, X., and Blobel, G. (2005) A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc. Natl. Acad. Sci. U.S.A. 102, 4777–4782

Ohuchi, T., Seki, M., Branzei, D., Maeda, D., Ui, A., Ogiwara, H., Tada, S., and Enomoto, T. (2008) Rad52 SUMOylation and its involvement in the efficient induction of homologous recombination. DNA Repair 7, 879–889

Kerscher, O. (2007) SUMO junction-what’s your function? New insights through SUMO-interacting motifs. EMBO Rep. 8, 550–555

Li, W., Hessabi, B., Babbo, A., Pacione, C., Liu, J., Chen, D. J., Nickoloff, J. A., and Shen, Z. (2000) Regulation of double-strand break-induced mammalian homologous recombination by UBL1, a RAD51-interacting protein. Nucleic Acids Res. 28, 1145–1153

Shen, Z., Panderton-Purtymun, P. E., Comeaux, J. C., Moyzis, R. K., and Chen, D. J. (1996) UBL1, a human ubiquitin-like protein associated with human RAD51/RAD52 proteins. Genomics 36, 271–279

Song, J., Durrin, L. K., Wilkinson, T. A., Krontiris, T. G., and Chen, Y. (2004) Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. Proc. Natl. Acad. Sci. U.S.A. 101, 14373–14378

Takahashi, H., Hatakeyama, S., Saitoh, H., and Nakayama, K. I. (2005) Noncovalent SUMO-1 binding activity of thymine DNA glycosylase (TDG) is required for its SUMO-1 modification and colocalization with the promyelocytic leukemia protein. J. Biol. Chem. 280, 5611–5621

Shima, H., Suzuki, H., Sun, J., Kono, K., Shi, L., Kinomura, A., Horiokshi, Y., Ikura, T., Ikura, M., Kanaar, R., Igarashi, K., Saitoh, H., Kurumizaka, H., and Tashiro, S. (2013) Activation of the SUMO modification system is required for the accumulation of RAD51 at sites of DNA damage. J. Cell Sci. 126, 5284–5292

Ransburgh, D. J., Chiba, N., Ishioka, C., Toland, A. E., and Parvin, J. D. (2010) Identification of breast tumor mutations in BRCA1 that abolish its function in homologous DNA recombination. Cancer Res. 70, 988–995

Towler, W. I., Zhang, J., Ransburgh, D. J., Toland, A. E., Ishioka, C., Chiba, N., and Parvin, J. D. (2013) Analysis of BRCA1 variants in double-strand break repair by homologous recombination and single-strand annealing. Hum. Mutat. 34, 439–445

32. Bennardo, N., Cheng, A., Huang, N., and Stark, J. M. (2008) Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosomal break repair. PLoS Genet. 4, e1000110

33. Zhuang, J., Jiang, G., Willers, H., and Xia, F. (2009) Exonuclease function of human Mre11 promotes deletional nonhomologous end joining. J. Biol. Chem. 284, 30565–30573

34. Liu, H. W., Zhang, J., Heine, G. F., Arora, M., Gulcin Ozer, H., Onti-Srinivasan, R., Huang, K., and Parvin, J. D. (2012) Chromatin modification by SUMO-1 stimulates the promoters of translation machinery genes. Nucleic Acids Res. 40, 10172–10186

35. Guo, W. Z., Sugaya, S., Satoh, M., Tomonaga, T., Nomura, F., Hiwasa, T., Takiguchi, M., Kita, K., and Suzuki, N. (2009) Nm23-H1 is responsible for SUMO-2-involved DNA synthesis induction after X-ray irradiation in human cells. Arch. Biochem. Biophys. 486, 81–87

36. Zheng, Z., Cai, C., Omwancha, J., Chen, S. Y., Baslan, T., and Shemshedin, L. (2006) SUMO-3 enhances androgen receptor transcriptional activity through a sumoylation-independent mechanism in prostate cancer cells. J. Biol. Chem. 281, 4002–4012

37. Critchlow, S. E., and Jackson, S. P. (1998) DNA end-joining: from yeast to man. Trends Biochem. Sci. 23, 394–398

38. Frank, K. M., Sekiguchi, J. M., Seidl, K. J., Swat, W., Rathbun, G. A., Chen, H. L., Davidson, L., Kangaloo, L., and Alt, F. W. (1998) Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. Nature 396, 173–180

39. Rosas-Acosta, G., Russell, W. K., Deyrieux, A., Russell, D. H., and Wilson, V. G. (2005) A universal strategy for protemic studies of SUMO and other ubiquitin-like modifiers. Mol. Cell. Proteomics 4, 56–72

40. Vertegaal, A. C., Andersen, J. S., Ogg, S. C., Hay, R. T., Mann, M., and Lamond, A. I. (2006) Distinct and overlapping sets of SUMO-1 and SUMO-2 target proteins revealed by quantitative proteomics. Mol. Cell Proteomics 5, 2298–2310

41. Ishiia, M., Kimura, M., Namikoshi, K., Yamazoe, M., Yamamoto, K., Arakawa, H., Agematsu, K., Matsuishi, N., Takeda, S., Buerstedde, J. M., and Takata, M. (2004) DNA cross-link repair protein SNM1A interacts with PIAS1 in nuclear focus formation. Mol. Cell. Biol. 24, 10733–10741

42. Mabb, A. M., Wuerzberger-Davis, S. M., and Miyamoto, S. (2006) PIASy mediates NEMO sumoylation and NF-κB activation in response to genotoxic stress. Nat. Cell Biol. 8, 986–993

43. Park, M. A., Seok, Y. J., Jeong, G., and Lee, J. I. (2008) SUMO1 negatively regulates BRCA1-mediated transcription, via modulation of promoter occupancy. Nucleic Acids Res. 36, 263–283

44. Desterro, J. M., Rodriguez, M. S., and Hay, R. T. (1998) SUMO-1 modification of IκBα inhibits NF-κB activation. Mol. Cell. 2, 233–239

45. Mahajan, R., Gerace, L., and Melchior, F. (1998) Molecular characterization of the SUMO-1 modification of RanGAP1 and its role in nuclear envelope association. J. Cell Biol. 140, 259–270

46. Yamada, K., Muramatsu, M., Saito, D., Sato-Oka, M., Saito, M., Moriyama, T., and Saitoh, H. (2012) Characterization of the C-terminal diglycine motif of SUMO-1/3. Biopol. Bioch. Biophys. 76, 1035–1037

47. Ouyang, K. J., Woo, L. L., Zhu, J., Huo, D., Matusin, M. J., and Ellis, N. A. (2009) SUMO modification regulates BLM and RAD51 interaction at damaged replication forks. PLoS Biol. 7, e1000252

48. Fontebasso, Y., Etheridge, T. J., Oliver, A. W., Murray, J. M., and Carr, A. M. (2013) The conserved Fanconi anemia nuclear Fan1 and the SUMO E3 ligase Pli1 act in two novel Pso2-independent pathways of DNA interstrand crosslink repair in yeast. DNA Repair 12, 1011–1023

49. Galanty, Y., Belotserkovskaya, R., Coates, J., and Jackson, S. P. (2012) RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. Genes Dev. 26, 1179–1195

50. Macris, M. A., and Sung, P. (2005) Multifaceted role of the Saccharomyces cerevisiae Srs2 helicase in homologous recombination regulation. Biochem. Soc. Trans. 33, 1447–1450