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Cell cycle-dependent SUMO-1 conjugation to nuclear mitotic apparatus protein (NuMA)

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ABSTRACT

Covalent conjugation of proteins with small ubiquitin-like modifier 1 (SUMO-1) plays a critical role in a variety of cellular functions including cell cycle control, replication, and transcriptional regulation. Nuclear mitotic apparatus protein (NuMA) localizes to spindle poles during mitosis, and is an essential component in the formation and maintenance of mitotic spindle poles. Here we show that NuMA is a target for covalent conjugation to SUMO-1. We find that the lysine 1766 residue is the primary NuMA acceptor site for SUMO-1 conjugation. Interestingly, SUMO modification of endogenous NuMA occurs at the entry into mitosis and this modification is reversed after exiting from mitosis. Knockdown of Ubc9 or forced expression of SENP1 results in impairment of the localization of NuMA to mitotic spindle poles during mitosis. The SUMOylation-deficient NuMA mutant is defective in microtubule bundling, and multiple spindles are induced during mitosis. The mitosis-dependent dynamic SUMO-1 modification of NuMA might contribute to NuMA-mediated formation and maintenance of mitotic spindle poles during mitosis.

1. Introduction

Chromosome segregation is a crucial step during cell cycle progression to ensure the partition of exactly the same amount of genetic material to the two daughter cells [1]. Mitotic apparatus are organized with bipolar spindle poles containing centrosomes and mitotic microtubules. The poles of the spindles are major focal points for the minus ends of spindle microtubules and serve as the final destination of chromosome segregation [2]. Cancer cells have been found to be defective in chromosome segregation resulting in multipolar spindles, chromosome instability, and aneuploidy [3].

The spindle protein NuMA has been shown to be critical for spindle assembly. NuMA was first identified as a mitotic centrosomal component [4]. The NuMA gene maps to chromosome 11q13, which is one of the most frequently amplified chromosomal segments in cancer cells [5], and NuMA is overexpressed in epithelial ovarian cancer [6]. NuMA localization is dynamic during cell cycle progression. In interphase cells, the NuMA protein is restricted to the nucleus and is a constituent of isolated nuclear matrices. After nuclear envelope breakdown, NuMA is solubilized and becomes concentrated at the polar regions of the mitotic apparatus. NuMA binds to microtubules through its C-terminal globular domain and is involved in spindle pole organization during early mitosis and the maintenance of spindle function [7]. Conditional loss of mitotic NuMA function resulted in defective centrosome attachment to spindle fibers, and disruption of the maintenance of focused kinetochore fibers at spindle poles throughout mitosis [8,9].

SUMOylation, a covalent modification of a target protein by small ubiquitin-like modifier (SUMO), plays a critical role in mitotic chromosome structure and segregation as well as transcription, DNA repair, and DNA recombination [10]. In budding and fission yeasts, cells that are mutant in Ubc9 or SUMO itself show defects in mitotic progression, in addition to defects in chromosome integrity, kinetochore function, and chromosome segregation [11]. Ubc9 knock-out mice die at an early post-implantation stage. In cell culture, loss of Ubc9 leads to major defects in chromosome condensation and segregation in addition to defects in nuclear organization [12]. In addition, a broad spectrum of mitotic defects were observed in SUMO mutant *Drosophila* embryos or *Drosophila* S2 cells with knocked-down SUMO, including defective mitotic spindle organization, defective spindle attachment to centrosomes, and multipolar spindles [13,14]. These results suggest that

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SUMOylation is important in coordinating multiple events in mitosis. Although many potential targets for SUMOylation have been suggested during kinetochore assembly [15], little is known about the role of SUMOylation in regulating spindle pole assembly during mitosis.

Here we show that NuMA is a target for covalent modification by SUMO-1 in a mitosis-specific manner. Lys 1766 of NuMA was identified as the primary SUMO-1 acceptor site by serial deletion analysis and site-directed mutagenesis. A SUMOylation-deficient NuMA mutant induces multipolar spindle formation during mitosis, but not the SUMO-NuMA fusion protein, which may mimic SUMOylated NuMA. These results provide insight into the regulation of NuMA-mediated establishment and maintenance of mitotic spindles via SUMOylation during mitosis.

2. Materials and methods

2.1. Cell culture and transfection

HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. For immunoblot analysis, HeLa cells were seeded into six-well plates and DNA transfection was carried out using the Fugene6 reagent (Roche Molecular Biochemicals).

2.2. Plasmid construction and site-directed mutagenesis

The constructs for *in vitro* SUMO modifications and the expression plasmid encoding SENP1 were described previously [16,17]. The GFP-NuMA expression plasmid was kindly provided by Duane Compton (Dartmouth Medical School, Hanover, USA). Various NuMA deletion constructs, and Myc-tagged NuMA expression plasmids were generated using Gateway Technology (Invitrogen). NuMA SUMOylation site point mutants were generated using the QuikChange mutagenesis kit (Stratagene). Mutations were verified by DNA sequencing.

2.3. Yeast two-hybrid screening

The yeast expression plasmid encoding SENP1 C-terminus (pGBK-SENP1 352–644 C603A) was transformed into the yeast strain AH109 for library screening. The transformants containing bait plasmid were mated with the pretransformed mouse 11 days embryo MATCHMAKER cDNA library cloned into the pACT2 vector (Clontech). Briefly, after the two yeast strains containing the bait and pretransformed library were mixed and incubated overnight in YPDA-kanamycin media, candidates for two-hybrid interaction were initially selected on Trp-/Leu-/Ade-/His-medium, and positive interactions were further confirmed by β-galactosidase activity. To characterize the SENP1-interacting proteins, plasmids were recovered from yeast strains showing positive interactions, and sequences were verified by DNA sequencing.

2.4. In vitro SUMO modification

In vitro SUMOylation assays were performed as described previously [16,17]. Briefly, assays were performed in a total volume of 20 μl in the presence of 50 mM Tris–HCl, pH 7.5, 2 mM DTT, 0.05% Tween-20 and 3 mM ATP at 30 °C. Myc-tagged NuMA or point mutants were synthesized using the TNT coupled Reticulocyte Lysate system (Promega), and mixed with purified 500 ng of E1(GST-SAE2/SAE1), 400 ng of His-Ubc9, 2 μg of GST-SUMO(GG), and incubated at 37 °C for 1 h. Reaction products were visualized by immunoblotting with anti-Myc antibody (Roche Molecular Biochemicals).

2.5. In vitro pull-down assays

Myc-tagged NuMA constructs were subjected to *in vitro* translation using the TNT coupled Reticulocyte Lysate system (Promega). Pull-down assays were performed by incubating equal amounts of GST or GST-SENP1 fusion proteins immobilized onto glutathione-Sepharose beads with *in vitro* translated, Myc-tagged NuMA, and bound proteins were resolved by 8% SDS–PAGE and detected with immunoblotting using anti-Myc antibody.

2.6. Immunocytochemistry

Immunocytochemistry for HeLa cells was performed as described previously [18]. Briefly, HeLa cells were grown on coverslips and transfected with GFP-NuMA plasmid and HA-SENP1 expression plasmid. Twenty-four hours after transfection, cells were fixed with 3% p*araformaldehyde* for 10 min, and permeabilized with 0.5% Triton X-100 in PBS for 5 min. Fluorescence microscopy was performed with a Zeiss Axiopgoto 2 microscope, using an excitation wavelength of 488 nm for GFP-NuMA. HA-SENP1 was detected using a rhodamine-conjugated secondary antibody against a mouse monoclonal HA antibody. For detection of endogenous NuMA, a mouse monoclonal anti-NuMA antibody (Calbiochem) was utilized. The acquired images were processed with Adobe Photoshop.

3. Results and discussion

3.1. Identification of NuMA as a SENP1-interacting protein

Increasing evidence reveals that the SUMO moiety is conjugated to a variety of cellular proteins, and this process is reversibly regulated by specific SUMO E3 ligases and SUMO deconjugating enzymes [19,20]. To identify cellular proteins which are SUMOylated and regulated by the SUMO-deconjugation enzyme SENP1, a yeast two-hybrid screen was carried out using the SENP1 catalytic domain corresponding to amino acids 352–644 as bait. Over twenty proteins were identified as SENP1 interacting proteins which are SUMOylated *in vitro* and *in vivo*. Among these, we focused on the nuclear mitotic apparatus protein (NuMA) which localizes to the mitotic spindle poles and nucleoplasm during mitosis and interphase, respectively. The specificity of the interaction between NuMA with SENP1 was confirmed again in a yeast two-hybrid assay. Both NuMA clones (clones 7 and 18) include the coding region spanning amino acids 637–894, and interacted specifically with SENP1 in yeast AH109 cells (Fig. 1A). The association of NuMA and SENP1 was verified by co-immunoprecipitation in HEK293 cells. Myc-NuMA was precipitated only when HA-SENP1 was co-expressed, indicating that NuMA can associate with SENP1 in mammalian cells (Fig. 1B). In addition, a GST pull-down assay revealed that *in vitro* synthesized NuMA binds to GST-SENP1 fusion protein, but not to GST protein (Fig. 1C). This indicates that NuMA can physically interact with SENP1.

3.2. Covalent modification of NuMA by SUMO-1 in vitro and in vivo

The physical interaction of NuMA with SENP1 led us to determine whether NuMA is a target of covalent modification by SUMO-1. Expression plasmids encoding Myc-tagged full-length NuMA and His-SUMO-1 were transfected into HeLa cells, and cell lysates were precipitated with nickel-affinity resin under denaturing conditions to exclude the possibility of non-covalent interactions between NuMA and SUMO-1. Immunoblotting showed that SUMO-conjugated NuMA is detected only when NuMA is co-expressed with SUMO-1 (Fig. 1D). Endogenous NuMA is also

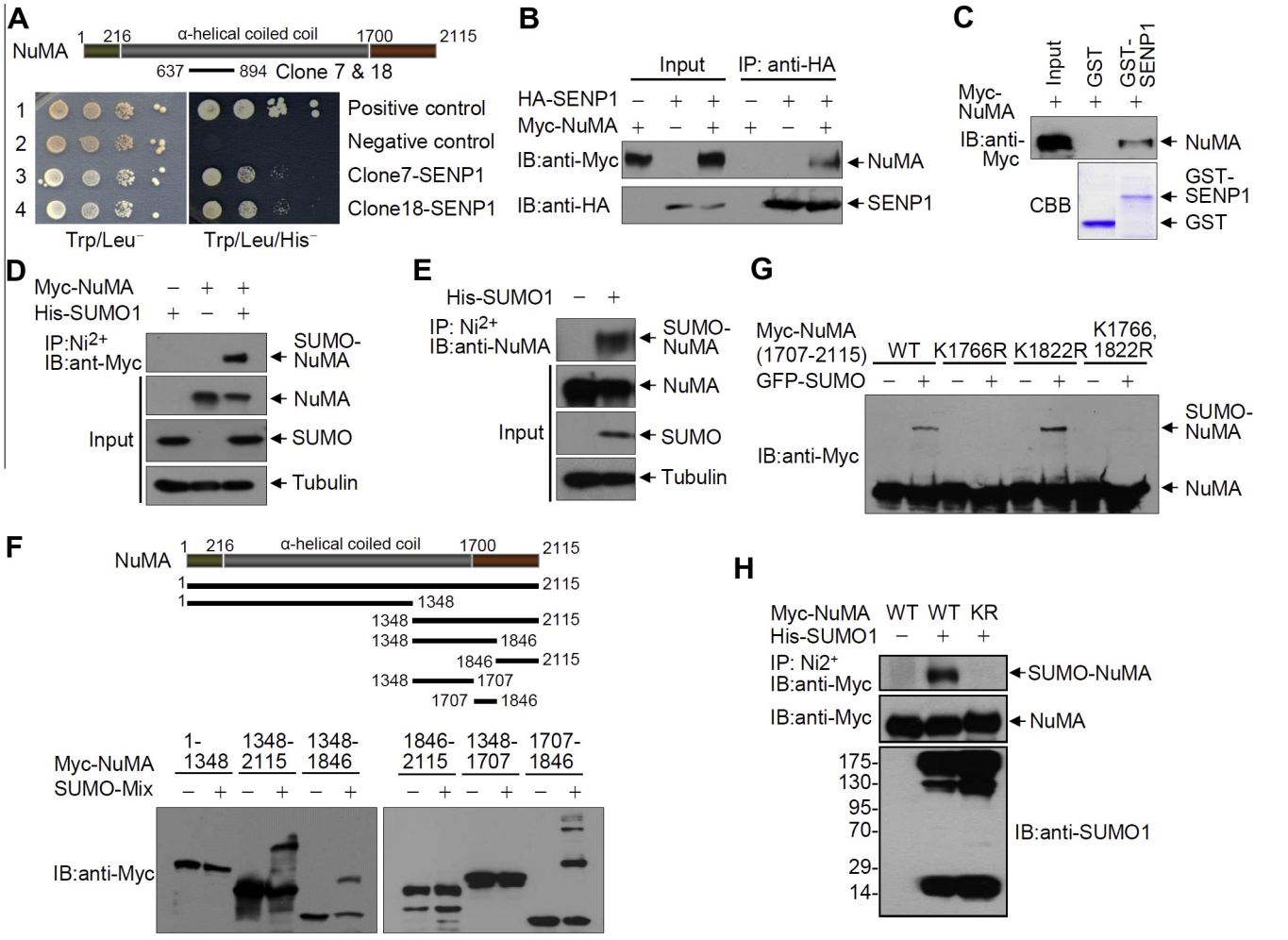

Fig. 1. SUMO modification of NuMA and identification of the SUMOylation site. (A) Identification of NuMA as a SENP1-interacting protein in yeast. A yeast expression plasmid encoding NuMA amino acids 637–894 fused to GAL4 activation domain (clones #7 and #18) was cotransformed with GAL4-SENP1 (aa 1–644) into the AH109 yeast strain, and the cells were dotted on synthetic medium lacking either Trp/Leu or Trp/Leu/His in a series of 5-fold dilutions. Positive and negative controls indicate interactions between the p53-SV40 T antigen and empty plasmids (pGBT9 and pGAD424), respectively. (B) Co-immunoprecipitation of SENP1 with NuMA. An expression plasmid encoding HA-SENP1 was transfected into HEK293 cells, with or without a Myc-NuMA expression plasmid. Transfected cells were lysed and immunoprecipitated with anti-HA antibody, followed by immunoblotting using anti-Myc antibody. (C) GST pull-down assay showing physical interaction of NuMA with GST-SENP1. *In vitro* translated NuMA was incubated with equal amounts of GST or GST-SENP1. Bound proteins were eluted and resolved by 8% SDS–PAGE, followed by immunoblotting using anti-Myc antibody. Affinity purified GST and GST-SENP1 used in the assay are shown in the lower panel. (D) The expression plasmids encoding Myc-NuMA and His-SUMO-1 were transfected into HeLa cells alone or together, and cell lysates were precipitated with a nickel-affinity column, followed by immunoblotting using anti-Myc antibody. (E) Expression plasmid encoding His-SUMO-1 was transfected into HeLa cells, and the cell lysates were precipitated with nickel-affinity column, followed by immunoblotting using anti-NuMA antibody. (F) Schematics for deletion mutants utilized in SUMOylation assays. The green, grey and orange regions in the schematic indicate the N-terminal domain, coiled-coil domain, and C-terminal globular domain, respectively. Myc-NuMA deletion mutants were subjected to *in vitro* SUMOylation with (+) or without (–) a SUMOylation mixture, followed by immunoblotting using anti-Myc antibody. (G) The plasmid encoding Myc-NuMA (1707–2115) or lysine substitution mutant was transfected into HeLa cells along with or without GFP-SUMO-1, and SUMOylated NuMA was determined by immunoblotting using anti-Myc antibody. (H) Expression plasmids encoding full-length Myc-NuMA or Myc-NuMA K1766R mutant were transfected into HeLa cells with (+) or without (–) His-SUMO-1, and the cell lysates were precipitated with a nickel-affinity column, followed by immunoblotting using the indicated antibodies. (For interpretation of color in this Figure, the reader is referred to the web version of this article.)

covalently modified by SUMO-1 (Fig. 1E). Deletion mutants of Myc-NuMA were generated to identify the NuMA SUMOylation site (Fig. 1F, top panel) and *in vitro* translated NuMA deletion mutants were subjected to *in vitro* SUMOylation assays. Immunoblotting indicated that NuMA mutant spanning amino acids 1707–1846 is conjugated to SUMO-1 (Fig. 1F). Analysis of amino acid sequences showed that both K1766 (PK^{1766}VE) and K1822 (KK^{1822}LD) match to the SUMO-conjugation site consensus sequence ψKXE/D (ψ, hydrophobic amino acid; X, any amino acid). An *in vivo* SUMOylation assay with either K1766R or K1822R mutant revealed that substitution of lysine 1766, but not lysine 1822, abrogates modification of NuMA by SUMO-1 (Fig. 1G). In addition, the full-length NuMA K1766R mutant was not conjugated by SUMO-1 in mammalian cells (Fig. 1H). Taken together, these results demonstrate that NuMA is a target for modification by SUMO-1 and lysine 1766 is the primary target site for SUMOylation.

3.3. Formation of multiple spindle poles by inhibition of NuMA SUMOylation

NuMA localization changes dynamically as the cell cycle progresses. To gain insight into the role of NuMA SUMOylation, we examined whether NuMA SUMOylation is dependent on cell cycle. HeLa cells were arrested at the beginning of S phase by a double thymidine block, and released into nocodazole-containing medium, followed by periodical analysis of endogenous NuMA

SUMOylation under denaturing conditions. SUMOylation of endogenous NuMA occurred at the onset of mitosis, and this modification was reversed after mitosis (Fig. 2A). These results suggest that SUMO modification of NuMA may be involved in its function during mitosis, such as mitotic spindle formation and maintenance.

To address the role of NuMA SUMO modification during mitosis, localization of endogenous NuMA was determined after blocking SUMOylation by knockdown of Ubc9 expression. Ubc9 shRNA plasmids were transfected into HeLa cells along with a GFP expression plasmid, and the localization of endogenous NuMA was observed by immunostaining using an anti-NuMA antibody. Cells expressing Ubc9 shRNA could be indirectly monitored by co-transfection of a GFP expression plasmid at a high ratio, at 3 μg (Ubc9 shRNA) to 0.2 μg (EGFP-C2). As shown in Fig. 2B, endogenous NuMA was prone to localize to multipolar spindles during metaphase, while nucleoplasmic localization during interphase was not changed. These results were verified by reversing the SUMOylation of NuMA through forced overexpression of the SUMO-deconjugating enzyme SENP1. The SUMO moiety was efficiently removed from NuMA-SUMO conjugates by forced expression of wild-type SENP1, but not by the catalytically inactive SENP1 C603A mutant (Fig. 2C). The expression of SENP1 in HeLa cells along with GFP-NuMA resulted in the formation of multipolar spindles associated with GFP-NuMA in addition to abnormal bipolar spindles (Fig. 2D). These results suggest that SUMOylation may be involved in NuMA-mediated formation and maintenance of mitotic spindle poles during mitosis.

3.4. Formation of multiple spindle poles by a SUMOylation defective NuMA mutant

Since inhibition of NuMA SUMOylation by knocking down Ubc9, an E2 conjugating enzyme for SUMOylation, or overexpressing SENP1 resulted in the formation of multiple spindle poles during mitosis (Fig. 2), the effects of a NuMA SUMOylation defective mutant on spindle pole formation was determined. To this end, full length wild-type NuMA or the NuMA K1766R mutant were expressed in HeLa cells, and the interphase and metaphase localization was analyzed by confocal microscopy (Fig. 3A). The NuMA K1766R mutant localized to the nucleoplasm excluding the nucleolus during interphase, the same as wild-type NuMA (Fig. 3A, the first and second row panel).

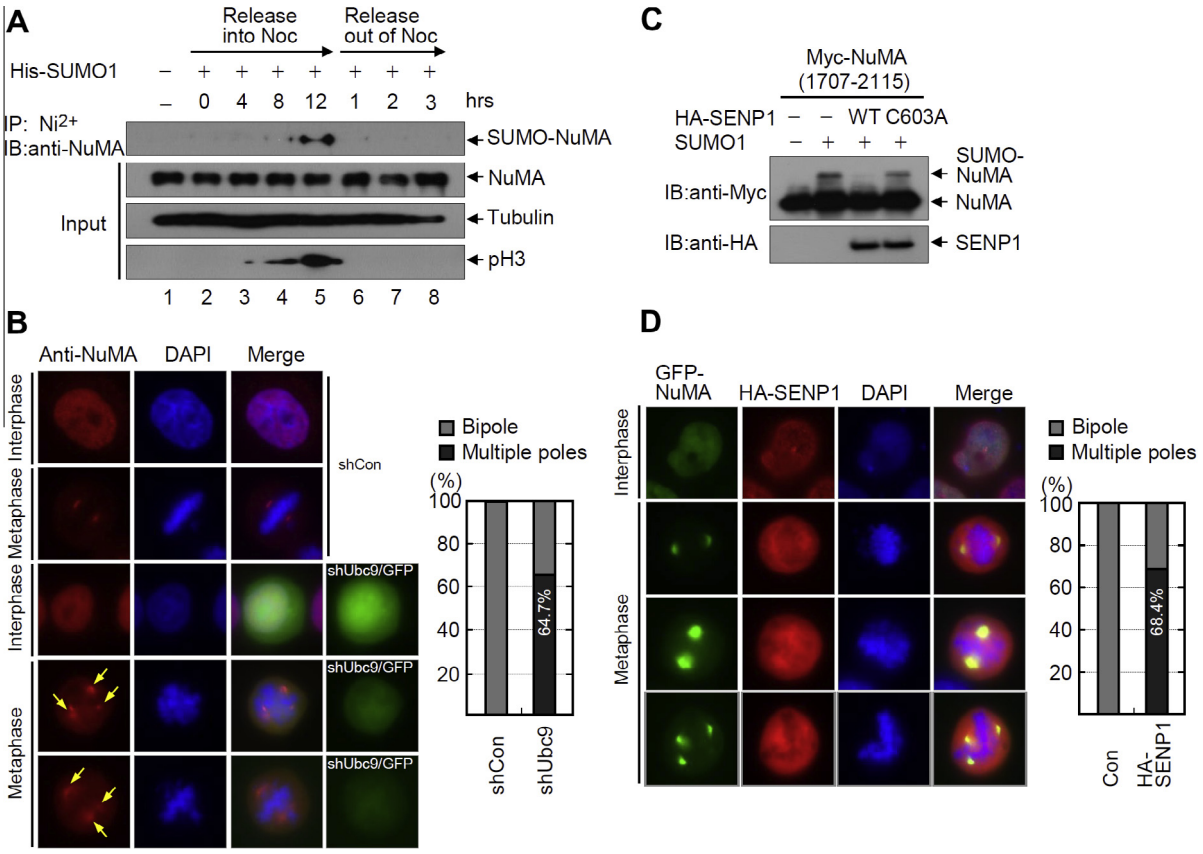

Fig. 2. Cell cycle-dependent SUMO modification of NuMA and formation of multipolar spindles by blocking SUMO modification of NuMA. (A) HeLa cells were transfected with His-SUMO-1 expression plasmid, and arrested at early S phase with a double thymidine block, and then released into culture medium containing nocodazole for 12 h and transferred to medium without nocodazole for 3 h. The arrested cells were harvested at the indicated times for immunoblotting using anti-NuMA antibody after precipitation of cell lysates with a metal-affinity column. pH3 denotes phosphorylated histone H3 which is a marker for mitosis. (B) The expression plasmid encoding control shRNA or shUbc9 was transfected into HeLa cells along with a GFP expression plasmid. The transfected cells were arrested at prometaphase by nocodazole treatment, and released 5 min before fixation. Cells expressing shUbc9 could be monitored indirectly by GFP expression. Endogenous NuMA was visualized by immunostaining of fixed cells with anti-NuMA antibody, followed by incubation of cells with anti-rabbit rhodamine red-conjugated secondary antibody, whereas the green signal was observed by GFP fluorescence. DAPI staining shows the nucleus during interphase or condensed chromatin during mitosis. Multiple poles are indicated by arrows. The percentage of cells containing multiple poles is presented in the graph to the right. (C) The Myc-NuMA (1707–2115) expression plasmid was transfected into HeLa cells along with either wild-type HA-SENP1 or the catalytically inactive HA-SENP1 mutant (C603A) plasmid in the presence of SUMO-1. The deconjugation of SUMO-modified NuMA was determined by immunoblotting using anti-Myc antibody. (D) The plasmids encoding GFP-NuMA and HA-SENP1 were transfected into HeLa cells, and cells were arrested at prometaphase with nocodazole treatment, and released 5 min before fixation. SENP1 expression was visualized by immunostaining with anti-HA antibody, and GFP-NuMA localization was determined by GFP fluorescence. DAPI staining indicates the nucleus during interphase and condensed chromatin during mitosis. The percentage of cells containing abnormal poles or multiple poles is presented in the graph to the right.

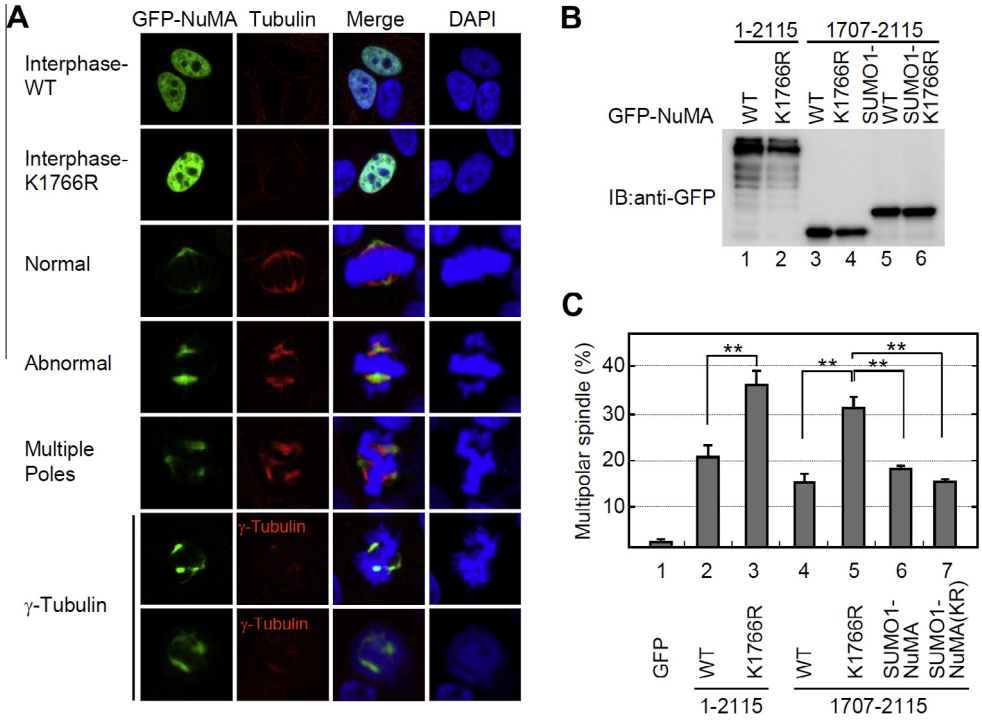

Fig. 3. Formation of multipolar spindles by expression of a SUMOylation-deficient NuMA mutant. (A) The GFP-NuMA K1766R mutant was transfected into HeLa cells and the transfected cells were stained with DAPI to determine the cell cycle stage of each cell. GFP-NuMA K1766R localization was visualized by GFP fluorescence. (B) Expression levels of wild-type GFP-NuMA, GFP-NuMA (K1766R), GFP-NuMA (1707–2115), GFP-NuMA (1707–2115, K1766R), SUMO-NuMA and SUMO-NuMA K1766R fusion protein were determined by immunoblotting using anti-GFP antibody. (C) The formation of multipolar spindles upon expression of wild-type GFP-NuMA, point mutants, and the SUMO-NuMA fusion protein was determined as describe above, and is presented as percentage of mitotic cells. Error bars indicate standard deviations from at least three independent experiments. $^{**}p < 0.01$ by paired Student's t test.

Forced expression of NuMA is known to result in the formation of multiple spindle poles during mitosis [21,22]. In addition, oral cancer cell lines that have high NuMA expression levels had ~20% multiploar spindles, and reduction of NuMA to 50% of the original level eliminated multipolar spindles [23]. Consistently, we observed multiple spindle poles in ~20% of cells expressing wild-type NuMA (Fig. 3C, lane 2). Interestingly, expression of the NuMA K1766R mutant increased the frequency of multipolarity to a higher level than wild-type NuMA (Fig. 3A, the 5th row panel; Fig. 3C, lane 3). The multiple spindle poles induced by NuMA K1766R were associated with γ-tubulin, indicating that the multiple spindle poles could be functional during mitosis (Fig. 3A, the 6th and 7th row panel). The C-terminal tail of NuMA was also analyzed to avoid association of tagged NuMA with endogenous NuMA through the central coiled-coil dimerization domain [24]. As expected, a C-terminal NuMA tail fragment tagged with GFP localized appropriately to nuclei during interphase and was present at spindle poles during mitosis in a crescent-shaped pattern typical of endogenous NuMA. The facilitation of multipolar spindle formation was also observed with expression of SUMOylation-defective C-terminal NuMA K1766R (Fig. 3C, lane 5). In order to determine whether an increase in the formation of multipolar spindles is caused by deficiency of NuMA SUMOylation, an expression plasmid encoding SUMO-fused C-terminal NuMA, which may mimic the effects of constitutively SUMOylated NuMA, was generated (Fig. 3B), and its effects on the formation of multiple spindle poles were determined. The formation of multiple spindle poles did not increase upon expression of SUMO-fused NuMA C-terminus or SUMO-fused NuMA K1766R, whereas it increased upon expression of NuMA K1766R (Fig. 3C, lanes 6 and 7). These results suggest that SUMOylation of NuMA contributes to organization of mitotic spindle poles.

3.5. Induction of microtubule bundling by SUMO modification of the NuMA C-terminus

Since the C-terminal globular domain of NuMA affects organization of mitotic spindle poles and contains a microtubule association domain in the vicinity of the SUMOylation site, we examined the correlation between the SUMO modification of NuMA and microtubule association. For this purpose, a SUMOylation deficient mutant and SUMO fusion C-terminal NuMA spanning amino acids 1707–1982 were generated (Fig. 4A) and their effects on association with microtubules were determined (Fig. 4B). These deletion mutants do not contain a nuclear localization sequence and thus localize to the cytoplasm where NuMA colocalizes and associates with microtubules. Wild-type NuMA (aa 1707–1982) strongly facilitated microtubule bundling, whereas the SUMOylation deficient NuMA (aa 1707–1982) K1766R mutant did not. Moreover, SUMO fusion to NuMA (aa 1707–1982) K1766R enhanced microtubule bundling the same as wild-type NuMA (aa 1707–1982) (Fig. 4B, arrow heads). These results demonstrate that NuMA SUMOylation at Lys1766 is involved in microtubule organization. To observe the effects of NuMA SUMOylation on microtubule stabilization, cells were incubated on ice for 1 h before fixation for immunofluorescence. Microtubules are depolymerized by incubating cells on ice [22]. We observed that microtubules were stabilized by expression of the wild-type NuMA C-terminus or the SUMOylation deficient mutant (Fig. 4C, compare microtubule intensity indicated by arrows and arrowheads). Altogether, these results indicate that SUMOylation of the NuMA C-terminus at Lys1766 induces microtubule bundling, but does not affect NuMA association with microtubules.

Overexpression of wild-type NuMA and the deletion mutant cause multipolar spindle formation. A NuMA-Rae1 balance was initially proposed as the underlying mechanism to explain NuMA- or

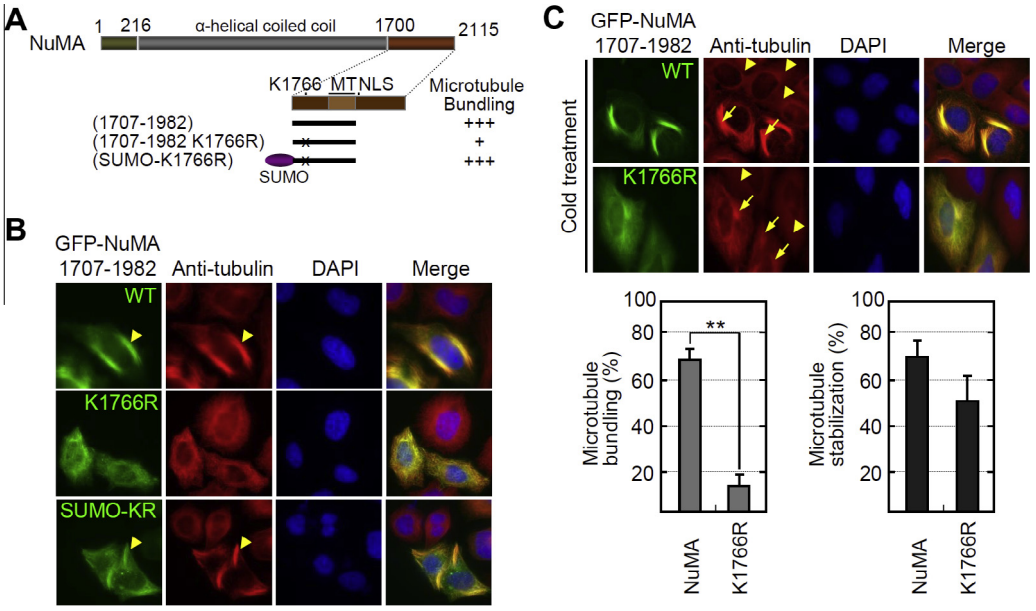

Fig. 4. Induction of microtubule bundling by NuMA SUMOylation. (A) Schematics for NuMA C-terminus deletion mutants and SUMO-NuMA fusion protein. (B) An expression plasmid encoding GFP-NuMA (1707–1982), GFP-NuMA (1707–1982) K1766R mutant, or GFP-SUMO-fused NuMA (1707–1982) was transfected into HeLa cells. Endogenous α-tubulin was visualized by immunostaining of fixed cells with anti-α-tubulin antibody, followed by incubation of cells with anti-rabbit rhodamine red-conjugated secondary antibody, whereas the green signal was obtained with GFP fluorescence. DAPI staining shows the nucleus during interphase. (C) The HeLa cells were prepared as described in (B) except that cells were incubated on ice for 1 h before fixation to induce destabilization of microtubules. The experiments were repeated three times and the number of cells displaying microtubule bundling and microtubule stabilization were counted. Data are presented as percentages of microtubule bundling and stabilization in the graphs below. Error bars indicate standard deviations from at least three independent experiments. $^{**}p < 0.01$ by paired Student's t test.

Rae1-mediated multipolarity, in which overexpression of NuMA or knock-down of Rae1 induced multiple spindle formation [21]. However, it appears that multiple mechanisms exist to regulate bipolar spindle formation in addition to the NuMA-Rae1 balance, because overexpression of the NuMA C-terminal region, which is absent of the Rae1-binding domain, recapitulated the effects on multipolarity induced by overexpression of full-length NuMA. Serial deletion analysis showed that 100 amino acids of the NuMA C-terminal microtubule binding domain are sufficient to induce multipolarity [22]. It remains unclear as to how NuMA SUMOylation influences multipolarity. One possibility is that SUMOylation of NuMA facilitates the association of essential spindle pole components or assembly of microtubule-associated proteins. Alternatively, but not mutually exclusively, NuMA oligomerization may be mediated by SUMOylation to directly participate in microtubule nucleation/assembly and stabilization. In support of this notion, the SUMO-NuMA fusion protein containing a SUMOylation domain in addition to the microtubule binding domain spanning amino acids 1707–1982 induced microtubule nucleation and stabilization, whereas the SUMOylation deficient mutant did not (Fig. 4B). Although the impact of SUMOylation on the biochemical function of NuMA remains unclear, these novel results provide the first step to elucidating how SUMOylation is involved in NuMA-mediated spindle formation and maintenance during mitosis.

Acknowledgments

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