Abstract of the dissertation submitted by MOE MOE HAN

Roles of the SUMO-related enzymes, PIAS1, PIAS4, and RNF4, in DNA double-strand break repair by homologous recombination

SUMO 関連酵素 PIAS1、PIAS4 及び RNF4 の DNA 二本鎖切断相同組換え修復における役割

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Introduction

Post-translational modification of proteins by small ubiquitin-like modifiers (SUMOs) is known to be involved in a variety of cellular events. This modification, called SUMOylation, is carried out by the E1 activating enzyme, the E2 conjugating enzyme, and multiple E3 ligases. Previous studies have demonstrated that the SUMO E3 ligases, protein inhibitors of activated STAT 1 (PIAS1) and 4 (PIAS4), and the SUMO-targeted ubiquitin ligase, RING finger protein 4 (RNF4), play important roles in the repair of DNA double-strand breaks (DSBs). However, the mechanism by which these SUMO-related enzymes promote DSB repair is still poorly understood. In the present study, we focused on homologous recombination (HR), the most accurate DSB repair pathway, and aimed to elucidate the mechanism by which PIAS1, PIAS4, and RNF4 promote HR.

Materials and Methods

BJ-hTERT cells (hTERT-immortalized normal human fibroblasts) were transfected with siRNA of PIAS1, PIAS4, RNF4, or 53BP1 by using Lipofectamine RNAiMAX (Thermo Fisher Scientific, MA, USA) and irradiated with 1Gy or 2Gy of γ-rays from ^{137}Cs source at room temperature at a dose rate of 1 Gy/min. To label the cells that had undergone DNA synthesis, cells were pretreated with 10μM 5-ethynyl-2′-deoxyuridine (EdU) for 30 min before irradiation. At several post-irradiation time, foci of RPA, RAD51, BRCA1 or RIF-1 were counted in G2 phase cells identified as EdU(-)/centromere protein F(CENPF)(+) by immunofluorescence. Statistical analyses were performed by Two-tailed Mann-Whitney U test and Dunn's multiple comparison test using Prism 7 software (GraphPad Software, CA, USA).

Results

Two essential early steps of HR are end resection of DSB indicated by accumulation of RPA and following replacement by RAD51. In γ-rays irradiated cells, both the number of RPA foci and RAD51 foci were significantly reduced by siRNA-mediated depletion of PIAS1, PIAS4, or RNF4. Thus, these SUMO-related enzymes promote DSB end resection and facilitate RAD51 loading on resected DSBs in the G_2 phase.

In processes of end resection and RAD51 loading, BRCA1 is known to play important roles. We found that the depletion of PIAS1, PIAS4 or RNF4 also reduced the number of BRCA1 foci in the cells irradiated in the G2 phase, which indicates that PIAS1, PIAS4, and RNF4

promote BRCA1 recruitment to DSBs. This finding let us hypothesize that these factors may help BRCA1 antagonize 53BP1 because it is known that BRCA1 facilitates resection by counteracting 53BP1-mediated resection blockade. In fact, 53BP1 depletion significantly increased the number of RPA foci and rescued RAD51 loading in PIAS1-, PIAS4- or RNF4-depleted cells.
Based on this finding, we speculated that SUMO-related enzyme help BRCA1 control RIF1 recruitment to DSBs, since RIF1 is known to be recruited to DSBs in a manner dependent on 53BP1 and functions as a resection inhibitor. We found that depletion of PIAS1, PIAS4, RNF4, and BRCA1 similarly increased the number of RIF1 foci after the irradiation. Moreover, the concomitant depletion of BRCA1 and either one of the SUMO-related enzymes did not further increase the number of RIF1 foci.
Collectively, these results suggest that PIAS1, PIAS4, RNF4, and BRCA1 work epistatically to counteract 53BP1/RIF1- mediated resection blockade, thereby promoting resection.

Discussion

In this study, we investigated the roles of the two SUMO E3 ligases (PIAS1 and PIAS4) and the SUMO-targeted ubiquitin ligase (RNF4) in HR of radiation-induced DSBs in G2 phase. We showed that the depletion of PIAS1, PIAS4, and RNF4 suppressed foci formation of RPA, RAD51, and BRCA1. Galanty et al. reported that RPA recruitment to the track of DSB-generating laser was decreased in PIAS1-, or PIAS4-depleted cells. Yin et al. showed that RPA70 recruitment to DSBs was diminished by RNF4 depletion. These studies and ours suggest that PIAS1, PIAS4, and RNF4 are generally involved in resection of DSBs induced by different kinds of DSB inducers. The two groups reported that RAD51 recruitment to laser track was compromised in RNF4-depleted cells. We found that the depletion of PIAS1 or PIAS4 reduced the number of RAD51 foci similarly to RNF4 depletion. Given that RNF4 recruitment to DNA damage is dependent on PIAS1 and PIAS4, it is likely that PIAS1 and PIAS4 promote RAD51 loading through RNF4. Previous studies reported that BRCA1 recruitment to DSBs induced by laser or hydroxyurea was decreased by the depletion of PIAS1 or PIAS4. Our results showed that RNF4 depletion impaired BRCA1 foci formation similarly to the depletion of PIAS1 or PIAS4. Considering the above studies and ours, it is suggested that PIAS1 and PIAS4 promote BRCA1 recruitment via RNF4.
We found that reduction of RPA-, or RAD51 foci in PIAS1-, PIAS4, or RNF4-depleted cells was partially rescued by concomitant depletion of 53BP1. Moreover, the depletion of PIAS1, PIAS4, or RNF4 promoted foci formation of RIF1. These results indicate that PIAS1, PIAS4, and RNF4 are all involved in antagonizing 53BP1/RIF1-mediated resection blockade. We propose that these SUMO-related enzymes counteract 53BP1/RIF1-mediated resection blockade through BRCA1, because (1) these enzymes promoted BRCA1 recruitment to DSBs, (2) RIF1 foci were increased similarly by BRCA1-depletion and the depletion of these enzymes, (3) the concomitant depletion of BRCA1 and any of PIAS1, PIAS4, or RNF4 did not further increase RIF1 foci, compared to the single depletion of BRCA1. To our best knowledge, this is the first study reporting epistatic relationship between BRCA1 and PIAS1/PIAS4/RNF4 in limiting RIF1 recruitment to DSBs.
In summary, we showed that PIAS1, PIAS4, and RNF4 promoted resection and RAD51 loading, the two critical steps in HR. Our results suggest that these SUMO-related enzymes help BRCA1 antagonizing 53BP1/RIF1-mediated resection blockade, thereby facilitating resection.

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