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SUMO Boosts the DNA Damage Response Barrier against Cancer

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Cells exposed to genotoxic insults such as ionizing radiation activate a signaling cascade to repair the damaged DNA. Two recent articles published in *Nature* show that such genome maintenance requires modifications of tumor suppressor proteins BRCA1 and 53BP1 by the *s*mall *u*biquitin-like *mo*difier SUMO.

Proper genome maintenance, ensured by the cellular DNA damage response (DDR) machinery, is a prerequisite for normal development and prevention of premature aging and diverse devastating diseases including cancer (Jackson and Bartek, 2009). Indeed, one reason for cancer incidence not being even higher appears to be the intrinsic ability of our cells to detect and deal with the DNA damage caused by exogenous genotoxic agents such as radiation or chemicals as well as endogenous sources such as oncogene-evoked replication stress and telomere erosion during the early stages of cancer development (Halazonetis et al., 2008; Jackson and Bartek, 2009). Even if some DNA lesions, such as subsets of DNA double-strand breaks (DSB) that occur commonly during tumorigenesis, remain unrepaired, sustained signaling and effector pathways within the DDR "anticancer barrier" machinery usually eliminate such hazardous, genetically unstable cells by inducing cell death or a permanent cell cycle arrest known as cellular senescence (Halazonetis et al., 2008).

From the mechanistic viewpoint, sensing, signaling, and repair of DSBs involve a plethora of proteins whose sequential accrual and function at the DNA damage sites is modulated by a myriad of post-translational modifications, including phosphorylation, acetylation, methylation, and ubiquitylation, which are highly dynamic and reversible. The phosphorylation/dephosphorylation events are performed by kinases such as the ATM, ATR, and DNA-PK, and several protein phosphatases (Jackson and Bartek, 2009). The emerging ubiquitylation cascade comprises the E3 ubiquitin ligases RNF8, RNF168, and BRCA1, as well as the E2 ubiquitin-conjugating enzyme UBC13 and the candidate assembly factor HERC2 (Bergink and Jentsch, 2009; Bekker-Jensen et al., 2010). Unlike the classical role of ubiquitylation in triggering protein degradation, however, this ubiquitin-mediated pathway orchestrates protein-protein interactions on damaged chromosomes and recruitment of the key DNA repair factors 53BP1 and BRCA1 to DSBs, thereby promoting genomic integrity (Figure 1).

Despite the rapid progress in understanding the molecular basis of DSB signaling and repair, more surprises are in store for us in this lively area of research, as illustrated by two recent reports in *Nature* (Galanty et al., 2009; Morris et al., 2009). These exciting studies provide evidence for a key role of yet another protein modification, sumoylation (covalent attachment of the small proteins known as SUMO1, SUMO2, and SUMO3), in coordinating the DNA damage response to DSBs (Figure 1). Processes critical for cell fate decisions including survival and some aspects of DNA repair have been linked to the sumoylation pathway, particularly in yeast (Bergink and Jentsch, 2009; Branzei and Foiani, 2008; Hay, 2005). However, the involvement of the sumoylation pathway in DSB response and its functional interplay with the ubiquitylation cascade that controls recruitment of 53BP1 and BRCA1 are novel and very relevant for genome maintenance and protection against cancer.

So what is revealed by the two new studies? First, in a complementary series of immunofluorescence and live-cell imaging experiments, they show that the SUMO1 and SUMO2/3 conjugates, as well as the E1 (SAE1), E2 (UBC9), and E3 (PIAS1 and PIAS4) sumoylation enzymes, all rapidly accumulate at the sites of DNA

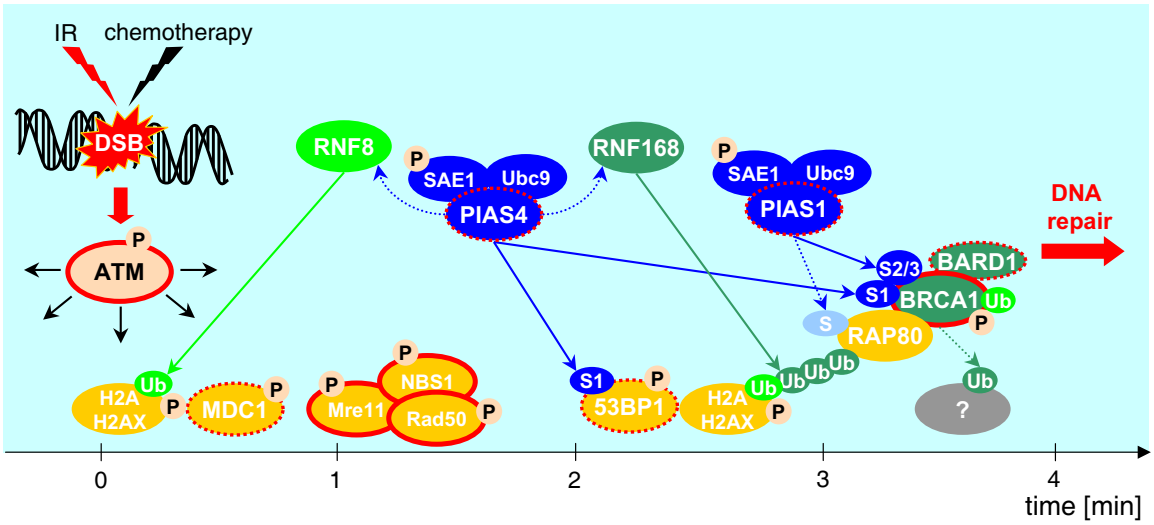

Figure 1. Role of SUMOylation in DSB Signaling and Repair

Induction of DNA double-strand breaks (DSBs) by, for example, ionizing radiation (IR) or genotoxic chemotherapeutics leads to activation of the ATM kinase, ATM-mediated phosphorylation (P) of histone H2AX, MDC1, Mre11-Rad50-NBSs1 complex and other proteins, and subsequent recruitment of, and signaling by, the ubiquitin ligases RNF8 and RNF168. Coordinated ubiquitylation- (green) and SUMOylation-mediated (blue) signaling leads to focal modification of chromatin and recruitment of repair proteins 53BP1 and the BRCA1/BARD1/Rap80 complex at DSBs, thereby setting the stage for efficient DNA repair. The approximate timing of accrual and modifications of targets of the SUMO ligases PIAS1 and PIAS4, as reported by Galanty et al. (2009) and Morris et al. (2009), are indicated. The role of this genome integrity mechanism as part of the intrinsic biological barrier against cancer is evident from the fact that numerous components of this cascade are established (encircled in solid red) or candidate (broken red outline) tumor suppressors.

damage induced by ionizing radiation, genotoxic anticancer drugs such as cisplatin and hydroxyurea, or laser (Figure 1). Second, functional assays to assess the phenotypes of human cells depleted of the individual E3 enzymes via RNA interference led to several conclusions. (1) PIAS1 is required for the SUMO2/3 modifications at DSB sites and sumoylation of the BRCA1 repair factor and tumor suppressor whereas PIAS4 mediates mainly SUMO1 modifications yet contributes also to SUMO2/3 conjugates and targets both the 53BP1 repair factor and BRCA1. (2) The E3 ligases PIAS1 and PIAS4 are also required for the DSB-induced ubiquitylation events mediated by the RNF8 and RNF168 ubiquitin ligases, whose activities are essential for efficient accrual of the downstream factors 53BP1 and the BRCA1/BARD1-Rap80 complex to DNA damage sites (Figure 1). (3) Consistent with the above observations, PIAS1 and PIAS4 are necessary for proficient DNA repair of DSBs, as documented by both impaired kinetics of DNA repair and lower survival (enhanced sensitivity) of PIAS1/4-depleted cells upon exposure to various genotoxic insults.

What else have we learned about the substrates and functional impact of the SUMO modifications of proteins at the DSB sites? Apart from evidence that both 53BP1 and BRCA1 become promptly sumoylated during the DDR, in a PIAS4- and PIAS1/4-dependent manner, respectively (Galanty et al., 2009), Morris et al. (2009) identified two consensus SUMO-conjugation sites of BRCA1 and used mutagenesis of these sites to document their importance for BRCA1's ubiquitin ligase activity. These results led Morris et al. (2009) to propose that BRCA1 is a SUMO-regulated ubiqutin ligase. Perhaps sumoylation could also guide BRCA1's ubiquitin ligase activity toward certain substrates. In addition, the effects of PIAS1/4 depletion on the activities of RNF8/RNA168 raise the possibility that these DSB-recruited ubiquitin ligases are also sumoylated (Figure 1).

A host of burning questions are raised by these new studies. How are the PIAS1/4 enzymes recruited to DSB sites? What is the significance of the E1 SAE1 phosphorylation by the damage-activated ATM/ATR kinases? What are potential additional SUMO substrates within the DSB signaling and repair machinery, and what is the functional significance of the SUMO modifications of such substrates? Furthermore, given the reversibility of sumoylation, how is desumoylation of 53BP1, BRCA1, and other putative SUMO substrates by SUMO-specific proteases at DSBs regulated? Sumoylation can also play a role in protein-protein interactions (through SUMO-interacting motifs), and therefore identification of such proteins at DNA damage sites may be anticipated.

Apart from the mechanistic insight into DSB processing, one striking feature of this emerging complex pathway is its intimate link with cancer. Thus, multiple components of this cascade are tumor suppressors (Figure 1), encoded by genes whose germline loss-of-function mutations predispose individuals to cancer (Jackson and Bartek, 2009). More work is needed to determine whether PIAS enzymes are tumor suppressors, not least because their cellular effects are pleiotropic and may be context specific (Hay, 2005; Rytinki et al., 2009). However, the prominent role of PIAS4 in stress-induced cellular senescence, the multiple ways that PIAS ligases promote genome stability, and SUMO-mediated regulation of major tumor suppressors implicate the sumoylation system in cellular defense against tumorigenesis (Bischof et al., 2006; Bergink and Jentsch, 2009; Rytinki et al., 2009). This notion is also supported by numerous reports on aberrant loss of PIAS1/4 in various types of human cancer

and by the data presented by Morris et al. (2009) and Galanty et al. (2009) on regulation of BRCA1 by PIAS1/4.

Last but not least, the new discoveries of sumoylation pathways in DNA damage response highlight the possibility to modulate these activities in order to either protect normal tissues from, or sensitize cancer cells to, effects of genotoxic anticancer therapies. The fact that the analogous ubiquitylation system appears to be "drugable" and that even drugs that affect pleiotropic mechanisms such as the proteasome are proving useful in cancer treatment offer some optimism for such potential future applications.

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