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A MEDIATOR METHYLATION MYSTERY: JMJD1C DEMETHYLATES MDC1 TO REGULATE DNA DAMAGE REPAIR

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Abstract

Mediator of DNA-Damage Checkpoint 1 (MDC1) has a central role in repair of DNA double-strand breaks (DSBs) by both homologous recombination and nonhomologous end joining, and its function is regulated by post-translational phosphorylation, ubiquitylation, and SUMOylation. In this issue, a new study by Watanabe et al. reveals that methylation of MDC1 is also critical for its function in DSB repair and specifically affects repair through BRCA1-dependent homologous recombination.

It is essential that DNA DSBs be repaired accurately and efficiently to prevent genome rearrangements that contribute to the development of cancer and other age-related diseases. During the S-G2 phase of the cell cycle, when sister chromatids are available as templates, DSBs can be repaired by homologous recombination (HR), a repair mechanism that relies on the breast and ovarian cancer predisposition gene product, BRCA1¹. In other phases of the cell cycle, when homologous DNA templates are not available, DSBs are repaired by non-homologous end joining (NHEJ), a process dependent on the repair factor 53BP1². Because the factors that commit repair to the HR or NHEJ pathways compete for recognition of DNA ends, it is important that these factors be regulated so that repair is channeled through the appropriate pathway. A new study in this issue of *Nature Structural & Molecular Biology*³ identifies the protein demethylase JMJD1C as the first branch-selective factor uniquely required for BRCA1-dependent HR-mediated repair (Figure 1).

The molecular signals that function to suppress or promote HR and NHEJ repair pathways at DSBs are only partially understood. Both HR and NHEJ repair pathways require initial recruitment of MDC1 to DSBs and subsequent ubiquitylation events mediated by the RNF8 and RNF168 ubiquitin E3 ligases⁴. Downstream of these ligases, however, recruitment of 53BP1 to sites of DNA damage is directed by ubiquitylation of histone H2A⁵, ubiquitin-dependent degradation of JMJD2A⁶ and removal of L3MBTL1 by p97 segregase⁷. In contrast, BRCA1 recruitment to DSBs relies on the synthesis of K63-linked polyubiquitin chains attached to histones, MCD1, or SUMO at DSBs. Ubiquitin and hybrid SUMO-ubiquitin chains are known to be recognized by the BRCA1-associated receptor, RAP80^{8,9}, but the factors that selectively promote the synthesis of K63-linked polyubiquitin chains and other signals required for RAP80-BRCA1 recruitment were unknown.

Many proteins involved in DSB repair undergo reversible posttranslational protein modifications (PTMs), including phosphorylation, ubiquitylation, and SUMOylation. For

example, the ATM kinase is activated in response to DSBs and phosphorylates histone H2AX, which in turn functions to recruit MDC1 to the damaged DNA (Figure 1). MDC1 is then itself phosphorylated and recruits the ubiquitin E3 ligases RNF8 and RNF168 which modify MDC1, histone H2A, and histone H2AX with polyubiquitin chains. Downstream factors, including RAP80-BRCA1 and 53BP1, are subsequently recruited to initiate DSB repair⁹. In addition to being phosphorylated and ubiquitylated, MDC1 is also acetylated¹⁰ and SUMO conjugated^{11–14}. In light of the number of PTMs that modulate MDC1 activity in DSB repair¹⁵, it is intriguing that Watanabe et al.³ have identified yet one more PTM, methylation, involved in its functional regulation.

In a search for RNF8 and RNF168 substrates, Watanabe et al. identified JMJD1C, a histone demethylase belonging to a family of demethylases containing Jumonji C domains¹⁶. JMJD1C was found to interact robustly with RNF8, and to be recruited to sites of DNA damage in a manner dependent on RNF8 and also its own protein demethylase activity. Depleting JMJD1C from cells reduced the levels of RNF8 and polyubiquitin at DSBs and impaired recruitment of RAP80-BRCA1. Surprisingly, despite the clear suppression of polyubiquitin at DSBs, RNF168 recruitment was not significantly affected. This suggests that polyubiquitylation of specific substrates by RNF168 may be suppressed in cells depleted of JMJD1C or that an imbalance may exist in ubiquitylation that favors deconjugation. Most notably, however, JMJD1C depletion had no effect on recruitment of 53BP1 to sites of DNA damage. Thus, JMJD1C is specific for the RAP80-BRCA1 branch of DSB repair.

So how does JMJD1C affect RAP80-BRCA1 recruitment to DSBs without affecting 53BP1 recruitment? MDC1 is among the earliest proteins detected at DSBs, where it serves as a platform for recruitment of downstream factors, including RNF8. Considering the decreased accumulation of RNF8 at DSBs in JMJD1C-depleted cells, the authors speculated that interactions between RNF8 and MDC1 might be modulated by JMJD1C activity. Consistent with this hypothesis, MDC1 was found to be methylated at multiple lysine residues, and methylation at one of these lysines, K45, is specifically reduced by the activity of JMJD1C. An MDC1 mutant that is unable to be methylated at this residue, K45A, displayed enhanced and constitutive interaction with RNF8, thus supporting a model in which demethylation of MDC1 promotes stable interactions with RNF8 (Figure 1). Interestingly, despite this increased RNF8 association, expression of the K45A MDC1 mutant was unable to support recruitment of RNF8 to chromatin following DNA damage, thus suggesting that both methylation and demethylation of MDC1 must be spatially and temporally controlled for proper assembly at sites of damage.

In summary, JMJD1C is the first protein identified that selectively promotes the RAP80-BRCA1 branch of DSB repair, and it does so by enhancing interactions between MDC1 and RNF8 through a mechanism that is dependent on demethylation of MDC1 at K45. How this demethylation affects MDC1 and RNF8 interactions at the molecular level remains to be fully determined. RNF8 binds to TQXF motifs in a central domain of MDC1¹⁷, so understanding of how methylation at K45 affects interaction with these distal sites will require structural studies. Additional important questions also remain to be addressed. Although MDC1 is the first nonhistone substrate of JMJD1C to be identified, other

substrates with roles in HR repair may exist. Consistent with a potential role of JMJD1C in regulating the activity of other factors in DSB repair, its depletion was found to have opposing effects on cellular sensitivity to PARP1 inhibitors and formation of RAD51 foci when compared to effects in cells depleted of BRCA1. Thus, it is certain that future studies of JMJD1C and its effects on HR-mediated DSB repair will reveal additional new and exciting discoveries. These findings could also have important clinical implications, given that JMJD1C expression is reduced or lost in a significant fraction of breast cancer carcinomas³.

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Figure 1. JMJD1C selectively regulates the RAP80-BRCA1 branch of double-strand break (DSB) repair

DSBs can be repaired by one of two pathways: homologous recombination (HR) or non-homologous end joining (NHEJ). Both pathways depend on recruitment of MDC1 and the RNF8 and RNF168 ubiquitin E3 ligases to sites of DNA damage (denoted by irradiation, IR). Pathways diverge through selective recruitment of downstream factors: 53BP1 recruitment leads to NHEJ repair (right), whereas HR repair requires BRCA1 recruitment (left). Watanabe et al.³ now show that recruitment of JMJD1C to sites of damage results in demethylation of MDC1 at K45 to promote interactions with RNF8 and consequent polyubiquitylation of MDC1 and other factors. The RAP80-BRCA1 complex is subsequently recruited through interactions with K63-linked polyubiquitin chains attached directly to MDC1 or through interactions with hybrid SUMO-ubiquitin chains whose synthesis depends on PIAS1, PIAS4 and RNF4. Me, methyl; P, phosphoryl; Ub, ubiquityl; S, SUMOyl; PIAS1/4, PIAS1 and PIAS4.