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Brd4 Shields Chromatin from ATM Kinase Signaling Storms

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Abstract

Upon activation, ataxia telangiectasia mutated (ATM) kinase rapidly phosphorylates hundreds of proteins, setting off chaotic signaling storms from areas of damaged chromatin. Recent work by Kaidi and Jackson and Floyd *et al*. advance our knowledge of the mechanisms that initiate or limit ATM kinase signaling storms at chromatin.

ATM Kinase Signaling Storms

Ataxia telangiectasia mutated (ATM) kinase signaling is tempestuous in nature. Capable of being activated within seconds of exposure to 10 cGy ionizing radiation (IR) and in response to as few as two DNA double-strand breaks (DSBs), ATM kinase is extraordinarily sensitive and reactive (1). Within 15 min of exposure to 50 cGy IR, more than 50% of ATM protein is phosphorylated on Ser^{1981}, and this phosphorylation is associated with a transition between inactive ATM dimers and active ATM monomers (1). Rather than functioning as a binary switch that selectively modifies the function of a privileged group of substrates, activated ATM rapidly phosphorylates hundreds of proteins, and extensive disorder spreads rapidly through the cell from focal points of DNA damage. Studies have identified in excess of a thousand substrates for ATM and the related kinase ataxia telangiectasia and Rad3–related (ATR), and the stoichiometry of the phosphorylations on many of these substrates is >50% (2, 3). The functional consequences of ATM kinase signaling are substantial and include cell cycle checkpoint activation, DNA repair and cell survival, senescence, or programmed cell death. However, it is reasonable to postulate that among the chaos of phosphorylations, functionally unimportant phosphorylations exist. What forces initiate the formation of ATM kinase signaling storms? In contrast, what factors protect areas of chromatin from chaotic ATM kinase signaling storms? Recent reports advance our knowledge of the mechanisms that initiate or limit ATM kinase signaling (4, 5).

The Eye of the Storm

ATM is activated by at least two independent mechanisms in chromatin. In one mechanism, ATM is activated by DNA damaging agents, such as ionizing radiation (IR), and involves allosteric activation by the MRE11 (meiotic recombination 11 homolog) complex (6, 7),

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which binds DSB ends directly (8). In a second mechanism, ATM is activated by agents that alter chromatin structure in the absence of detectable DSBs, such as trichostatin A or chloroquine (1, 4). When chromatin is relaxed, the binding of lysine acetyltransferase 5 (KAT5, also known as TIP60) to histone H3 trimethylated at Lys^9 (H3K9me3) promotes KAT5-dependent ATM acetylation and ATM kinase activity (9-11). Kaidi and Jackson recently discovered a transient, c-Abl–mediated phosphorylation of KAT5 Tyr^{44} that is required for its binding to H3K9me3 (4). KAT5 Tyr^{44} phosphorylation is induced and then stabilized as phosphorylated KAT5 accumulates in the chromatin of cells in which H3K9me3 is exposed by treatment with TSA or chloroquine, or after small interfering RNA–mediated depletion of heterochromatin protein 1α, and ATM acetylation and activation follow independently of the MRE11 complex (4). As such, alterations in chromatin structure, which are yet to be defined beyond exposed H3K9me3, can activate ATM signaling in the absence of DSBs, perhaps at DNA lesions including DNA single-strand breaks and bulky lesions that expose H3K9me3 in chromatin structure, as well as around DSBs that result from clustered sites of damage. ATM kinase signaling storms have the potential to converge and be intolerable to the cell, leading to cell death.

Shelter from the Storm

Like a violent thunderstorm (Fig. 1), ATM kinase signaling bombards regions of DNA damage, which are adjacent to regions of undamaged DNA. Recently, the bromodomain protein Brd4 isoform B was identified as a chromatin factor that functions to shield chromatin from ATM kinase signaling. Brd4 isoform B contains both a bromodomain that binds acetylated histones and an extra-terminal domain that interacts with several chromatin-binding proteins, including SMC2 (structural maintenance of chromosomes protein 2) and SMC4 (5). Using a high-through put quantitative microscopy assay applied to an RNAi (RNA interference) library, Floyd *et al*. identified an increased number, size, and intensity of γH2AX (phosphorylated H2A histone family member X), 53BP1, and Ser^{1981}-phosphorylated ATM foci in Brd4 isoform B knockdown cells after exposure to 2 Gy IR. Brd4 isoform B–knockdown cells did not demonstrate any changes in either the incidence or repair kinetics of DSBs after irradiation compared with that of control cells, suggesting that the enhanced signaling seen in irradiated Brd4 isoform B–knockdown cells is not because of either increased DNA damage or deficient DNA repair (5). Brd4 isoform B–knockdown cells did, however, show increased sensitivity to micrococcal nuclease, suggesting that Brd4 isoform B functions in the condensation of global chromatin structure (5). Furthermore, SMC2- and SMC4-knockdown cells were refractory to the effects of Brd4 isoform B overexpression, indicating that these proteins function in the same pathway to shield ATM kinase signaling from extending to neighboring chromatin (5). Brd4 isoform B binding to acetylated regions of chromatin recruits SMC2 and SMC4 to compact the chromatin and limit DNA damage signaling, whereas KAT5 binding to exposed H3K9me3 induces ATM acetylation, ATM kinase activation, and DNA damage signaling; thus, changes to global chromatin structure dynamically regulate ATM kinase signaling. Mechanisms that control the binding of Brd4 isoform B to acetylated proteins are yet to be identified and may determine the extent to which Brd4 isoform B can limit ATM kinase signaling. It is tempting to envision a model in which Brd4 functions to shelter territories of undamaged chromatin from ATM kinase signaling. However, Brd4-mediated global chromatin compaction is likely unfavorable to cell survival when occurring in areas of chromatin damage. Indeed, Floyd *et. al*. showed that Brd4 isoform B knockdown enhanced cell survival after irradiation and that overexpression of Brd4 isoform B resulted in increased cell death after radiation. These findings are consistent with a model in which global chromatin condensation induced by Brd4 isoform B inhibits access of the DNA repair and cell cycle checkpoint machinery to areas of damaged chromatin. In the context of supraphysiological amounts of DNA damage, Brd4-mediated global chromatin compaction

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at sites of damaged chromatin is deleterious to cell survival. Nevertheless, the evidence for a mechanism that limits unruly ATM kinase signaling storms suggests an evolutionary need to insulate at least some nuclear activities from disorder either to maintain certain nuclear functions or to direct repair enzymes to the incident DNA lesions. Brd4-like mechanisms may also function to limit the ATM kinase-dependent signaling initiated during programmed DSB formation, such as that which occurs during meiosis (12). In either event, these new data suggest that there are two early and distinct IR-induced nuclear environments in which ATM kinase signaling storms are initiated by at least two independent mechanisms through MRE11 at sites of damage or KAT5 at regions of relaxed chromatin. Concomitantly, in undamaged areas, chromatin is maintained in a compacted state by Brd4 and sheltered from the ensuing ATM storm.

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Fig. 1. IR is commonly drawn as a lightning bolt

The lightning bolt is associated with a violent storm of ATM kinase activity that rains phosphorylations on surrounding clouds of proteins. Brd4 shields neighboring regions of chromatin from the damage-response storm.