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All Stressed Out Without ATM Kinase

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Abstract

Ataxia-telangiectasia (A-T) is a rare, neurodegenerative, inherited disease arising from mutations in the kinase A-T mutated (ATM), which promotes cell cycle checkpoints and DNA double-strand break repair. Puzzlingly, these ATM activities fail to fully explain A-T neuropathologies, which instead have links to stress induced by reactive oxygen species (ROS). However, a landmark discovery reveals an unexpected intersection of ROS and kinase signaling: ATM can be directly activated by oxidation to form a disulfide-linked dimer in a mechanism distinct from DNA damage activation. When combined with notable structural-based insights into the ATM homolog DNA-PK (DNA-protein kinase) and mTOR (mammalian target of rapamycin), these results suggest conformation and assembly mechanisms to signal oxidative stress through an ATM nodal point. These findings fundamentally affect our understanding of ROS and ATM signaling and of the A-T phenotype, with implications for altering signaling in cancer cells to increase sensitivities to current therapeutic interventions.

Cellular responses to DNA damage or oxidative stress require interrelated pathways that are critical to survival (1, 2). The inter-relationship arises because reactive oxygen species (ROS) can cause oxidative DNA damage, resulting in base mutations and even chromosomal rearrangements. ROS can furthermore inactivate DNA repair enzymes through oxidation, increasing genome instability. Thus, defects in cellular responses to ROS can lead to carcinogenesis and also to neurodegenerative syndromes resulting from the relatively higher amounts of oxidative stress in the central nervous system (CNS), which consumes about 20% of inhaled oxygen. One such ROS-related neurodegenerative disorder is ataxia-telangiectasia (A-T), caused by mutations in the kinase A-T mutated (ATM), which has signaling functions for not only ROS responses but also for DNA doublestrand break (DSB) repair. However, despite its clear importance, the roles and mechanisms for ATM-mediated ROS signaling were undefined until the notable discovery of a direct mode of ATM activation by ROS (3, 4). Here, we discuss this discovery and its implications, which define how the interrelated ROS and DNA damage signaling pathways are connected and help to clarify apparent paradoxes in the A-T phenotype.

The kinase ATM was first identified by A-T mutations mapping to the *ATM* gene (5), and now over 400 A-T mutations have been characterized, most of which result in truncated forms of ATM. A-T is an autosomal recessive inherited disorder with a rare occurrence of 1 in 40,000 to 100,000 individuals worldwide (6). A spectrum of clinical phenotypes is

observed in A-T individuals and includes neurodegeneration with progressive cerebellar ataxia, accelerated aging, increased risk of type II diabetes, enlarged ocular blood vessels, and immunodeficiency. A-T individuals also have a marked tumorigenesis risk, with increased frequencies for most cancers with lymphomas and leukemias being particularly common (6).

The first described and major cellular A-T phenotype is an impaired activation of the DNA damage–induced checkpoint, where A-T cells were defined as having “radioresistant DNA synthesis,” which means that the cells fail to arrest DNA replication after irradiation-induced DNA damage (7). ATM is now known to phosphorylate many protein targets, including p53, the checkpoint kinase CHK2, and breast cancer susceptibility protein 1, to control the cell cycle at the G₁/S, intra-S, and G₂/M phases (8). A-T cells also display genome instability, a hypersensitivity to ionizing radiation and radiomimetic chemicals, and abundant unresolved DNA breaks that are increased during V(D)J and class switch recombination (6). In line with these phenotypes, the kinase activity of ATM functions in chromatin remodeling and DNA damage signaling and activates DNA DSB repair machinery, coordinating these different processes. ATM is rapidly recruited to a DSB by the complex of Mre11-Rad50-Nbs1 (MRN), which is a DNA damage sensor, through a direct interaction with the Nbs1 subunit (9). At the repair site, ATM phosphorylates the histone variant H2AX, leading to the recruitment of the scaffold protein, mediator of DNA damage checkpoint protein 1 (MDC1), and its phosphorylation by ATM. Together, phosphorylated H2AX and MDC1 provide a stage for repair and signaling components, allowing for DNA damage repair to proceed. Yet, despite these key contributions of ATM to DNA repair, only about 10% of DSBs remain unrepaired in A-T cells after ionizing radiation. This limited overall impact could be due to functional overlaps with other repair proteins, because some of the roles of ATM are shared with the related repair kinases. This includes the ataxia-telangiectasia and Rad3-related protein kinase and the DNA-dependent protein kinase (DNA-PK), as well as a functional redundancy with XRCC4-like factor, which is involved in the ligation step of nonhomologous end joining recombination and V(D)J recombination (10–13). Also, ATM may have specialized repair roles, such as at DNA damage sites within heterochromatin. ATM-mediated KAP1 phosphorylation results in relaxation of heterochromatin, potentially allowing access for the DNA repair machinery (9). Additionally, A-T cells display chromosomal end-to-end fusions, indicating that ATM has undefined roles in maintaining telomeres, which may involve regulating Mre11 nuclease-mediated DNA end degradation. This degradation promotes error-prone microhomology-mediated end joining (14).

Which signaling pathways are disrupted to generate the A-T neurodegenerative pathology has been debated. Arguments for genomic instability promoting cell death, as discussed in (15), and therefore an A-T phenotype are not fully reconciled with how DNA repair defects affect genome maintenance in postreplicative neuronal cells. The comparatively high amounts of oxidative stress that occur in the CNS relative to other organs suggest a hypothesis of neuronal damage in A-T cells that occurs through loss of the normal cellular redox balance (16, 17). Support for a role of ROS in neurodegeneration associated with A-T includes clinical findings, where A-T individuals have significantly reduced concentrations of plasma antioxidants (18). A-T fibroblasts are more susceptible to the effects of ROS, as indicated by an increase in micronucleus induction upon exposure to H₂O₂, as compared with wild-type cells (19). A-T cells also have decreased amounts of various antioxidant molecules and diminished glutathione biosynthesis (19, 20). *ATM*^{−/−} mice have increased concentrations of superoxide anions in the cerebella. Additionally, studies on the stem cells of *ATM*^{−/−} mice reveal a progressive loss of redox balance (20). Although an A-T–like neuropathology is absent in *ATM*^{−/−} mice, these mice exhibit bone marrow failure that is linked to excessive ROS occurs and that can be ameliorated through the use of antioxidants

(21). Antioxidants can also extend tumor latency in these mice and, in cultured *ATM*^{-/-} cells, provide enhanced survival and reduced amounts of oxidative stress and DNA deletions.

How ATM responded to ROS was not apparent until the key observation that ATM is directly activated through oxidation at specific cystines (3, 4). Treatment of human fibroblasts with hydrogen peroxide resulted in activation of ATM through autophosphorylation and phosphorylation of its substrates p53 and CHK2, but not its DNA DSB repair substrates H2AX and KAP1. The activation mechanism in response to ROS is distinct from that in DSB repair response (Fig. 1). DSBs result in the inactive dimeric forms of ATM dissociating into active monomers (22), whereas hydrogen peroxide treatment results in catalytically active dimers. Cys²⁹⁹¹ is critical to the formation of these active dimers through disulfide bond formation, and a Cys²⁹⁹¹→Leu²⁹⁹¹ (Cys2991Leu) substitution results in a mutant form of ATM that cannot be activated in vitro. Incorporation of this Cys2991Leu mutant into human lymphocytes lacking wild-type ATM results in cells that do not undergo apoptosis in response to hydrogen peroxide treatment. However, these Cys2991Leu mutant-expressing lymphocyte cells still exhibit an apoptotic response to DNA damage. An A-T-causing mutation, Arg3047X (R3047X, where X represents a premature termination), results in a protein lacking the 10 C-terminal residues. Similar to the Cys2991Leu mutant, the R3047X mutant form of ATM cannot be activated by oxidation in vitro or in immortalized lymphoblasts that were derived from an A-T individual. The R3047X mutant retains a DNA DSB repair response, and individuals with this R3047X mutation are described as “A-T variant.” Although they have some of the classical A-T symptoms, including telangiectasia and a neurodegenerative phenotype, they display mild radiosensitivity, and their immune response appears relatively normal (23–25). Thus, the biochemical and the clinical phenotypes of the truncation mutant further support the idea that oxidative damage underlies the central neurodegenerative phenotype of A-T.

An understanding of how mutation affects ATM structure and function comes in part from analyses of the structurally related kinase DNA-PK. Together, ATM and DNA-PK critically control DSB repair processes in the cell, and they both belong to the phosphatidylinositol-3-kinase related protein kinase (PIKK) family. PIKKs are relatively large serine/threonine kinases that have signaling functions following cellular stress (26). The overall domain architecture of PIKKs was revealed by the crystal structure of the DNA-PK catalytic subunit (DNA-PKcs) (27). This break-through determined that DNA-PKcs has a ringlike architecture that is 160 Å long by 120 Å wide (Fig. 2). The ringlike structure is built from the N-terminal α-helical heat repeats and contains a cavity in the center large enough to accommodate double-stranded DNA and an opening at its N-terminal base. At the top of the ring is a “crown” that is formed by the C-terminal kinase domain, FAT, and the FAT-C domains. The Cys2991Leu and R3047X ATM mutations reside in the FAT-C domain, indicative of its functional importance to the holo-enzyme. Complementing the crystallographic studies, small-angle x-ray scattering (SAXS) data suggest that recruitment and dimerization mechanisms activate holo-DNA-PK, which also contains the Ku70 and Ku80 DNA-binding subunits (28, 29). SAXS reveals that a flexible C-terminal arm of Ku80 recruits and maintains DNA-PKcs at DSBs. The presence of Ku70, Ku80, or DNA causes DNA-PKcs dimerization that promotes trans-autophosphorylation, resulting in a sufficiently large opening in the ring structure that potentially promotes either the entry or the release of DNA ends. Thus, these structural-based studies raise interesting questions, such as whether there are commonalities in dimerization and activation mechanisms among PIKK family members. Similarities between DNA-PK and ATM are suggested, because DNA-PK dimerization occurs through the FAT-C-containing crown region in the physiologically relevant SAXS dimer, and the oxidatively activated ATM dimer is also formed in the FAT-C region, through a disulfide bond. Furthermore, the nuclear magnetic resonance structure of the FAT-C domain from the kinase mTOR, a PIKK with regulatory functions in cellular

proliferation, cell motility, cell survival, and protein synthesis and transcription, revealed that the structure contains an intramolecular disulfide bond. Mutational studies of the yeast analog TOR2 suggest that this disulfide is redox-sensitive, and its reduction promotes TOR2 degradation and hence provides a mechanism for controlling the activity of TOR2 (30). Thus, the discovery of the activation of ATM by oxidation and the DNA-PKcs and mTOR structural analyses now provide a means for defining roles, mechanisms, and pathways of PIKK-mediated redox signaling in the maintenance of genomic integrity.

Notably, ionizing radiation that generates DNA DSBs can also produce ROS that inactivate key DNA repair proteins. This includes (i) major sensors of DNA DSBs, either the MRN complex or the Ku70 or Ku80 subunits of holo-DNA-PK, and (ii) the DNA repair helicase XPD and likely the DNA repair helicase FANCD1, because of its similarities to XPD (4, 31, 32). Hence, ATM oxidative activation may allow cells to respond to DNA DSBs and maintain genetic integrity under these toxic conditions. A DSB response is critical because even a single unrepaired DSB can promote apoptosis. The importance of this redox-sensing function by ATM in promoting genomic integrity is also highlighted by the presence of redox regulation in other processes that promote genomic stability. This includes the cell cycle, where increased ROS results in degradation of the cell-cycle phosphatase CDC25C (33). Additionally, increased ROS activates the base excision repair phosphodiesterase APE1, resulting in increased cellular resistance to oxidizing agents (34). APE1 forms a central signaling point for certain repair and transcriptional pathways (35). Similarly, ATM appears to function as a key nodal point, bringing together DNA damage response and also the response to oxidative stress. Additional support for the redox role for ATM includes that ATM activates the pentose phosphate pathway to increase antioxidant generation and promote DNA repair (36). Also, it has been observed that a co-factor protein, ATMIN (ATM interactor), is critically required for the ATM redox response (37).

What does this newly identified ROS-to-kinase connection mean going forward? Forty years of ROS investigations are finally revealing how, despite broad reactivity and short half-lives, ROS signaling is regulated and propagated to control biological outcomes. Because the cysteine thiol has high reactivity in oxygen and nitric oxide (NO)-dependent reactions, it is a prototypic biological target for ROS signaling and regulation, as shown by the autoregulation of inducible NO synthase (38). The landmark ATM results discussed here reveal that ROS signaling is transduced by modifying ATM cysteine residues into a kinase signaling cascade. The architecture of the ATM dimer may create specificity for ROS reactivity, because the dimer may form an acidic environment that lowers the cysteinyl pK_a (where K_a is the acid dissociation constant), making it a good nucleophile at neutral pH for increased ROS reactivity. Thus, the detailed definition of the structures of the DNA damage response kinases will be of importance for understanding and controlling this activation mechanism.

ATM in redox signaling and sensing has important relevance to health. Loss of ATM's activity toward p53 contributes to insulin resistance and metabolic syndrome in A-T patients, and reduced ATM activity promotes tumor angiogenesis. Therefore, modulation of certain aspects of ATM function that include redox sensing may have implications for combating diabetes or cancer growth. ATM is an attractive therapeutic target for the treatment of cancer, because ATM inhibitors dramatically sensitize cancer cells to ionizing radiation. Improving on initial ATM-based inhibitors may aid current cancer therapeutics that largely include DNA-damaging agents, because ATM inhibitors could remove signals for DNA repair that potentially limit chemotherapeutic cytotoxicity. Regarding the A-T phenotype, there is no effective treatment for the syndrome, and therapies are focused on treating cancers and providing immunoglobulins to enhance immune function. Thus, the

discovery of a mechanism for ATM redox sensing now lends increased support to the development of antioxidants as therapies for treating A-T.

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Fig. 1.

Distinct ATM signaling mechanisms. A DSB results in the inactive dimeric forms of ATM dissociating into active monomer and recruitment of the monomers to a DSB by an interaction with the Nbs1 subunit of MRN damage sensor. At the DSB, ATM phosphorylates the histone variant H2AX and other key targets, leading to activation of the DNA repair machinery. Oxidative stress instead results in catalytically active ATM dimers that do not target DNA repair machinery but instead trigger cellular ROS responses. G6PDH, glucose-6-phosphate dehydrogenase; Hsp, heat shock protein; IR, ionizing radiation; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate.

Fig. 2.

The 6.6-Å crystal structure of the catalytic subunit of DNA-PK, an ATM homolog. DNA-PKcs [Protein Data Bank accession identification 3KGV] consists of a ring structure of heat repeats (dark green) and several domains that create a “crown” on top of the ring. The kinase domain is depicted in yellow, “forehead” domain in dark blue, and FAT and FAT-C domains in magenta. The ring contains a large central cavity that would readily accommodate double-stranded DNA, and the domain highlighted in cyan is a putative DNA binding domain. Right image depicts a 90° rotation of the left view.