ATM and Genome Maintenance: Defining Its Role in Breast Cancer Susceptibility

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The ATM gene is mutated in ataxia-telangiectasia (A-T), a genetic instability syndrome characterized by increased cancer risk, as well as other features. Recent studies have shown that the ATM protein kinase plays a critical role in maintaining genome integrity by activating a biochemical chain reaction that in turn leads to cell cycle checkpoint activation and repair of DNA damage. ATM targets include well-known tumor suppressor genes such as p53 and BRCA1, both of which play an important role in predisposition to breast cancer. Studies of A-T families have consistently reported an increased risk of breast cancer in women with one mutated ATM gene, but so far an increased frequency of ATM mutations has not been found in women with breast cancer. Some specific missense and protein truncating variants of ATM have been reported to confer increased breast cancer risk, but the magnitude of this risk remains uncertain. A more comprehensive analysis of ATM is needed in large case-control studies, and in multiple-case breast cancer families.

KEY WORDS: ATM; breast cancer; cell cycle checkpoints; DNA damage; genome integrity.

INTRODUCTION

The maintenance of genomic stability requires cells to duplicate and segregate their genome precisely and coordinately to the next generation of daughter cells. The intrinsic process associated with these DNA transactions presents a persistent threat to genome stability in dividing cells. The risk of this damage is further compounded by constant encounters with environmental insults, such as ultraviolet light (UV), chemical carcinogens, and free radicals. A particularly lethal form of DNA damage is DNA double strand breaks (DSBs). In a unicellular organism such as yeast, as little as one DNA DSB can be sufficient to kill a cell if it inactivates an essential gene (1) or, in metazoa, leads to triggering of apoptosis. A faulty or inaccurate rejoining of broken DNA DSBs may occur, leading to the loss or amplification of chromosomal material, or under certain circumstances to translocations in which segments of chromosomal arms are exchanged, sometimes in a reciprocal fashion. These events can, in turn, favor tumorigenesis.

Given the disastrous consequences of DNA damage, it is anticipated that an effective DNA damage response will be utilized by multicellular organisms to maintain the integrity of the genome. This surveillance network monitors the genome for the presence of abnormal DNA structures, including DNA DSBs. The presence of DSBs in the DNA

Abbreviations Used: A-T, Ataxia-telangiectasia; ATM, Ataxia-telangiectasia mutated; NBS, Nimegen breakage syndrome; AT-LD, A-T like disease; ATR, Ataxia-telangiectasia and Rad3-related; ATR-IP, ATR interacting protein; FRAP1, FKBP12 and rapamycin associated protein; DNA-PKcs, Catalytic subunit of DNA-dependent protein kinase; Cdk, Cyclin-dependent kinase; DSBs, DNA double strand breaks; IR, Ionizing radiation; UV, Ultraviolet light; RDS, Radiosensitive DNA synthesis; MRN, MRE11-Rad50-NBS1 complex; Rad complex Rad9-Rad1-HUS1.

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activates the ATM protein kinase, which results in the coordinated induction of a complex cellular program of events that in turn leads to cell cycle checkpoint activation, DNA repair, gene transcription, and, in some instances, apoptotic cell death.

This review will selectively discuss the current state of our understanding of the role of ATM in genome maintenance. Moreover, we comment on the contradictory and supporting data on the role of ATM in breast cancer susceptibility to provide a foundation for assembly of available data into a coherent picture.

**ATM AND ITS FUNCTIONS**

ATM was identified as the product of the gene mutated/inactivated in ataxia-telangiectasia (A-T), a genetic instability syndrome characterized by progressive neuronal degeneration, immunological deficiency, radiosensitivity, and increased cancer risk, particularly for lymphoid malignancies (2). ATM spans more than 150 kb, is composed of 66 exons (62 coding), and is expressed in a wide range of tissues as a 13-kb transcript encoding a protein of 3056 amino acids. Disruption of the ATM gene in mice gives rise to a phenotype remarkably similar to A-T except for neurodegeneration, which is largely absent (3–5).

Notably, cells from A-T patients or ATM-nulilzygous mice are exquisitely sensitive to IR and other agents that induce DSBs, and fail to activate the IR-induced G1/S or G2/M checkpoints (6). In addition, A-T cells exhibit radioresistant DNA synthesis, the failure to transiently downregulate DNA replication in response to IR, which is indicative of an S-phase checkpoint defect.

Most of the cellular functions of ATM are tightly linked to the carboxy terminal catalytic domain, which bears significant sequence similarity to the catalytic domains of phosphoinositide 3-kinases (PIK) (2). The sequence homology to lipid kinases indicates that ATM is a member of phosphatidylinositol-3 kinase (PIK) related kinases family and has conserved orthologs from lower eukaryotes to mammals including Drosophila melanogaster (mei-41), Saccharomyces cerevisiae (Tel1p and Mec1p), and Schizosaccharomyces pombe (Tel1 and Rad3), and in mammals the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), ataxia-telangiectasia and Rad3-related (ATR), and FKBP12 and rapamycin associated protein (FRAP1). Most of these proteins are involved in cell cycle control and/or detection of DNA damage (7,8). The prototype PI3-kinase has a dual specificity for both proteins and lipids because it phosphorylates p85 (its regulatory subunit) and the inositol ring of phosphatidylinositol. No lipid kinase activity has been reported for DNA-PKcs, ATR, FRAP, and ATM, although these enzymes have been shown to have protein kinase activity against a range of substrates with specificity for serine/threonine followed by glutamine (9–11). Tel1p, Mec1p, and Rad3 in yeast also demonstrate protein kinase activity, providing further evidence of similarity (12,13).

In mammals, ATM and ATR play a central role in DNA damage recognition to initiate checkpoint responses to DNA damage (14). Despite their structural similarity and overlapping substrate specificities (Ser/Thr-Gln), ATM and ATR are functionally nonredundant protein kinases that are activated in response to distinct, as well as partially overlapping, types of genotoxic stimuli (14,15). ATR mediates responses to a broad spectrum of genotoxic stimuli, including IR, DNA replication inhibitors (e.g., hydroxyurea, HU), UV light, and agents such as cis-platinum that induce DNA interstrand crosslinks, whereas ATM is evolved selectively to deal with IR and other radiomimetic agents that cause double strand breaks in DNA (1,6,15,16).

Recent studies have begun to elucidate the molecular events that lead to activation of these kinases during genotoxic stress. In the case of ATR, it has been suggested that ATR-IP, the regulatory partner of ATR, specifically recognises RPA bound to single strand DNA and thereby localizes the ATR-ATRIP complex to sites of DNA damage (17). ATM’s activation has recently been shown to involve autophosphorylation. It has been proposed that ATM molecules are inactive in undamaged cells, being held as dimers and high order multimers (18). Upon DNA damage, ATM is activated by intermolecular autophosphorylation on Ser1981. Furthermore, it has also been postulated that changes in chromatin structure ensuing from DNA damage are the initiating events in ATM activation (18). A prediction of the model would be that ATM would not be required to recognise or directly contact the DSB. Contrary to this prediction, there is recent evidence that suggests that ATM does act at the DSB site to phosphorylate various targets (19). Phosphorylated Nibrin and H2Ax, a variant of histone H2A, are exclusively located at the DSB site whereas phosphorylated CHK2 is globally distributed; however, the chromatin-tethered form of CHK2 was