Cancer Risk and the ATM Gene: a Continuing Debate

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Deficiencies in the ability of cells to sense and repair damage in individuals with rare genetic instability syndromes increase the risk of developing cancer. Ataxia-telangiectasia (A-T), such a condition, is associated with a high incidence of leukemia and lymphoma that develop in childhood. Although A-T is an autosomal recessive disorder, some penetrance appears in individuals with one mutated ATM gene (A-T carriers), namely, an increased risk of developing breast cancer. The gene mutated in A-T, designated ATM, is homologous to several DNA damage recognition and cell cycle checkpoint control genes from other organisms. Recent studies suggest that ATM is activated primarily in response to double-strand breaks, the major cytotoxic lesion caused by ionizing radiation, and can directly bind to and phosphorylate c-Abl, p53, and replication protein A (RPA). Analysis of ATM mutations in patients with A-T or with sporadic non-A-T cancers has suggested the existence of two classes of ATM mutation: null mutations leading to A-T and dominant negative missense mutations predisposing to cancer in the heterozygous state. Studies with A-T mouse models have helped determine the basis of lymphoid tumorigenesis in A-T and have shown that ATM plays a critical role in maintaining genetic stability by ensuring high-fidelity execution of chromosomal events. Thus, ATM appears to act as a caretaker of the genome. [J Natl Cancer Inst 2000;92:795–802]

Ataxia-telangiectasia (A-T), caused by mutations in the ATM (A-T mutated) gene, is a rare autosomal recessive disorder characterized by progressive neuronal degeneration, immunologic deficiency, radiosensitivity, premature aging, and an increased risk of cancer (1,2). As many as one third of A-T patients develop cancer, with the majority of these cancers being of the lymphoid type (3). Lymphoid malignancies in patients with A-T are of both B-cell and T-cell origin and include non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, and several forms of leukemia (4). Long before the gene was cloned, an association with breast cancer was suggested by Swift et al. (5,6), who reported an excess of breast cancers in the relatives of A-T patients. These results were supported by the analysis of several published studies by Easton et al. (7). On the basis of early estimates that the frequency of A-T carriers (individuals with one mutated ATM gene) was 1%, it was suggested that A-T heterozygosity was the best candidate for a common predisposing condition and could be associated with about 5% of all breast cancers (8). This is substantially greater than the 1%–2% associated with mutations in BRCA1 and BRCA2 genes. Interest in this observation was rejuvenated when the ATM gene was cloned in 1995 (9).

ATM AND ITS FUNCTION

The ATM gene is located at 11q22–23. It spans about 150 kilobases of genomic DNA, has 66 exons, and is expressed in a wide range of tissues as a 13-kilobase transcript (9). Construction of the full-length, 10,140-base-pair complementary DNA proved to be difficult, but the gene was eventually cloned in two laboratories (10,11). The gene encodes a 350-kd (3056 amino acid) protein (12,13). The first distinctive feature of ATM recognized was the phosphatidylinositol 3-kinase (PI3-kinase) domain close to the C terminus of the protein (9). ATM is related to this domain to a family of proteins involved in cell cycle control and DNA-damage recognition (14–16). It seems likely that these proteins phosphorylate one or more substrates (including p53, c-Abl, RPA [i.e., replication protein A], and PHAS-1 [i.e., phosphorylated heat- and acid-stable protein regulated by insulin] proteins) in response to DNA damage to activate radiation signal transduction pathways and/or recruit proteins to sites of DNA repair (reviewed in (17)). ATM levels are not altered by radiation exposure (13,18), but the kinase activity of ATM increases severalfold after exposure to ionizing radiation (19,20). The mechanism of activation of ATM is equally uncertain but may involve a modification, such as phosphorylation, or a conformational change in response to an alteration in chromatin structure after irradiation.

The kinase domain represents less than 10% of the total ATM protein. Surprisingly few other functional domains or interacting sequences have been identified in ATM. There is a leucine zipper consensus sequence in the N terminus, but there is no clear-cut evidence that this motif is responsible for interaction of ATM with itself or with other proteins. We have identified a proline-rich region that is a site for interaction with c-Abl, the non-receptor protein tyrosine kinase (21). The radiation-induced activation of c-Abl tyrosine kinase is defective in A-T cells and in fibroblasts from Atm–/– mice, but ectopic expression of the ATM kinase domain activates c-Abl kinase and corrects these defects (21,22). The signaling steps from ATM to stress-activated protein kinase might involve the action of c-Abl (21,23); in addition, c-Abl could act as a positive regulator of p53 by relieving the inhibitory effect of the MDM2 protein (24). ATM is also required for the c-Abl-mediated assembly of the Rad51 repair protein complex after ionizing radiation. In this process, c-Abl directly phosphorylates Rad51, resulting in an enhanced association between Rad51 and Rad52 that may lead to increased repair proficiency (25).

It is becoming increasingly apparent that ATM plays a central role in signaling DNA damage, predominantly double-strand breaks in DNA, and in activating checkpoints to slow the progress of cells carrying DNA damage through the cell cycle (Fig. 1). ATM is implicated in DNA-damage recognition pathways...
Fig. 1. Simplified model for the function of the ATM protein. Double-strand breaks (DSBs) in the DNA caused by treatment of cells with ionizing radiation (IR) or with radiomimetic drug activate the ATM kinase by an undescribed mechanism. As a consequence, different target proteins are phosphorylated by ATM to promote cell cycle arrest at G1/S and G2/M checkpoints, possibly through formation and utilization of different complexes containing ATM at different phases of the cell cycle. ATM also signals to repair machinery through its interaction with the BRCA1/Rad51 complex and the nibrin/Mre11/Rad50 complex. BRCA1 and nibrin have also been implicated in regulation of cell cycle checkpoints. Activation of c-Abl is also ATM dependent, and it is likely that c-Abl and BRCA1 control transcription of stress response genes.

involving both homologous recombination and nonhomologous end joining; as a part of this recognition, it overlaps with nibrin or p95, the gene mutated in another chromosomal instability disorder, Nijmegen breakage syndrome (26,27). Strong parallels exist between the pattern of radiosensitivity, chromosomal instability, defective double-strand break repair, and cell cycle anomalies, particularly radioresistant DNA synthesis, in patients with A-T or Nijmegen breakage syndrome. The gene defective in Nijmegen breakage syndrome encodes p95 or nibrin (26,27), which is a part of a complex with human (h) Mre11 and hRad50 proteins. This complex has been proposed to act as a sensor of DNA damage because it localizes to double-strand breaks within 30 minutes of irradiation and remains associated with radiation-induced foci for long periods after irradiation (28). A recent study (29) has identified mutations in the hMre11 gene in two families clinically diagnosed with a variant form of A-T. These studies strengthen the link between damage recognition by the hMre11/hRad50/nibrin complex and the ability of cells to activate ATM-dependent DNA damage response pathways.

Recent data obtained by Cortez et al. (30) and data from our laboratory provide evidence that ATM is involved directly in the regulation of the breast cancer gene product BRCA1 (Gatei M, Scott S, Filippovich I, Soronika N, Lavin MF, Weber B, et al.: unpublished data), which is involved in the regulation of certain DNA-repair processes through its association with Rad51 (31). This functional interaction of ATM with the BRCA1 protein argues in favor of the involvement of particular aspects of ATM function in predisposition to breast cancer. In addition to its role in response to DNA damage, the ATM protein clearly plays a role in many physiologic pathways, as is apparent from the phenotype of A-T patients (32). It is possible that ATM has different functions in different cell types and at different stages of development, and it remains to be established how these different functions contribute to the clinical features of A-T.

DO A-T HETEROZYGOTES HAVE AN INCREASED RISK OF CANCER?

With the ability to screen for ATM mutations and to identify A-T carriers, many studies have been initiated to determine whether A-T carriers have an increased susceptibility to breast cancer. However, few studies have yet been published on the frequency of breast cancer in patients with A-T and carriers with defined ATM mutations. Athma et al. (33) studied the haplotype of 99 A-T families, included the original families reported by Swift et al. (5,6) to have an increased rate of breast cancer, and reported a fourfold increased risk overall for breast cancer. However, the mutations in these families and patients with breast cancer have yet to be reported, so it is not known exactly which mutations confer this increased risk. More recently, Stankovic et al. (34) found that the T7271G variant is associated with a 12.7-fold (95% confidence interval = 3.53–45.9) increased risk of breast cancer in both heterozygotes and homozygotes. This finding suggests that the risk of breast cancer may depend on the particular ATM mutation.

A few more studies have examined the frequency of ATM mutations in patients with breast cancer (usually of early onset or exhibiting radiosensitivity) compared with control subjects. Most of these studies have used mutation detection methods that are biased toward detecting protein-truncating mutations—often the protein truncation test or alternatively single-strand conformation polymorphism analysis. Most studies have been too small to have adequate power or have focused only on women with early-onset breast cancer (35–38). FitzGerald et al. (37) used the protein truncation test to examine germline DNA from 401 women with early-onset breast cancer and 202 control subjects but did not find an increase in A-T carriers among the patients with cancer. This result does not conflict with that reported by Athma et al. (33) because the confidence limits of risk estimates (33,37) overlap in these two studies (39). Overall, the results appear to be contradictory, but nonetheless most studies do not support the claims of Athma et al. (33) of a fourfold increased risk of breast cancer in A-T carriers. Instead, the increased risk may be closer to twofold, but the confidence intervals are still wide (40). Similarly, if patients with radiosensitive breast cancer are selected, the A-T carrier frequency is not any higher in these patients than it is in control subjects (41–43). However, there is increasing evidence that, although protein-truncating ATM mutations may not be more common in patients...
with breast cancer than in control subjects, allelic variants that lead to amino acid substitutions or in-frame deletions/insertions may be more common. In 1996, Vorechovsky et al. (44) found the same five rare amino acid substitutions in ATM among 88 patients with breast cancer and among control subjects. Teraoka et al. (45) found possible missense ATM mutations in 11 of 142 patients with breast cancer but in only one of 80 control subjects; all 11 individuals carrying missense mutations had a family history of breast cancer. Dork (46) also found a high rate of amino acid substitutions in ATM of patients with breast cancer, some in the PI3-kinase domain of ATM. In 140 patients with breast cancer tested, Schubert et al. (47) found a Glu999Gly substitution in ATM from a patient with breast cancer but no truncating mutations. A recent ATM analysis of 103 patients with breast cancer in which fluorescent chemical cleavage of mismatched DNA was used (48) found one putative missense mutation (G8293A) in the PI3-kinase domain and five rare variants. To date, there have been no large case–control association studies of these ATM variants.

**ATM Mutations in A-T Patients**

ATM mutations have now been described in more than 300 A-T families (for more information, see http://www.vmmc.org/vmrc/atm.htm). Most of the patients are compound heterozygotes and, in many populations, there is a strong founder effect (49,50). Currently, about 70% of the ATM mutations identified result in premature protein truncations (34,51), but there are also about 30% missense mutations and small in-frame deletions/insertions. In addition, a further 36 “polymorphisms” have been described. In many studies, only the protein truncation test has been used to identify mutations, inevitably creating a bias; thus, the prediction that 70% of the ATM mutations result from a truncation could be an overestimate. It is interesting that missense mutations tend to be clustered at the 3’ end of the ATM gene, the region that codes for the regulatory and PI3-kinase domains. There is some evidence that missense mutations may have a different functional outcome. Only a few missense homozygotes have been described, but one of them (T7271G) has a valine-to-glycine substitution and results in a milder phenotype (34).

**ATM Mutation in Cancers**

Deficiencies in the ability of cells to sense and repair damage in individuals with rare genetic instability syndromes increase the risk of developing cancer. Genetic instability is a cardinal feature of A-T, and this defect results in the inability to activate cellular functions that promote genetic stability by temporarily arresting the cell’s division cycle in response to damage. The ATM-dependent responses allow cells to react to double-strand breaks that result from endogenous oxidative DNA damage and environmental mutagens, as well as the breaks created during meiosis and immunologically important gene rearrangements. Thus, one might expect that the ATM protein would, like p53, play a key role as a guardian of the genome. Inactivation of the ATM gene may be a frequent event in the development of certain common sporadic cancers. In support of this argument, the loss of heterozygosity at 11q22–23 that includes the ATM locus is a frequent event in many tumors. Mutations in the ATM gene have also been found in patients with T-cell prolymphocytic leukemia and B-cell chronic lymphocytic leukemia (36,52–56). It has been difficult to prove whether the patients with T-cell prolymphocytic leukemia were A-T heterozygotes; however, studies of patients with B-cell chronic lymphocytic leukemia suggest that as many as 20% may be constitutional A-T heterozygotes [reviewed by Gatti et al. (57)]. It is interesting that missense mutations are found in a high proportion of patients with T-cell prolymphocytic leukemia and B-cell chronic lymphocytic leukemia. Most missense mutations occur in the PI3-kinase domain, and some missense mutations have been reported more than once. This observation is in stark contrast with the predominance (70%–80%) of truncating mutations observed in A-T-affected families, as discussed above. Therefore, it would appear that cancer patients with ATM missense mutations are not A-T heterozygotes.

To resolve this paradox, Gatti et al. (57) and Meyn (58) have hypothesized that there are two populations of A-T carriers, one group with a truncating allele coupled with a normal allele (ATM trun/w) and a second group with a missense mutation coupled with a normal allele (ATM mis/w), and that the latter group might be more prone to carcinogenesis. Truncating mutations act effectively as null mutations because they produce an unstable ATM protein that is present in very small amounts within a cell and ATM trun/w carriers would have 50% of wild-type ATM activity and, hence, a nearly normal phenotype. ATM trun/trun homozygotes or compound heterozygotes would be expected to have A-T because they lack functional ATM protein. Some of the ATM missense mutations, on the other hand, encode stable, functionally abnormal proteins that are present at normal intracellular levels. Overexpression of a mutant ATM polypeptide that lacks the PI3-kinase domain has been shown to increase genetic instability in normal cells grown in culture and, thus, have a dominant negative cellular phenotype (59). One possible mechanism by which this might occur is if the ATM polypeptide forms a functional complex with itself and/or with other proteins. The missense polypeptides will compete with normal ATM polypeptides in complex formation, and the functionally abnormal missense polypeptides will sequester key regulators or substrates into nonfunctional complexes, resulting in a dominant negative cellular phenotype. The loss of function in ATM mis/w heterozygotes would be more severe than in ATM trun/w heterozygotes and, thus, would be associated with an increased risk of cancer. The missense alleles, when combined with themselves or with other ATM alleles, would not be sufficiently compromised to give the complete clinical A-T phenotype. To date, few A-T patients have been found in whom both ATM-mutated alleles carry missense mutations. Stankovic et al. (34) described an A-T-variant family with a homozygous missense ATM mutation (T7271G). In this family, affected members had an atypical A-T-like condition characterized by intermediate radiosensitivity, unusual longevity, and absence of typical telangiectasia. Of further interest, patients with breast cancer in this family carried an ATM allele with the missense mutation [reviewed by Gatti et al. (57)]. This underscores the importance of determining the frequency of ATM mis/w in the general population, which is being pursued in a number of laboratories. The existence of two distinct A-T carrier populations (ATM trun/w and ATM mis/w) might explain some of the seemingly contradictory data on cancer risk associated with the ATM gene.
Several murine models for A-T have been developed by disruption of the ATM gene, the mouse homologue of ATM, by gene targeting (60–62). These murine models have lost functional ATM protein because the truncated forms of ATM are highly unstable. Disruption of the ATM gene produces a model with characteristics that are similar in many aspects to those of human A-T, including a predisposition to develop malignant thymic lymphomas. All published reports (60–62) show that ATM−/− mice develop thymic lymphomas by the age of 4 months. Spectral karyotyping of the tumor cells indicated multiple and recurrent chromosomal translocations (60). In addition, chromosomal abnormalities involving the locus for the T-cell receptor-β have also been identified in monoclonal cells derived from ATM−/− mice (61). Therefore, similar to the lymphoid tumorigenesis in A-T patients, genetic instability appears to play a role in the early onset of the lymphoid tumors in ATM−/− mice.

More recently, an Atm mutant mouse has been developed that harbors a 7673del9 mutation, a common in-frame deletion in exon 54. This mouse shows a delayed development of lymphoma and the occurrence of other tumor types (Spring K: unpublished data). This mutation represents a “hot spot” because it has been reported in as many as 10% of patients with A-T in studies of various populations (50). It should also be noted that this mutation has been observed in a breast cancer patient of Swedish origin (35). A-T cells homozygous for this three-amino acid deletion express a relatively stable, near full-length ATM protein that appears to be functionally inactive because, when immunoprecipitated, it cannot phosphorylate p53 (Khanna KK: unpublished results). The region of the ATM protein encoded by exon 54 may provide an essential regulatory element for the ATM kinase activity because it is immediately adjacent to the PI3-kinase domain. This type of regulation has been demonstrated for other members of the ATM subgroup of the PI3-kinase family (63). However, thus far, no regulatory proteins have been implicated in the control of ATM kinase activity. Alternatively, the loss of the amino acids in this region of ATM could alter the conformation of the molecule and could inactivate the kinase domain.

Mouse models of A-T have also been valuable in elucidating the basis of lymphoid defects and tumorigenesis in A-T. Recent studies have provided genetic evidence suggesting that ATM plays a role in the regulation of V(D)J recombination and that abnormality in this process could contribute to genetic instability and tumorigenesis in ATM−/− lymphocytes. In support of this hypothesis, the onset of thymic lymphoma is substantially suppressed in ATM−/− and RAG2−/− double null mice, which are completely deficient in V(D)J recombination (64). The suppression of tumors cannot be solely caused by greatly reduced thymocyte numbers in RAG2−/− mice because p53−/− and RAG2−/− double null mice and p53−/− mice develop lymphoma with the same kinetics and at the same frequency. This also suggests that the underlying mechanism for development of thymic lymphoma in ATM−/− and p53−/− mice may be distinct. This observation is consistent with studies showing that ATM−/− and p53−/− double-mutant mice develop tumors much earlier than the single-mutant mice and indicating that ATM and p53 cooperate in suppression of tumorigenesis. In this context, it is important to note that ATM is mainly responsible for directing the p53 response to double-strand breaks in DNA but is dispensable in the p53 responses to other genotoxic and cellular stresses, such as DNA damage induced by UV irradiation (65).

**How the Defect in ATM Might Promote Tumorigenesis**

Loss of normal control of genomic stability is thought to be a necessary step in carcinogenesis that allows clonal populations of cells to accumulate a sufficient number of mutations to acquire a fully malignant phenotype. Genomic integrity is maintained by monitoring and/or repairing DNA lesions that occur either physiologically (recombination) or accidentally (mutations and replication errors). Recent studies have provided evidence that ATM operates via the phosphorylation of signaling molecules that link the detection of DNA damage to the maintenance of genomic stability by modulation of cell cycle progression (Fig. 2). ATM acts very early in this signal transduction pathway, because the ATM kinase is required for the immediate response to damage (66,67). In ATM null cells or heterozygotic cells, the loss of functional ATM protein leads to a relaxation in the monitoring of DNA lesions, and the cells become receptive to unrepaired damage and the accumulation of mutations (Fig. 2). These mutations might be repaired if a second level of monitoring (gatekeepers), such as the p53 pathway, is activated and causes cell cycle arrest. However, if this second level is defective, the cell becomes permissive for a flow of oncogenic events that ultimately leads to cancer.

The past 5 years have seen rapid progress in linking checkpoint functions with genomic stability and prevention of cancers in humans. A number of cancer-prone diseases have now been shown to be caused by mutations of genes involved in checkpoint functions. The p53 gene is mutated in patients with Li–Fraumeni syndrome (68), and the p16INK4a gene is mutated in patients with familial melanoma (69). Furthermore, two novel DNA helicases have been identified as the genes responsible for Bloom’s syndrome (70) and for Werner’s syndrome (71), both of which are characterized as cancer-prone diseases. Protein products of the breast cancer genes BRCA1 and BRCA2, which associate with the Rad51 protein, appear to be involved in checkpoint function as well (72,73).

ATM is involved in the regulation of multiple cell cycle checkpoints (G1/S, S, and G2/M phases) after DNA damage (Fig. 1). Kastan et al. (74) demonstrated the importance of p53 in activating the G1/S checkpoint after irradiation; subsequently, it was demonstrated that the p53 response to ionizing radiation was defective in A-T cells (65,75). More recently, we (66) have provided evidence for a direct interaction between ATM and p53. ATM is initially activated in response to DNA damage and, in turn, activates p53. At least part of this activation mechanism involves phosphorylation of p53 on serine-15 by ATM (1,19,20). Phosphorylation at this site may contribute to the increased half-life of p53 by facilitating its dissociation from MDM2 (76), a protein that promotes p53 proteolysis (77,78). These studies show a direct link between ATM and p53 and shed light on the molecular mechanisms that underlie the greater susceptibility of A-T patients to cancer.

In addition to the G1/S checkpoint, the induction of S- and G2/M-phase checkpoints after ionizing radiation is also dependent on the presence of functional ATM protein [reviewed in (1)]. Checkpoint controls in G1 and M phases are important in preventing clastogenic damage, and there are multiple potentially redundant controls to prevent errors in mitosis. The rapid
ATM-dependent phosphorylation of Chk2/hCds1 (79) and Chk1 (80) proteins in response to radiation provides an attractive mechanism for the G2/M delay (Fig. 1). Chk1 and hCds1 mediate phosphorylation of Cdc25C on serine-216, which promotes the binding of Cdc25C protein to 14-3-3 protein, leading to its export from the nucleus and sequestration in the cytoplasm (79,81). This would prevent Cdc25C from activating the nuclear Cdc2/cyclinB complex required for entry into mitosis. Furthermore, the failure to activate Chk2 in A-T cells in response to a replication block could account for the defective function of the S-phase checkpoint in these cells. In budding yeast, the homologues of ATM (Mec1) and Chk2 (Rad53) are required for the function of the S-phase checkpoint and might exert their effect by blocking the initiation at late origins of replication (82,83). ATM has also been implicated in DNA-damage-induced phosphorylation of RPA, an additional potential effector of the S-phase checkpoint (84). RPA is a heterotrimeric complex, and ionizing radiation-induced hyperphosphorylation of the 34-kd subunit of RPA is considerably reduced and delayed in A-T cells (85). Furthermore, ATM phosphorylates RPA (34-kd subunit) in vitro on residues that are similar to those targeted in vivo (86). The ATM-dependent S-phase checkpoint would allow double-strand breaks to be repaired before the lesions are permanently fixed by DNA replication into chromosomal breaks that cannot be repaired.

The DNA-damage-associated spindle checkpoint is also defective in A-T cells (87). A defect in this mitotic checkpoint leads to chromosomal instability due to mis-segregation of chromosomes during cell division. The mitotic checkpoint or spindle assembly checkpoint controls the ploidy of the cell and senses a multitude of spindle defects, ranging from the presence of a single, unattached kinetochore to massive structural defects induced by microtubule-depolymerizing drugs, such as nocodazole [reviewed in (88)]. This checkpoint appears to require the products of a number of genes acting in a linear pathway. In yeast with an unattached kinetochore, the Mad, Bub, and Mps1 proteins act to block the onset of anaphase by inhibiting the activity of the anaphase-promoting complex/cyclosome (89,90). hsBUB1, the human homologue of at least one of these proteins, has been shown to be mutated in colorectal tumors (91). Cells with a mutation in any of the genes encoding a component of the spindle checkpoint will attempt sister chromatid segregation despite mitotic spindle defects, but the attempt will result in a catastrophic mitosis, with daughter cells either losing or acquiring extra chromosomes. Studies with p53 null cells suggest that the DNA-damage and spindle surveillance pathways may intersect. After DNA damage, p53 null cells have defects in the G1/S checkpoint and in the spindle structure surveillance pathway. When exposed to spindle depolymerizing agents, p53 null mouse embryonic fibroblasts undergo repeated rounds of DNA synthesis without chromatid segregation, generating polyploid cells (92). The loss of p53 may also cause genetic instability through centrosome amplification (93). Gadd45a null mice have several characteristics of p53-deficient mice, including genomic instability (exemplified by aneuploidy, chromosomal aberrations, and centrosome amplification), accompanied by abnormalities in mitosis and cytokinesis (94). p21 (cip1/waf1) has also been shown to be required for spindle assembly checkpoint in mitosis. Reduced p21 expression also results in gross nuclear abnormalities and centriole overduplication (95). An intriguing recent observation from the study of mice homozygous for truncated BRCA2 mutation (96) suggests that the mitotic checkpoint defect cooperates with BRCA2 deficiency to foster transformation in these cells. In these experiments, tumors from BRCA2-deficient animals had a dysfunctional spindle assembly checkpoint, as well as mutations in p53, Bub1, and Mad3L. Similar cooperativity may also initiate tumorigenesis in ATM-deficient individuals.

It is possible that cancer proneness in the absence of ATM results from a combination of checkpoint and DNA repair defects. ATM has been proposed to play a critical role in the DNA-damage response network by arresting cells in the cell cycle and activating relevant DNA-damage repair genes (97). A-T cells are three to five times more sensitive to agents that

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**Fig. 2.** Model to explain how mutational alterations of the ATM protein might promote carcinogenesis. ATM maintains the integrity of the genome by activating multiple cellular functions that prevent DNA damage from causing genetic instability. A defect in ATM leads to a lack of damage-activated functions that monitor genomic stability resulting in susceptibility to cancer (see text).
generate double-strand breaks, and this sensitivity is in part due to persistence of a higher residual level of double-strand breaks that result from inefficient repair of the double-strand breaks (98). The mechanism by which ATM regulates repair is largely unknown. Recent studies (99,100) in yeasts have provided evidence that the ATM homologue Mec1 is required to mobilize the components of the nonhomologous end joining machinery in response to double-strand breaks. An important component of nonhomologous end joining is the Ku heterodimer (Ku70/Ku80) in Saccharomyces cerevisiae. The Ku heterodimer is constitutively present at the telomeres, along with the telomeric silencing factors termed the “Sir proteins” (Sir2/3/4), and is essential for maintaining telomere integrity [reviewed in (100,101)]. The presence of DNA damage (through in vivo induction of EcoRI activity or a single chromosomal break) releases Ku70/Ku80 and Sir proteins from telomeres in S phase and specifically relocates them to the site of DNA damage (99). Importantly, this relocation is dependent on the checkpoint proteins Mec1 and Rad9. Thus, in this model, recognition of DNA damage by Mec1 and Rad9 is required before recruitment and relocation of Ku70, Ku80, and Sir proteins from telomeres to double-strand breaks. A similar mechanism may implicate ATM in nonhomologous end joining in mammalian cells.

Genomic instability observed in the absence of ATM may partly arise from alterations of telomere structure and enhanced rates of recombination or vice versa. It has been shown that lack of functional ATM in A-T cells leads to telomere shortening and telomeric fusions (102). This observation is quite important because of the high degree of homology between the 3' end of the ATM complementary DNA and the yeast Tel1 gene (103,104). Mutation of the Tel1 gene in yeast reduces telomere length and, just like ATM inactivation, elevates aneuploidy and recombination rates (105). Although the precise mechanism by which ATM maintains telomere length is not yet understood, it is clear that lack of ATM leads to enhanced rates of recombination. Because recombination-based mechanisms have been proposed to have a role in the control of telomere length, an antirecombinogenic role of ATM in controlling the stability of long stretches of repetitive telomeric tracts is possible. Moreover, a lack of ATM might affect the chromatin structure, reducing the accessibility of telomeric DNA to telomerase or other proteins involved in telomeric length maintenance. It is also conceivable that ATM may bind a regulator of telomere length. It is possible that loss of telomere function and a dysfunctional p53 pathway might cooperate to initiate the transformation process in the absence of functional ATM.

**Future Directions**

Although recent studies provide evidence for a preponderance of ATM inactivation in various cancers, the degree to which the cancer risk is increased in A-T heterozygotes will require further studies. Intriguing differences in the pattern of mutation of the ATM gene in A-T compared with cancers (e.g., T-cell prolymphocytic leukemia and B-cell chronic lymphocytic leukemia) have been reported. These studies suggest that, in general, ATM missense variants in the germline do not give a recognizable A-T phenotype, although they may substantially elevate the risk of cancer. However, a more comprehensive analysis of ATM variants is needed by molecular epidemiologic studies using case–case, case–control, or cohort designs to estimate precisely the risk of cancer associated with missense variants. The use of an appropriate study design is crucial, and consideration should be given to gene–gene and gene–environment interactions. It is likely that certain environmental exposures, such as exposure to radiation, may have a greater effect on certain ATM genotypes/variants. If this is the case, the importance of radiation exposure as a risk factor should be examined in epidemiologic analyses that are stratified by genotype. Clearly, much additional research is needed to address these questions, and these issues can be resolved as considerable advances have been made for the detection of mutations in ATM gene.

We are now much closer to elucidating the unifying role of ATM in DNA-damage response, maintenance of genetic stability, regulation of multiple cell cycle checkpoints, and modification of cellular responses to ionizing radiation. These studies should greatly enhance our understanding of these fundamental biologic processes and should shed light on the molecular basis of proneness to cancer. Loss of ATM decreases the genetic stability of malignant cells but, at the same time, affects the sensitivity of malignant cells to radiation and thereby could provide a therapeutic gain. For example, inactivation of ATM could sensitize tumor cells to ionizing radiation and radiomimetic drugs, like cells from A-T homozygotes, and eventually it may be possible to develop individualized treatment strategies that are tailored to exploit the differences between a patient’s malignant cells and normal somatic cells. New drugs could also be screened for their ability to exploit the differences between normal cells and malignant cells in cell cycle checkpoints and DNA repair.

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Notes

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