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ATM: from gene to function

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The identification of *ATM*, the gene responsible for the pleiotropic recessive disease ataxia telangiectasia, has initiated extensive research to determine the functions of its multifaceted protein product. The ATM protein belongs to a family of protein kinases that share similarities at their C-terminal region with the catalytic domain of phosphatidylinositol 3-kinases. Studies with ataxia telangiectasia (A-T) cells and Atm-deficient mice have shown that ATM is a key regulator of multiple signaling cascades which respond to DNA strand breaks induced by damaging agents or by normal processes, such as meiotic or V(D)J recombination. These responses involve the activation of cell cycle checkpoints, DNA repair and apoptosis. Other roles outside the cell nucleus might be carried out by the cytoplasmic fraction of ATM. In addition, ATM appears to function as a 'caretaker', suppressing tumorigenesis in specific T cell lineages.

ATAXIA TELANGIECTASIA AND THE *ATM* GENE

Ataxia telangiectasia

Genetic disorders often flag unknown proteins and provide initial clues to their presumed functions. Identification of a culprit gene and subsequent analysis of its product, coupled with the creation of the corresponding animal model of the disease, may illuminate a novel physiological pathway. Ataxia telangiectasia (A-T) is a prominent example of a genetic disorder whose investigation currently is leading to the elucidation of a central junction of multiple signaling pathways that play major roles in the biology of the cell.

A-T (MIM 208900) is an autosomal recessive disorder expressed as an extensive combination of somatic and physiological defects (for recent reviews see refs 1–3). The clinical hallmark of the disease is progressive neuromotor dysfunction resulting from several neuropathological processes dominated by gradual cerebellar cortical atrophy. Telangiectases (dilated blood vessels) in the eyes and sometimes on the facial skin are typical, but show variable appearance. Additional features are thymic degeneration, immune deficiency, recurrent sinopulmonary infections in some patients, retarded somatic growth, premature aging, gonadal dysgenesis, extreme predisposition to lymphoreticular malignancies and acute sensitivity to ionizing radiation. A-T carriers were suspected to be cancer-prone.

A-T cells

The cellular phenotype of A-T points to a complex defect in handling DNA discontinuities formed either by specific types of externally inflicted damage or by normal processes such as the maturation of the immune system genes or meiotic recombination. A-T cells show increased chromosomal instability, extreme sensitivity to ionizing radiation (IR) and various radiomimetic chemicals, and defective activation of several signal transduction pathways which normally are induced by these agents.

The best documented defects are in the radiation-induced cell cycle checkpoints (reviewed in refs 4–6). A-T cells do not arrest at the G_1 phase of the cell cycle in response to DNA damage. The typical stabilization of the p53 protein, a major activator of the G_1/S checkpoint and several other damage-induced pathways, is retarded in A-T cells, which in turn delays the activation of downstream genes (7,8). A-T cells show less inhibition of DNA synthesis in S-phase upon irradiation, displaying radioresistant DNA synthesis (RDS). These cells are also deficient in the IR-induced phosphorylation of the replication protein A (RPA) (9,10). RPA phosphorylation has been thought to play a role in the S-phase checkpoint in response to DNA damage, although a dissociation between deficient phosphorylation of RPA and RDS suggests that RPA may not be involved in this checkpoint (11). In keeping with the absence of the typical IR-induced G_2 delay in A-T cells, the kinase activity of the cyclin B–cdc2 complex is not attenuated at short times post-irradiation in these cells (12,13). Other radiation-induced responses defective in A-T cells are stimulation of the stress-activated protein kinase (SAPK) (14) and activation of the c-Abl tyrosine kinase (15,16).

Several studies have shown that the overall kinetics of DNA strand break repair in A-T cells is not deficient (reviewed in ref. 17). However, a recombination-based mechanism responsible for repair of double strand breaks (DSBs) seems to be defective in A-T cells (18,19). This might account for the higher residual amounts of unrepaired strand breaks detected in irradiated A-T cells (20 and references therein).

Critical examination of the A-T cellular phenotype, as well as results of *in vitro* manipulations of A-T cells, indicated that the radiosensitivity of these cells cannot be attributed to the cell cycle checkpoint defects (see refs 21,22 and references therein). Rather, a defect in the recognition or repair of a subclass of DSBs might underlie at least part of this hypersensitivity (20,22,23).

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A significant feature of the genomic instability of A-T cells is increased frequency of end-to-end chromosomal associations and reduced telomere length in A-T cell lines and peripheral lymphocytes. However, telomerase activity in these cells is normal (24–26).

It is important to note that not only DNA damage-induced pathways seem to be defective in A-T cells. Khanna *et al.* (27) observed a general defect in signaling pathways induced by cross-linking the B-cell receptor of A-T lymphoblasts *in vitro*. Kondo *et al.* (28) described defective calcium-dependent signal transduction in T cells of A-T patients, and Rhodes *et al.* (29) recently reported defective activity of potassium ion channels in A-T fibroblasts. These observations point to impairment of functions associated with cytoplasmic and membranal organelles in A-T cells, and call attention to the possible roles of the protein defective in A-T in these cellular compartments.

The *ATM* gene

The gene responsible for A-T, *ATM*, was identified using the positional cloning approach (30,31). This gene occupies 150 kb on chromosome 11q22–23, and contains 66 exons encoding a 13 kb transcript. Numerous *ATM* transcripts were identified, all of which share the same 9.2 kb open reading frame while exhibiting a variety of 5′-untranslated regions (5′-UTRs) formed by complex alternative splicing, and several alternative 3′-UTRs (32). The significance of the multiple 5′-UTRs is still unclear, but may point to the regulation of ATM protein levels by post-transcriptional mechanisms. The *ATM* gene shares a bidirectional promoter with another gene, *NPAT/E14/CAN3* (33–35). Interestingly, the *NPAT* gene recently was found to encode a substrate of the cyclin E–CDK2 kinase which is involved in the regulation of S-phase entry (36).

Ectopic expression of a full-length *ATM* cDNA complements many features of the cellular A-T phenotype (37,38), while the corresponding antisense construct imposes an A-T-like phenotype in normal cells (39). Similarly, expression of the C-terminal portion of ATM, which contains the putative catalytic domain, complements certain features of the phenotype of A-T cells, while overexpression of ATM fragments in non-A-T cells induces a dominant-negative effect creating features of the A-T phenotype (40). On the other hand, inactivation of the corresponding mouse gene, *Atm*, creates an organismal and cellular phenotype which, by and large, recapitulates the human disorder (see below). Thus, *ATM* is clearly the gene responsible for the A-T phenotype.

THE ATM PROTEIN: A BIG MOLECULE WITH MANY FUNCTIONS

ATM's physical characteristics and cellular location

The predicted ATM protein contains 3056 residues, and its C-terminal region of ~400 amino acids is highly similar to the catalytic subunit of phosphatidylinositol 3-kinases (PI 3-kinases). This PI 3-kinase-related region is common to a series of large proteins identified in various organisms, which are involved to different extents in cell cycle progression, cellular responses to DNA damage and maintenance of genome stability (for a recent review see ref. 41). An extensively studied member of this family is the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), which is involved in the processing of DSBs (reviewed in ref. 42). Another human member of this protein family is the ATR/FRP1 protein, encoded by a gene on chromosome 3q22–23, which is the homolog of the Rad3 protein of *Schizosaccharomyces pombe* (43,44). No human phenotype has been associated with either protein, but deficiency of DNA-PKcs in the mouse leads to the severe combined immune deficiency (SCID) phenotype (reviewed in ref. 42). Despite the resemblance of all members of this family to lipid kinases, it is believed that all or most of them are protein kinases of the serine-threonine type. This activity is clearly characteristic of DNA-PK (42) and FRAP/mTOR/RAFT1, the mammalian homolog of the *Saccharomyces cerevisiae* TOR proteins (45,46; reviewed in ref. 47). There is also evidence of associated protein kinase activity for ATR (48). ATM's catalytic activity is discussed below.

ATM is a 370 kDa highly phosphorylated protein which is constitutively expressed in numerous tissues, but is present at elevated levels in testis, spleen and thymus of adult mice (49). Although the relative ATM level is not high in adult mice brains, expression of *ATM* mRNA is highest in the embryonic mouse nervous system, primarily in regions undergoing mitosis (49,50).

ATM resides primarily in the nucleus, however, with varying amounts identified in microsomal fractions (49,51–53); Watters *et al.* (53) showed that ATM is associated with cytoplasmic vesicles. Some of these vesicles have been identified as peroxisomes, subcellular organelles involved in oxidative metabolism (M. Lavin, personal communication). Interestingly, ATM's levels and cellular distribution are not altered following treatment with ionizing radiation or at different phases of the cell cycle (51–53). Recombinant ATM was produced in insect and human cells (37,38,54). Similarly to endogenous ATM, most of the recombinant protein is found in the nucleus, in keeping with its ability to complement the defective A-T response to DNA damage (37,38).

The ATM protein plays an essential role in meiosis, and its absence in A-T patients and Atm-deficient mice results in complete absence of germ cells (55–57). Spermatocytes of these mice show meiotic arrest in early prophase I, followed by severe chromosome fragmentation (57). Both ATM and ATR are expressed at high levels in mouse testes (48,49), and they were shown to associate with meiotic chromosomes. ATR is seen as discrete foci along unpaired (asynapsed) axial elements, whereas ATM is found along synapsed chromosomal axes (48). RPA and ATM co-localize along the synaptonemal complex, the specialized structure in which meiotic recombination occurs, implying a possible functional interaction between these two proteins (58).

Another protein, Chk1, also plays an important role in the response of cell cycle checkpoints to DNA damage and, like ATM and ATR, it is highly expressed in testes (reviewed in refs 59,60). The association of Chk1 along meiotic chromosomes and its accumulation during prophase I of spermatogenesis are dependent upon a functional *ATM* gene product (61). ATM is also required for the proper assembly of Rad51 onto the chromosomal axial elements during meiosis (62).

ATM's catalytic activity

Most or all members of the PI 3-kinase-related family are expected to be serine-threonine kinases, with DNA-PK being a paradigm. Protein kinase activity was reported to be associated with immunoprecipitates obtained with anti-ATM antibodies (48). Jung *et al.* (63) reported the phosphorylation of the NF-κB

inhibitor, IκB-α, by such activity. The biological significance of this observation is not clear.

An essential control in such studies is a kinase-defective ATM, which would rule out co-immunoprecipitation of another kinase with the ATM protein. Such controls recently were obtained by S. Banin *et al*. (submitted for publication) and C.E. Canman *et al*. (submitted for publication), using patient cell lines and recombinant ATM in which inactivating mutations had been induced. ATM-specific protein kinase activity *in vitro* was found in these studies to be directed against the translation regulator PHAS-I (64) and the p53 protein. Importantly, the major site of phosphorylation by ATM on p53, *in vitro*, is a serine residue at position 15, whose phosphorylation *in vivo* is induced by treatment of cultured cells with ionizing radiation (65,66). The kinetics of this phosphorylation are delayed in A-T cells (65). ATM's kinase activity is enhanced within several minutes following treatment of the cells serving as ATM source with IR or radiomimetic chemicals, while the level of the protein remains unchanged (S. Banin *et al*., submitted for publication; C.E. Canman *et al*., submitted for publication). The mechanism of this activation is not clear yet.

ATM's interactions

The catalytic site of ATM is believed to reside within the C-terminal PI 3-kinase-related domain, while the rest of this big molecule is probably responsible for receiving the stimuli for its activity and identifying downstream targets. Several proteins have been shown to interact with ATM. Watters *et al*. (57) provided evidence for *in vivo* interaction between ATM and p53. This interaction and the ability of ATM to phosphorylate p53 *in vitro* suggest that ATM activates p53 directly, thereby triggering the various p53-dependent signaling pathways in which both proteins participate.

Baskaran *et al*. (15) and Shafman *et al*. (16) demonstrated interaction between ATM and the protein product of the c-*abl* proto-oncogene. This non-receptor tyrosine kinase, which is involved in several stress responses including activation of the SAPK (67), binds to a specific proline-rich motif in ATM via its SH3 domain (16). Baskaran *et al*. (15) also found evidence for phosphorylation and activation of c-Abl by the PI 3-kinase fragment of ATM. Thus, the identification of c-Abl, itself a junction of several signaling pathways, as a downstream target of ATM demonstrates how ATM can serve as a master controller of numerous processes.

This observation was extended recently (E. Lee, personal communication) by the finding of a trimolecular complex between ATM, c-Abl and the human RAD51 protein, a homolog of the bacterial RecA protein involved in strand break repair and recombination (for a recent review see ref. 68). These investigators further observed that the increase in tyrosine phosphorylation on Rad51, presumably by c-Abl, and its association with the Rad52 protein are also ATM dependent. These findings support the notion that ATM is involved in actual repair and recombination processes.

A somewhat unexpected ATM-interacting protein was identified recently by Lim *et al*. (69): β-adaptin, a component of the AP-2 adaptor complex which is involved in clathrin-mediated endocytosis of receptors. The interaction, which was confirmed *in vivo* and *in vitro*, points to at least one function of the cytoplasmic fraction of ATM, possibly in vesicle and/or protein transport. This finding sheds new light on the previously observed association of ATM with cytoplasmic vesicles (53). Lim *et al*. (69) also identified an *in vitro* interaction between ATM and β-NAP, a neuronal homolog of β-adaptin thought to be involved in synaptic vesicle transport in neuronal cells. These authors suggested that this interaction could explain at least some of the neuropathological features of A-T.

ATM MUTATIONS AND GENOTYPE–PHENOTYPE CORRELATION

Over 250 *ATM* mutations have been identified to date in A-T patients (reviewed in ref. 70, see also refs 71–73; a web site created by P. Concannon and R. Gatti contains a recent list of these mutations: http://www.vmmc.org/vmrc/atm.htm). Most of these mutations are private, with different extents of founder effects identified among Moroccan-Jewish, Costa Rican, Norwegian, Polish and British Isles patients (72–75). Despite the considerable variability in the severity of the clinical features among patients with the classical A-T phenotype, most of the numerous different mutations seem to be quite uniform in outcome: the large majority truncate the ATM protein or leave large deletions in it. In fact, the truncated ATM derivatives appear to be very unstable, leaving no protein product of the *ATM* gene in these patients (51; R. Khosravi *et al*., unpublished data). This mutation profile indicates that the typical A-T phenotype is defined by null *ATM* alleles, while mutations with less severe effects on the protein might cause other phenotypes (76).

Milder variants of A-T exist, although they are difficult to identify at early ages, and their definition as 'variants' is often retrospective. The mutations identified in such cases are either very small in-frame deletions or leaky splicing mutations which allow correct splicing part of the time, leading to considerably reduced but still detectable levels of ATM rather than complete absence of the protein (77,78).

These observations form the basis for easy and rapid diagnosis of A-T and A-T variants. Since the ATM level is reduced to various extents in almost all patients with these disorders, western blotting analysis can provide a firm diagnosis long before it is established by other laboratory tests.

ATM: A TUMOR SUPPRESSOR GENE?

Extensive efforts are underway to elucidate the possible role of the *ATM* gene in cancers among the general population. While the presumed cancer predisposition of A-T carriers is still an open question, recent studies have implicated *ATM* as a tumor suppressor gene in at least one malignancy, T-prolymphocytic leukemia (T-PLL) (79–82). These studies were initiated in view of the high frequency of this rare malignancy among A-T patients (83). Tumor tissues from patients with this malignancy, who are unrelated to A-T families, show somatic inactivation of both *ATM* alleles by rearrangements or point mutations in >50% of the cases. Interestingly, patients in whom the constitutional genotype was tested were not A-T carriers, indicating that in these patients both events leading to *ATM* inactivation appear to occur in somatic cells (82; M. Yuille, personal communication). These findings, together with the extreme propensity of A-T patients to lymphoreticular malignancies, support the possible role of *ATM* as a tumor suppressor gene and tie the biology of T-PLL to ATM functions.

ATM-DEFICIENT MICE

Mutant mice in which the *Atm* gene was disrupted through homologous recombination were created in several laboratories, and have already proved extremely useful in clarifying some of the functions of the ATM protein. These mice are viable and display most of the pleiotropic features of the A-T phenotype (56,57,84,85; Y. Gu, personal communication).

Growth defects

Atm-deficient mice exhibit growth retardation. Although their growth rate appears normal, they are 10–25% smaller and weigh less than wild-type or heterozygous littermates of the same sex, and this difference persists through nursing, weaning and adulthood (56,57,84).

Unlike normal mouse embryo fibroblasts (MEFs) explanted into culture, cells derived from tails or embryos of homozygous mutant mice proliferate very slowly and reach a senescence-like growth arrest after only a few passages (56,86). Similarly to primary fibroblast cell lines derived from A-T patients, $Atm^{-/-}$ MEFs display high levels of chromosomal breaks (84). In addition, these cells exhibit increased constitutive levels of the cell cycle inhibitor $p21^{WAF1/CIP1}$ (hereafter, p21) (86,87), which is normally up-regulated by p53 as part of the growth arrest response in the G_1 phase following DNA damage (88).

Atm/p53 and Atm/p21 double null MEFs proliferate rapidly and do not exhibit the premature growth arrest observed in Atm single null MEFs (87,89). In addition, Atm/p53 double null MEFs show undetectable levels of p21 (87). Taken together, these observations suggest that p21 is activated in a p53-dependent manner in Atm-deficient MEFs, and activation of the p53–p21 pathway contributes to the growth retardation observed in mouse fibroblasts lacking functional Atm, probably due to the accumulation of unrepaired DNA damage.

Meiotic defects

Both male and female Atm-deficient mice are infertile. Although they have grossly normal reproductive organs, the gonads of both sexes are extremely small and show a complete absence of mature gametes (56,57,84). Analysis of spermatogenesis in these mice revealed a meiotic arrest at the zygotene–pachytene stage of prophase I due to abnormal chromosomal synapsis and subsequent chromosomal fragmentation, resulting in apoptotic degeneration and meiotic disruption (57,62). Interestingly, the time when the initial disruption of meiotic events seems to occur in $Atm^{-/-}$ spermatocytes correlates with the association of the Atm protein along synapsed chromosomal axes of homologous chromosomes (48). Moreover, assembly of Rad51 on chromosomal axial elements during early prophase I of meiosis is defective in Atm-deficient mice (62).

These observations suggest involvement of Atm in the repair of DNA breaks that normally occur during meiotic recombination. Lack of functional Atm compromises the integrity of synapsed meiotic chromosomes, resulting in chromosome fragmentation and meiotic failure. Unrepaired DNA breaks might be the trigger for the higher baseline levels of p53 and its downstream genes, p21 and Bax1, which are observed in testes of Atm-deficient mice (62).

A partial rescue of meiosis was observed in Atm/p53 and Atm/p21 double null mice. These animals are still infertile and lack mature sperm, but testes of male double mutants are larger than those of Atm single null mice, although they do not reach the size of wild-type testes. Accordingly, the degree of apoptosis detected in seminiferous tubules from double null mice was considerably reduced, and spermatogenesis was improved, progressing through later stages of meiosis. No normal pachytene synaptonemal complexes were seen in spermatocytes of Atm-deficient mice, but nearly normal pachytene complexes were observed in double null spermatocytes, although later stages of meiosis were not achieved. The Rad51 assembly defects were not rescued in spermatocytes of double mutants (62).

Surprisingly, the basal levels of p21 and Bax in testes of Atm/p53 double mutant mice were as high as those in $Atm^{-/-}$ mice. This suggests that in these cells, as opposed to MEFs, p21 can be activated via a p53-independent pathway. Basal levels of p53 and Bax in testes of Atm/p21 double null mice were also high. These higher baseline levels were dependent on loss of Atm, since p53 and p21 single mutants show no basal elevation in the expression of these gene products (62).

Immunologic abnormalities

Although the structure of lymphoid organs from $Atm^{-/-}$ mice is histologically normal, they are generally smaller than those of normal littermates, and the number of thymocytes is considerably reduced (56,57,84). Atm is probably required for T-cell development, since its absence leads to a marked reduction in the relative number of mature thymocytes, in particular $CD4^+$ single positive thymocytes (56,57; Y. Gu, personal communication). A defective maturation of thymocytes probably predisposes to the large reduction of mature lymphocytes in the peripheral lymphoid organs of $Atm^{-/-}$ mice (56,57). A decrease in the number of pre- and immature B cells in the bone marrow of $Atm^{-/-}$ cells was observed as well, although the composition of mature peripheral B cells was not affected (56,57,84; Y. Gu, personal communication).

The abnormal maturation of lymphocytes in $Atm^{-/-}$ mice most likely results from defective processing of V(D)J recombination. Atm activity might be required to halt the cell cycle until completion of V(D)J recombination, which appears to be restricted to the G_0/G_1 phase (90). In the absence of Atm, lymphocytes could enter S phase prematurely, leading to a decreased frequency of productive V(D)J recombination, and eventually to a reduction in mature lymphocytes.

Cancer predisposition

$Atm^{-/-}$ mice develop aggressive malignant thymic lymphomas and usually succumb to the disease by 4 months of age (56,57,84,91). In a recently developed Atm-deficient mouse model, the average onset of such tumors was considerably later, with >50% of these animals surviving 10 months or longer (Y. Gu, personal communication). The difference in cancer susceptibility between these and previously reported Atm-deficient mice is not clear, but might be due to a different genetic background or a different environment.

The tumors in Atm-deficient mice invariably develop from clonal expansions of immature $CD4^+/CD8^+$ double positive thymocytes that bear translocations involving chromosome 12 or 14, near the T cell receptor (TCR) loci (56,57; C. Barlow, personal communication). These findings are very similar to those observed in lymphoid tumors of A-T patients (83).

Defective processing of V(D)J recombination in $Atm^{-/-}$ thymocytes may contribute to an increased frequency of chromosomal translocations involving these loci, leading eventually to deregulated expression of proto-oncogenes and tumorigenesis (57). In support of this idea, loss of RAG2, which abolishes V(D)J recombination, resulted in a complete absence of T cell lymphomas in Atm-deficient mice (C. Barlow, personal communication; Y. Xu, personal communication).

p53 null mice, like Atm null mice, primarily develop T cell lymphomas (92). While many Atm or p53 null animals survive beyond 100 days of age, Atm/p53 double null mice die before 70 days of age and the spectrum of tumors in these animals is altered, with appearance of B cell lymphomas, sarcomas and teratomas, in addition to T cell lymphomas. Thus, loss of p53 in an Atm null background dramatically accelerates tumorigenesis (91). However, loss of p21 delays the onset of lymphomas in Atm-deficient mice, probably because of the higher levels of apoptotic cells present in tumors from Atm/p21 double null mice (89).

Radiation responses

Mice homozygous for Atm disruption display acute radiation sensitivity, which is manifested selectively in certain tissues, including the gastrointestinal tract, salivary glands and skin. Irradiated mutant mice die rapidly from gastrointestinal toxicity, rather than bone marrow failure, following exposures that have no deleterious effects on control mice (56,91). Radiation resulted in a similar depletion of the lymphoid system in both types of mice, but the gastrointestinal tract of Atm mutant mice displayed severe degeneration (56,91), and their skin showed radiation dermatitis and hair loss not observed in control mice (91). Increased radiation sensitivity of salivary gland tissues was also noted (56).

Loss of p53 in an Atm null background does not alter the radiation sensitivity further, suggesting that the radiosensitivity observed in $Atm^{-/-}$ mice is most likely not mediated by p53 (91). In agreement, p53 null mice are not radiosensitive (91,93). Surprisingly, loss of p21 in Atm-deficient mice increases their sensitivity to IR. p21 single null mice show a certain degree of sensitivity to radiation due to toxic effects on their intestines, but their radiosensitivity is not nearly as acute as that of Atm null animals. It seems then, that p21 and Atm normally cooperate in preventing radiation-induced toxicity of the intestinal epithelium, so that the absence of both proteins leads to an accelerated radiation toxicity (89).

Defects in the activation of the G_1/S and S phase checkpoints by irradiation were detected in Atm-deficient fibroblasts, embryonic stem cells and thymocytes (56,86,87,94). Concordantly, the up-regulation of p53 in response to IR, but not to UV, was defective in $Atm^{-/-}$ MEFs (86). The IR-induced G_1/S checkpoint of MEFs deficient for both Atm and p53 is as impaired as in MEFs lacking either gene, suggesting that Atm and p53 function in a common pathway mediating the activation of the G_1/S checkpoint in response to IR (87).

Detailed studies of the apoptotic response of Atm-deficient thymocytes showed them to be as sensitive as controls after irradiation *in vivo* (56) or *in vitro* (84). However, a high percentage of immature $CD4^+/CD8^+$ double positive thymocytes, which are the most susceptible to apoptosis in wild-type mice, were found to be radioresistant (86). This may account for the partial resistance of $Atm^{-/-}$ thymocytes to radiation-induced apoptosis reported by Westphal (91).

p53 has been shown to be required for the activation of apoptosis after DNA damage in cultures of thymocytes (95,96), cerebellar neurons (97) and hippocampal and cortical neurons (98). Thymic apoptosis is inhibited in p53 null mice (96). In stark contrast, thymi of Atm null mice are as sensitive as controls (85,94). Absence of p53 most likely accounts for the radioresistance of Atm/p53 double null thymi, while those of Atm/p21 mice display a normal apoptotic response. Radiation-induced apoptosis in the lung of wild-type, Atm and p53 single null mice was similar (94).

In contrast to the thymus, pronounced resistance to radiation-induced apoptosis was observed in the developing central nervous system (CNS) of Atm null mice, including the cerebellum (85). p53 null mice show a similar lack of radiation-induced apoptosis in the developing CNS (85,99). Consistent with a requirement for p53 in Atm-dependent apoptosis, p53 stabilization in the thymus and developing CNS coincided with cell death (85). Interestingly, certain regions of the CNS of Atm null mice exhibit a normal apoptotic response (85). These regions were the subventricular zone of the lateral ventricle and the marginal zone of the developing retina. Notably, these regions contain relatively undifferentiated multipotent precursor cells, whereas the regions of the CNS of $Atm^{-/-}$ mice that were radioresistant contain neuroblasts that generally give rise to a single lineage. This suggests that Atm function following irradiation might become apparent as neuronal cells move towards a more differentiated stage (85).

Neurodegeneration

Unlike A-T patients, Atm-deficient mice are not grossly ataxic. However, the performance of these mice was impaired in several motor function tests, such as the rota-rod and the open-field tests (56), as well as motor learning on an accelerating rotating rod (R. Segal, personal communication). Furthermore, the hind-paw footprint analysis showed that these mice had significantly shorter stride lengths than controls, and less consistency in their stepping patterns (56). These results are indicative of neurological deficiencies, although no histological abnormalities were observed in the brains of Atm-deficient mice in most laboratories that analyzed them (56,57,84; P. McKinnon, personal communication). However, electron microscopic evidence for neuronal degeneration in mutant mice was reported by Kuljis *et al*. (100), who noticed degeneration of granule cells, Purkinje neurons and molecular layer neurons in the cerebellar cortex. Other alterations in their cerebellum included glial activation, deterioration of neuropil structure, and both pre- and postsynaptic degeneration. The reason for the discrepancy between these findings and those of other laboratories, is not clear. Abnormal morphology and location of Purkinje cells, as well as thinning of the cerebellar molecular layer, were observed in Atm-deficient mice developed in one laboratory (Y. Gu and R. Segal, personal communication). As mentioned before, these particular Atm mutant mice survive longer due to a later onset of lymphomas, allowing the analysis of age-dependent progression of the neurological deficits. The abnormalities described above did not progress with age in these mutant animals.

A selective loss of dopaminergic neurons restricted to the striatum was observed recently in Atm-deficient mice (R. Eilam *et al*., submitted for publication). The reduction in nigrostriatal neurons was reflected in the supersensitivity of these mice to

treatment with the dopaminergic drug amphetamine. Treatment of these mice with the dopamine precursor L-DOPA, which has been shown to enhance dopaminergic transmission, corrected the stride-length asymmetry observed in the animals (R. Eilam *et al*., submitted for publication).

CONCLUSIONS

ATM is involved in the cellular response to DNA breaks at several levels, including cell cycle checkpoint activation, DNA repair and induction of apoptosis. It most likely acts as a sensor for a specific type of strand break, such as those created by free radicals or during DNA recombination, subsequently activating several key regulators of the DNA damage response and triggering multiple signaling cascades.

Cells that normally maintain certain levels of DSBs, such as those created during meiotic or V(D)J recombination, are likely to be more sensitive to the absence of this function. The abnormal chromosomal synapsis and subsequent fragmentation that lead to meiotic arrest in Atm-deficient mice can be explained in this context. Similarly, defective processing of V(D)J recombination intermediates in A-T patients and Atm-deficient mice would result in abnormal maturation of lymphocytes on the one hand, and in increased frequency of translocations involving the TCR locus on the other, eventually resulting in clonal expansion of immature thymocytes and the development of thymic lymphomas. The rescue of the predisposition for these tumors in Atm/RAG2-deficient mice supports this notion.

RAD51 is involved in DNA DSB repair, and physically associates with proteins that participate in this type of repair, such as BRCA1, BRCA2 (reviewed in ref. 101) and ATM (E. Lee, personal communication). Mice homozygous for null mutations in Rad51, Brca1 or Brca2 display early embryonic death. Similarly to loss of Atm function, the absence of Brca1, Brca2 or Rad51 results in reduced levels of cellular proliferation, radiation hypersensitivity, genomic instability and elevated baseline levels of p21 (in the case of Brca1 and Brca2 deficiencies) (reviewed in refs 102,103).

Mutations in either p53 or p21 partially rescue the embryonic lethality of Rad51, Brca1 or Brca2 mutants (102), as well as the defective spermatogenesis of $Atm^{-/-}$ mice (62). Defects in DNA damage repair would result in massive DNA strand breaks during meiotic recombination or as a consequence of the frequent mitotic events that typify early embryogenesis, activating a p53/p21-mediated growth arrest. Relief of this arrest by disruption of either p53 or p21 rescues the defective processes only partially, since the DNA breaks remain unrepaired and are incompatible with normal completion of embryogenesis or meiosis.

The recent development of viable Brca2 mutant mice is of particular significance in the analogy between Brca2 and Atm. These animals, which probably have only a partial loss of function of Brca2, exhibit a wide range of defects; most of them show a striking resemblance to those of Atm-deficient mice, such as growth retardation, absence of mature gametes, development of thymic lymphomas, defective proliferation of MEFs and overexpression of p53 and p21 (104).

Complete inactivation of the *Prkdc* gene in mice, which encodes DNA-PKcs, results in profound immune deficiency and a strong predisposition for thymic lymphomas (105). Scid mice, which carry a nonsense mutation at the 3′ end of the *Prkdc* gene, have only a low incidence of lymphomas (106). These mice and cells derived from them are highly radiosensitive. The hypersensitivity and cancer susceptibility observed in the case of DNA-PK deficiency must stem from defects in repair of DSBs, since IR-induced cell cycle checkpoints and apoptosis are normal in Scid cells and mice, and in other cell lines lacking DNA-PK activity (42).

The phenotype of Atm null mice and humans suggests that, unlike Rad51, Brca1 and Brca2, Atm is not required for repair of DNA damage during embryonic development, but is crucial during meiosis, and plays an important role during lymphocyte maturation. DNA-PK is essential for V(D)J recombination but is not required for embryogenesis or meiosis.

In addition, like ATM, DNA-PK functions as a tumor suppressor of T cell lineage. These two proteins, as well as the cancer susceptibility gene products BRCA1 and BRCA2, participate in the DSB repair pathways (101). These gene products and those involved in other types of DNA repair, namely excision repair and mismatch repair, are responsible for maintenance of the genome integrity through DNA repair function. Their loss is expected to result in genetic instability and additional mutations, ultimately increasing the likelihood of neoplasia. Cancer susceptibility genes of this type, which include the *ATM* gene, were defined recently as ‘caretakers’ (107).

Unlike the ‘caretakers’ described above (BRCA1, BRCA2 and DNA-PK, whose association with the DNA damage response is primarily through their role in DNA repair), ATM is also involved in activation of cell cycle checkpoints or apoptosis in response to DNA damage. Like p53, ATM-dependent activation of either pathway probably depends on cell type and degree of DNA damage. ATM most likely acts upstream of p53 in a signal transduction pathway leading to the activation of the G_1/S checkpoint. However, the apoptotic response of different cell types and tissues lacking Atm or p53 is not entirely equivalent. IR-induced apoptosis in most of the CNS seems to depend on both Atm and p53. However, while thymic apoptosis *in vivo* and *in vitro* depends heavily on p53, it is only partially dependent on Atm. Radiation-induced apoptosis of the lung does not depend on either gene, but rather on a ceramide-dependent pathway (108).

The recent observations of defects in receptor function and ion channels in A-T cells, together with ATM’s localization to cytoplasmic vesicles and its interaction with proteins of the β-adaptin family, draw attention to roles for ATM beyond responses to DNA damage. Recently we proposed a more general role for ATM in coordinating acute phase stress responses with cell cycle checkpoint control and repair of oxidative damage to macromolecules, compatible with the broad phenotype of A-T (21). Absence of ATM could limit the repair of oxidative damage that occurs under normal physiological conditions, creating an increased state of oxidative stress which might underlie the premature aging observed in A-T, as well as the preferential loss of sensitive cells, such as thymocytes and certain neurons.

The editorial that accompanied the report of the discovery of the *ATM* gene stated that it ‘sparked a biomedical research bonanza’ (109). The fierce efforts to elucidate the functions of the ATM protein were spurred by the striking A-T phenotype, the involvement of the *ATM* gene in cancer predisposition and the functional ramifications of the ATM protein’s sequence. While these efforts are still far from reaching final conclusions, it is clear at this point that ATM is a key regulator of basic, diverse cellular functions whose complete understanding should have implications for many fields in biomedical research.

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