INTRODUCTION

The rare human autosomal-recessive inherited disorder ataxia telangiectasia has a complex clinical phenotype affecting multiple organ systems in the body. It is characterized by extreme sensitivity to ionising radiation and susceptibility to cancer, immunodeficiency, oculocutaneous telangiectasias, progressive neurodegeneration, growth retardation, developmental abnormalities and premature ageing. Death is usually from lymphomas or respiratory infections; however, the most debilitating aspect of the disease is progressive neurodegeneration particularly of Purkinje and granule cells in the cerebellum, but also including dopaminergic neurons. Fibroblasts derived from ataxia telangiectasia patients are characterised by extreme sensitivity to ionising radiation, failure to arrest the cell cycle after DNA damage, increased chromosome breakage, and telomere end fusions. Both human fibroblasts and cells from Atm null mouse embryos grow poorly in culture and then senesce.

The gene which is defective in this disease, ATM (ataxia-telangiectasia-mutated), codes for a protein kinase (ATM) shown in Figure 1, that participates in multiple signal-transduction pathways, which are activated after DNA double-strand breaks caused by ionising radiation. It has recently been shown that ATM is also activated in response to DNA alkylation. The response of ataxia telangiectasia cells to UV is normal. Several mouse models of the ataxia telangiectasia disease are now available: knockout mice where the ATM protein is completely absent (reviewed by Shiloh & Kastan) and a knockin mouse (Atm ΔSRI) with a mutation found in human patients. This mutation results in the production of near full-length, but inactive (kinase dead), protein albeit at lower than normal levels. These mice provide an excellent model of the human disease; however, there are some differences in neurological features. In contrast to humans, the various mouse models display no overt ataxia or gross neurodegeneration of Purkinje cells; however, there are ectopic cells and cells with abnormal and diminished dendritic arborization. An explanation for these species differences is currently lacking.

In humans, carriers of the disease, while otherwise normal, have an intermediate sensitivity to ionising radiation and an increased susceptibility to cancer and ischaemic
heart disease. Heterozygous mice show increased cataract formation, radiation-induced mammary ductal dysplasia (precursor to mammary cancer), and increased susceptibility to cancer.

Ataxia telangiectasia cells are in a constant state of oxidative stress even in the absence of irradiation. The pleiotropic effects of reactive oxygen species (ROS) have been reviewed by Droge and include signal transduction, ageing/senescence, apoptosis, neuromodulation, and antioxidant gene modulation. Moreover, ROS have been linked to atherosclerosis and several neurodegenerative diseases such as Parkinson’s disease and Alzheimer’s disease. This review will focus primarily on oxidative stress and how it contributes to the phenotype of ataxia telangiectasia.

ROLE OF ATM IN THE DNA DAMAGE RESPONSE

The most studied aspect of ATM function is its role in response to DNA damage. ATM is activated by double-strand breaks and meiotic recombination, and sits at the apex of a range of cellular responses, phosphorylating proteins involved in cell cycle checkpoint control, DNA replication and repair, and apoptosis (Fig. 2). Several substrates of ATM have been identified to date (Fig. 3, reviewed by Kastan & Shiloh). However, the mechanism of activation of ATM itself is still not understood.

ATM exists in a large multiprotein complex, the BRCA-1 associated genome surveillance complex (BASC). This complex includes many proteins involved in the maintenance of genomic stability, which when defective give rise to a number of clinical disorders. These proteins include Mre11 (ataxia telangiectasia-like disorder, ATLD) and NBS-1 (Nijmegen breakage syndrome), Bloom’s syndrome), FANCD2 (Fanconi anaemia), BRCA1/2 (breast cancer susceptibility), MSH2/3/6, MLH1 (mismatch repair proteins). Many of these proteins are also substrates of ATM and exist in a complex with the Rad50 protein (MRN complex) and this complex forms foci after DNA damage involving dsb (reviewed by D’Amours & Jackson). ATM co-localises with the MRN complex and the histone H2AX after ionising radiation and ATM kinase activity is rapidly enhanced. ATM then phosphorylates Mre11, H2AX and NBS-1. BRCA1 is also constitutively associated with the c-Abl tyrosine kinase and this interaction is disrupted by ATM.

Fig. 1. Schematic diagram of the primary structure of the ATM protein indicating motifs and domains. In addition, ATM has multiple phosphorylation sites for various kinases (not shown).

Fig. 2. Central role of ATM in the cellular response to DNA damage.
after ionising radiation. ATM has also been shown to bind to histone deacetylase and could, therefore, control chromatin structure and DNA exposure.

ATM is predominantly present in the nucleus of proliferating cells, in keeping with its role in the response to DNA damage; however, a significant proportion of the protein is present outside the nucleus in cytoplasmic vesicles and peroxisomes. There is evidence that the localisation of ATM depends on the cell type and it is noteworthy that in post-mitotic neurones such as the Purkinje cells, the majority of ATM protein is outside the nucleus in neuronal endosomes. While the DNA damage response functions of ATM are well documented, very little is known about its functions outside the nucleus.

A ROLE FOR ATM IN THE MAINTENANCE OF REDOX BALANCE?

Ataxia telangiectasia cells are in a state of oxidative stress, and are unusually sensitive to treatment with oxidants such as hydrogen peroxide, superoxide anion and nitric oxide. Reichenbach and co-workers reported a diminished antioxidant capacity in the plasma of 10 ataxia telangiectasia patients compared to healthy, age-matched controls. Another study reported that red blood cell membranes from ataxia telangiectasia patients and heterozygotes had increased fluidity, decreased microviscosity and a decreased content of sulphhydryl groups consistent with increased oxidative stress and lipid oxidation. Rotman and Shiloh reviewed the evidence showing constitutive activation of oxidative stress response pathways involving NF-κB and p21 in ataxia telangiectasia cells, and they hypothesised that oxidative stress might underlie the pleiotropic phenotype. The chronic activation of these pathways can be ameliorated by treatment with an antioxidant such as α-lipoic acid.

We have localised a portion of the extranuclear ATM to peroxisomes, organelles involved in redox homeostasis and lipid metabolism. This finding led us to investigate the activity of the major peroxisomal enzyme catalase, which is important in the detoxification of hydrogen peroxide. We found decreased levels of catalase in ataxia telangiectasia fibroblasts compared to control cells and corresponding increased levels of lipid hydroperoxides. There have been several other reports of reduced catalase activity in ataxia telangiectasia cells as reviewed by Rotman and Shiloh and also reported by Takao et al. We, and others, have found no change in the level of catalase protein in ataxia telangiectasia cells or tissues from Atm−/− mice. Furthermore, catalase does not appear to be a substrate for ATM despite the presence of an ATM consensus phosphorylation sequence, LSQE (Watters et al., unpublished observations). It has been reported that the decreased activity of catalase in SV40-transformed fibroblasts and cells from xeroderma pigmentosum patients is related to decreased levels of the cofactor, NADPH.
Oxidative damage has been demonstrated in specific neuronal populations in Atm−/− mice, particularly the Purkinje cells in the cerebellum and the striatum; however, cortical neurones and hepatocytes appear unaffected. In addition, elevated levels of superoxide were found in the affected neuronal populations and increased levels of hemoxgenase 1 (HO-1) and 3-nitrotyrosine were seen in Purkinje cells of Atm−/− mice. In a detailed study of redox balance in mouse brain, Kamsler and co-workers reported decreased catalase activity in the cerebellum at 4 months but not in the cerebrum, as well as elevated activity of superoxide dismutase (SOD), and thioredoxin, all indicative of oxidative stress. Elevated SOD combined with diminished catalase activity would result in increased levels of intracellular hydrogen peroxide. It was proposed that differences in antioxidant defences between brain regions could determine the sensitivity of different neuronal populations.

Double mutant mice are shedding more light on the role of oxidative stress in the pathology of ataxia telangiectasia. Atm−/− mice engineered to overproduce Cu/Zn SOD (SAT mice) display a marked exacerbation of growth retardation, defective maturation of T cells and radiosensitivity, but no enhancement of the predisposition to thymic lymphomas, indicating that oxidative stress does not contribute to tumorigenesis in the ATM knockout mouse. Similarly to ATM deficient mice, there was also no gross abnormality in cerebellar pathology in the SAT mice.

Several studies support a role for ATM in modulating/sensing oxidative stress; however, the biochemical mechanisms remain to be elucidated. ATM may modulate DNA turnover and control cell fate in lymphocytes by regulating the cellular redox state. Thymocytes and splenocytes are under genomic stress because of rapid recombinational events postnatally. In freshly isolated thymocytes from Atm−/− mice, there is greatly increased thiol redox activity (as measured by reduction of the tetrazolium MTS) and DNA turnover activity ([3H]-thymidine incorporation) compared to Atm+/+ mice. The elevated DNA turnover activity quickly leads to cell death. These activities could be suppressed completely by the permeant thiol, N-acetylcysteine, (NAC). The authors postulate that a primary function of ATM may be to regulate thiol redox activity and thereby suppress the DNA turnover activity.

More reactive oxygen species are generated in ATM−/− DT40 cells than in wild-type DT40 cells following treatment with several apoptotic inducers supporting the hypothesis that ATM has a role in the maintenance of cellular redox homeostasis. ATM-deficient fibroblasts fail to exhibit normal G1 and G2 checkpoint responses following exposure to oxidative stress induced by t-butylhydroperoxide; however, HO-1 induction was normal in ataxia telangiectasia cells in response to this agent. HO-1 is also elevated in Purkinje cells as mentioned above; therefore, some responses to oxidative stress in ataxia telangiectasia cells appear to be normal.

In mammalian systems, the transcription factors NF-κB and AP-1 are involved in regulating the oxidative stress response. Defects in ionising radiation-induced signalling in ataxia telangiectasia include impaired activation of NF-κB and AP-1. ATM is essential for activation of NF-κB after double-strand breaks and this activity is mediated by IkB kinase. ATM is, however, not required for NF-κB activation after pro-inflammatory stimuli.

Lee et al. have analysed c-Jun phosphorylation in ataxia telangiectasia cells in response to ionising radiation- and CdCl₂-induced oxidative stress. They showed that CdCl₂ generates a greater extent of oxidative stress in ataxia telangiectasia cells compared to normal cells, as measured by HO-1 induction, and that ataxia telangiectasia cells were unable to detect ionising radiation-induced oxidative stress but exhibited a hypersensitive response to CdCl₂, presumably because of impaired antioxidant function. The phosphorylation/dephosphorylation cycle of JNK and p38 by the radiomimetic agent neocarzinostatin was attenuated in ataxia telangiectasia cells. Phosphorylation of JNK and p38 was reduced and delayed with a delay also in dephosphorylation.

The ATM-dependent up-regulation of the dual specificity phosphatase of the MAP kinase phosphatase family, MKP-5, which dephosphorylates and inactivates JNK and p38, was demonstrated in that study.

**SIGNAL TRANSDUCTION DEFECTS IN ATAXIA TELANGIECTASIA**

Elevated ROS levels could also disrupt other cellular signalling pathways and there is a growing body of evidence for a more general role for ATM in signal transduction and cellular homeostasis. This includes poor growth and a greater demand for growth factors in cultured ataxia telangiectasia cells, defective response to PHA in ataxia telangiectasia lymphocytes, defective potassium currents in ataxia telangiectasia fibroblasts and calcium-spike bursts in Purkinje cells of Atm−/− mice, and defective signalling through the EGF receptor. Gosink et al. showed that ATM is required for astrocyte growth and that the response of Atm−/− astrocytes to ionising radiation was indistinguishable from normal, suggesting a distinct role for ATM in cell growth in this cell type. ATM is also essential for the normal development, differentiation and survival of adult neural progenitor cells and Atm−/− neural progenitor cells are less able to respond to environmental cues. Yang and Kastan reported that ATM kinase is activated by insulin treatment and phosphorylates 4EBP-1.
leading to the release of eIF-4E with subsequent initiation of mRNA translation for protein synthesis.\textsuperscript{49} It is noteworthy that the 4EBP-1 protein has an entirely cytoplasmic location.\textsuperscript{50} Fibroblasts from ataxia telangiectasia patients also express lower levels of cell surface IGF1R than normal cells.\textsuperscript{51} IGF1R down-regulation is associated with radiosensitivity and impaired activation of ATM kinase in mouse melanoma cells.\textsuperscript{52} Interestingly, the levels of IGF1R in NBS cells are normal.\textsuperscript{53}

The immunological deficiencies in ataxia telangiectasia have been attributed not only to reduced numbers of naïve T cells, but also to defective signalling via the T-cell receptors.\textsuperscript{54,55} Defective activation of protein kinase C in T lymphocytes of ataxia telangiectasia patients has also been observed: proliferation responses were severely impaired when PMA was used as a co-stimulus with pokeweed mitogen, concanavalin A, anti-CD69 or anti-CD26.\textsuperscript{56}

To what extent the signalling and electrophysiological defects in ataxia telangiectasia cells can be attributed to elevated oxidative stress remains to be determined.

\textbf{CAN THE NEUROLOGICAL AND IMMUNOLOGICAL SYMPTOMS BE EXPLAINED SOLELY BY DEFECTIVE DNA DSB REPAIR DURING DEVELOPMENT?}

The lymphopenia in ataxia telangiectasia is most likely the result of defective DNA dsb repair during development; however, the defective activation and cytokine production of T lymphocytes from ataxia telangiectasia patients\textsuperscript{55,56} still requires adequate explanation.

It has been postulated that the neurodegeneration in ataxia telangiectasia is a result of cumulative damage during development, which impacts progressively with age.\textsuperscript{57} In support of this hypothesis is the observation of Stern and colleagues using TUNEL analysis, that DNA damage accumulates progressively in the cerebellum.\textsuperscript{58} The cerebrum, however, recovers from an early onset of DNA breaks. They also found a significant decrease in NAD⁺, NADH and NADP⁺ and NADPH levels in the cerebellum but not the cerebrum of Atm\textsuperscript{-/-} mice. This was interpreted as being due to increased energetic or oxidative stress in ataxia telangiectasia cells. It was also suggested that NAD⁺ depletion stems from progressive accumulation of DNA damage since NAD⁺ is a substrate of PARP, which accumulates early at sites of DNA damage. Interestingly, the inhibition of PARP has been shown to increase the growth rate of ataxia telangiectasia cells.\textsuperscript{59} The reduced NADH levels could also then lead to diminished catalase activity as discussed above. Oxidative stress is known to result from other DNA repair defects, for example, mice heterozygous for mutations of the \textit{Apex} gene, exhibit oxidative stress.\textsuperscript{60} The \textit{Apex} gene codes for apurinic/apyrimidinic endonuclease and mutation leads to a deficiency in base excision repair.

The spectrum of neurological symptoms among the genomic instability syndromes varies, for example patients with Nijmegen breakage syndrome exhibit microcephaly but no progressive neurodegeneration. The \textit{Apex\textsuperscript{+/+}} mice do not exhibit any gross neuropathology, nor do mice with targeted disruption of the NBS gene.\textsuperscript{61} This again highlights the differences between mice and humans in neurological outcomes. It has been suggested that the lack of neurodegeneration in Atm\textsuperscript{-/-} mice is due to the short life-span of mice compared to humans (P. McKinnon, personal communication). The question also still remains as to why the oxidative stress and DNA damage accumulation in ataxia telangiectasia is cell/tissue specific.

The cumulative damage hypothesis also ignores the evidence for other functions of ATM, and the fact that ATM kinase can be activated by other triggers besides double-strand breaks, for example insulin, which likely contributes to nervous system maintenance.

While much has been learnt about ATM function since the discovery of the gene by Shiloh’s group in 1995, there are still many unanswered questions about this complex disease; for example, how ATM is activated, the role of ATM outside the nucleus, specific roles of ATM in the neuronal system, cell type specificity of ATM function, further ATM interacting proteins, the role of ATM in cellular homeostasis and redox balance and in signalling networks, and the aetiology of telangiectasias to name a few.

Oxidative stress undoubtedly plays a significant role in the pathology of ataxia telangiectasia. Preliminary data by Lavin et al. (unpublished) show that antioxidants can enhance the poor dendritic arborization of cultured Purkinje cells from Atm\textsuperscript{-/-} mice. This provides hope that antioxidant treatments may be useful in halting or slowing the progression of neurological symptoms in this devastating disease.

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\textbf{REFERENCES}


