Ataxia-telangiectasia mutated plays an important role in cerebellar integrity and functionality

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Abstract

Accumulating evidence indicates that ataxia-telangiectasia mutated kinase is critical for maintaining cellular homeostasis and that it has both nuclear and cytoplasmic functions. However, the functions of ataxia-telangiectasia mutated that when lost lead to cerebellar degeneration are still unknown. In this review, we first describe the role of ataxia-telangiectasia mutated in cerebellar pathology. In addition to its canonical nuclear functions in DNA damage response circuits, ataxia-telangiectasia mutated functions in various cytoplasmic and mitochondrial processes that are critically important for cellular homeostasis. We discuss these functions with a focus on the role of ataxia-telangiectasia mutated in maintaining the homeostatic redox state. Finally, we describe the unique functions of ataxia-telangiectasia mutated in various types of neuronal and glial cells including cerebellar granule neurons, astrocytes, and microglial cells.

Key Words: ataxia telangiectasia; ATM; cerebellum; DNA damage response; double-strand breaks; mitochondrial dysfunction; oxidative stress; single-strand breaks

Introduction

DNA damage is caused in living organisms primarily by endogenous oxygen radicals produced during metabolism, which induce tens of thousands of DNA lesions per cell per day (Shiloh, 2010). Ataxia-telangiectasia mutated (ATM) is critical responses to genotoxic stresses, maintenance of the cellular redox balance, and regulation of mitochondrial metabolism (Agudo et al., 2022). When ATM is missing, as it is in ataxia-telangiectasia (A-T) patients, modulation of these pathways is defective, which results in a complex, progressive, disease. The aim of this review is to outline the current state of knowledge of the roles of ATM in tissue regeneration and in regulation of the cellular redox state and on the functions of ATM in nuclear and the non-nuclear compartments.

Search Strategy and Selection Criteria

Studies cited in this review were published from 1926 to 2022, with a predominant emphasis from 2017 to 2022 (more than 50%). All studies cited were searched on the PubMed database using the following keywords: ataxia telangiectasia, ATM, cerebellum, glia, microglia, astrocytes, granule neurons, Purkinje neurons, DNA damage response, oxidative stress, mitochondria, neural networks, and metabolism. All studies were cited due to their relevance to the review.

Ataxia-Telangiectasia

A-T (OMIM #209800) is a highly pleiotropic, autosomal recessive disorder (Rothblum-Oviatt et al., 2016) caused by mutations in the gene that encodes the ATM protein kinase (Shiloh, 2020). The first cases of A-T were documented in 1926 (Syllaba, 1926). More than four decades later, in 1958, A-T was described as a familial syndrome with progressive ataxia, oculocutaneous telangiectasia, and frequent pulmonary infection (Boder and Sedgwick, 1958). Many A-T patients develop peripheral neuropathy during the second decade of life. Oculocutaneous telangiectasia, marked immunodeficiency, neutromotor dysfunction, and gonadal and thymic dysgenesis are also typical symptoms. Cancer predisposition is manifested as an increased tendency to develop lymphoreticular malignancies, and various carcinomas appear in older patients [reviewed in (Rothblum-Oviatt et al., 2016; van Os et al., 2017; Levy and Lang, 2018; Amirfar et al., 2019)]. Growth retardation and occasional endocrine abnormalities are also seen, particularly insulin-resistant diabetes. Premature aging has recently been recognized in A-T patients (Tal et al., 2018; Agudo et al., 2022). Major laboratory findings include elevated serum levels of a-fetoprotein and carcinoembryonic antigen.

All A-T patients have mutations in the ATM gene (Savitsky et al., 1995). Various ATM mutations that cause classical and milder forms of A-T have been identified. A-T patients show a striking sensitivity to the cytotoxic effect of ionizing radiation (IR), and ATM-deficient cells exhibit marked chromosomal instability, sensitivity to IR and radiomimetic chemicals, and reduced telomere length (Rothblum-Oviatt et al., 2016). IR sensitivity results from a profound defect in the cellular response to DNA double-strand breaks (DSBs) due to the lack of ATM protein kinase (Rothblum-Oviatt et al., 2016). Chromosomal instability observed in patients is also suggestive of defects in V(D)J recombination (Menolfi and Zha, 2020). Mouse cerebellar cells lacking Atm have also been shown to have impaired mitochondrial functionality (Stern et al., 2002). Furthermore, mammalian cells lacking Atm have increased levels of reactive oxygen species (ROS) and deletions in mitochondrial DNA in the region encoding cytochrome c oxidase (Lee and Pauli, 2020). Post-mortem studies revealed a significant loss of Purkinje and granule neurons in the cerebella of children with A-T (Tal et al., 2018).

Cerebellar Vulnerability in Ataxia-Telangiectasia

Accumulating evidence indicates that ATM has numerous functions in the nucleus and in the cytoplasm that help maintain cellular homeostasis. The reason for cerebellar vulnerability in A-T is still unknown (Rothblum-Oviatt et al., 2016; Shiloh, 2020; Kwak et al., 2021; Lee et al., 2021). Possibilities include its regulatory roles in the dynamics of cerebellar networks, transcription, vasculature integrity, or neural-glial-vascular-immune interactions. Despite extensive research, it is still unclear why ATM deficiency affects the cerebellum and the dopaminergic system more severely than the rest of the brain.

Ataxia is defined as a loss of coordination that in most cases affects gait and...
A-T is characterized by the attrition of Purkinje neurons, which are specifically cerebellar cells. The firing rate of Purkinje neurons is among the highest in the neuronal cells in the brain, and these neurons operate as a fast pacemaker with a mean half width of 0.23 ms (Masoli et al., 2015). The vulnerability of Purkinje neurons to ATM deficiency may lead to cerebellar attrition. Alternatively, ATM loss may impact other cell types that in turn cause Purkinje cell attrition. Of note, ATM deficiency leads to contraction light on the behavior of Purkinje neurons in animal behavior. Mutation in an ATM/GTP binding protein, Agtb1, led to the loss of all Purkinje neurons within 3 weeks while sparing the granule neurons. Surprisingly, these mutant mice had only mild behavioral defects (Ben-Asher et al., 2007). A more pronounced behavioral effect, the lurcher phenotype, was detected in the Grid2 mutant mice in which the gene encoding Grid2, a glutamate receptor, has been deleted. This mutation leads to the loss of 100% of Purkinje neurons, at least 90% of granule neurons, 30% of deep nuclei, and 60–75% of inferior olivary neurons (60–75%) within 2 weeks of birth (Hilber and Caston, 2001; Lalonde and Strazielle, 2007). Mice lacking Grid2, an inward rectifying K-channel, lose 40% of the Purkinje neurons and 100% of granule and dopaminergic neurons of the midbrain and have severe behavioral deficits (Lalonde and Strazielle, 2007). Kim et al. (2020) showed that loss of both Atm and Polymerase b results in severe ataxia but not Purkinje cell loss. Collectively, these results suggest that there is not a simple relationship between the degree of behavioral impairment and the severity of cell loss. For example, mutant mice lacking Atp2b1-null mice have better motor capabilities than Grid2 mutants, despite the total absence of Purkinje neurons in the Grid2- and Atp2b1-null strains, suggesting that partial loss of Purkinje neurons promotes worse dysfunction than a complete absence.

The average glucose consumption per neuron is nearly 20 times higher in the cerebral cortex than in the cerebellum (Herculano-Houzel, 2011). This stems from the fact that about 80% of all brain neurons are in the cerebellum, and the average glucose use per cerebellar neuron is markedly lower than that of a cortical neuron (Herculano-Houzel, 2011). The neuron to glia ratio depends on the brain region and varies among species (Lalonde and Strazielle, 2007). It is 4.35:1 in the cerebellum (Herculano-Houzel, 2014). In contrast, in the cerebral cortex, which is mostly composed of non-neuronal cells, the neuron to glia ratio is 1.14 (von Bartheil et al., 2016). Astrocyes account for around 20% of the total number of neurons in the cerebral cortex (Herculano-Houzel, 2011). Recent data implicate microglia in many brain functions, but not the cerebral cortex (Krishner et al., 2012), similar to the relentless process in A-T patients.

Microglia are the resident immune cells in the brain and much mystery has surrounded their function. Long viewed as the brain’s defenders against biological threats and injury, these chameleonic-like cells transform from a resting to an activated, macrophage-like state when challenged by injury or infection. Microglia are rapidly recruited at sites of damage as phagocytes and debris and undying and dying cells. Although critical for the immune response to infection and trauma, microglia also contribute to pathological neuroinflammation by releasing cytokines and neurotoxic products (Daniel et al., 2020). Recent evidence suggests that cerebellar network dynamics of granule neuronal cultures are heavily dependent on astrocytes (Kanner et al., 2018). Furthermore, in the mouse brain, ATM deficiency leads to age-dependent attrition of the cerebellum and dopaminergic system but not the cerebral cortex (Krishner et al., 2012), similar to the relentless process in A-T patients.

DNA damage is caused in living organisms primarily by endogenous ROS produced during metabolism, which induces tens of thousands of DNA lesions per cell per day (Shihol, 2020). Defense against threats to genome integrity is critical for cellular homeostasis and prevention of undue cell death, cancer, and age-related diseases (Mouches et al., 2013). The major targets of reactive oxygen species and reactive nitrogen species and their neutralization by deleterious ROS or reactive nitrogen species and their neutralization by oxidative damage to macromolecules and also disrupt signaling pathways in addition to those associated with DNA damage, some of which are cytoplastic (Tal et al., 2018). Thus, when ATM is missing, as it is in A-T patients, the modulation of numerous pathways becomes suboptimal.

Oxidative stress occurs due to an imbalance between the generation of deleterious ROS or reactive nitrogen species and their neutralization by antioxidant enzymes or low-molecular-weight antioxidants. The main antioxidant enzymes are superoxide dismutases, catalases, glutathione peroxidase, and glutathione reductase (Wei et al., 2018). Low-molecular-weight antioxidants include reduced glutathione, uric acid, melatonin, and vitamin D (George and Abrahamse, 2020). ROS can be generated by the reduction of molecular oxygen to superoxide anion, hydroxyl radical, alkyl radical, and peroxy radicals (Vijayan and Jackson, 2007), both of which are involved in the response to genotoxic and other stresses. ATM phosphorylates more than 1000 proteins, which are involved in cell-cycle control, DNA repair, apoptosis, modulation of chromatin structure, and other cellular processes (Shihol and Ziv, 2013; Stracker et al., 2013; Schlam-Babayev et al., 2021). A summary of ATM cellular functions is presented in Figure 1.

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ATM Deletion Impairs the Redox State of the Central Nervous System

In 1997, Rotman and Shihol (1997) proposed that most of the pleiotropic nature of A-T can be explained by elevated oxidative stress. In support of this hypothesis, ATM has been implicated as a key player in the maintenance of the cellular redox balance (Lee and Paul, 2021) and mitochondrial metabolism (Stern et al., 2017). The role of ATM in the maintenance of genome stability as it supports the repair of single-strand breaks and double-strand breaks (SSB and DSB) repair system (Oberdoerffer and Miller, 2002). ATM Deletion Impairs the Redox State of the Central Nervous System

ATM is a master regulator of key nuclear and cytoplasmic processes.

ATM is a serine/threonine kinase that belongs to a family of PI-3 kinase-like protein kinases (Shihol and Ziv, 2013; Shihol, 2020) that includes the catalytic subunit of the DNA-dependent protein kinase (A-T; Blackford and Jackson, 2017), both of which are involved in the response to genotoxic and other stresses. ATM phosphorylates more than 1000 proteins, which are involved in cell-cycle control, DNA repair, apoptosis, modulation of chromatin structure, and other cellular processes (Shihol and Ziv, 2013; Stracker et al., 2013; Schlam-Babayev et al., 2021). A summary of ATM cellular functions is presented in Figure 1.

Important downstream targets of ATM are the genome guardian p53 and the histone H2AX. p53 responds to diverse cellular stresses to regulate cell cycle arrest, senescence, DNA repair, changes in metabolism, apoptosis, and cell death (Blackford and Jackson, 2017). AKT is a crucial survival signaling protein resistant to ATM and dependent H2AX phosphorylation is one of the earliest signs of DNA damage. H2AX phosphorylation recruits other proteins that initiate the chromatin-modulating process essential for efficient DSB repair (Obertoederer and Miller, 2022).
A direct connection between the accumulation of DNA breaks and oxidative stress was demonstrated by Stern et al. (2002) who showed that Atm deficiency resulted in reductions in the reduced and the oxidized forms of NADH and NADPH. Atm-deficient mice are increased in numerous catabolic and biosynthetic reactions in living organisms, and NADH and NADPH play critical roles in the antioxidant machinery. NADH and NADPH regulate the activity of PARP and SIRT2 (Covarrubias et al., 2021), factors critical in DNA damage repair and cell survival. The reduction in the mitochondrial respiration rate specifically in the cerebellum but not in the cerebrum (Stern et al., 2002). Elevated mitochondrial respiration can lead to the generation of superoxide anions and oxidative stress. Taken together, these findings point out that oxidative stress can lead to upregulation of DNA breaks, which in turn impose stress on DNA damage response mechanisms. Overactivation of these systems depletes the cells of pyridine nucleotides, disrupting critical signaling pathways and cellular energetic homeostasis.

Ziv et al. (2005) generated Atm-deficient mice in the background of either Scid or Nocr. The combination of Atm and Scid led to increased oxidative stress, whereas combined inactivation of Atm and Nocr, which encodes the mismatch repair enzyme, leading to increased genomic instability. Increasing oxidative stress exacerbated growth retardation and markedly reduced the mean survival time of mice following IR. In contrast, increasing genomic instability caused a moderate increase in radiation sensitivity but a dramatic increase in aggressive lymphomas compared to Atm+/ mice. These results demonstrate that Atm deficiency-induced oxidative stress and genomic instability are responsible for different phenotypes of A-T.

In addition to its canonical activation, which is dependent on the MRN complex, DNA damage can also be activated independently of ATR and ATM. Using purified recombinant human ATM, Paul and her colleagues showed that ATM can be directly activated by the addition of hydrogen peroxide in the absence of the MRN complex or DNA (Lee and Paul, 2020). Under these conditions, ATM is not inhibited by Nbs1, a subunit of the MRN complex, suggesting that ATM can be directly activated by hydrogen peroxide. Activation of ATM by ROS regulates protein homeostasis, mitochondrial function, and ROS levels and activity. Cell-cycle checkpoint activation of various types of autophagy can be regulated either by an activated monomeric ATM or the covalent dimer (Lee et al., 2018).

The Role of ATM in Tissue Regeneration

Is there a connection between genome instability, DNA damage, and tissue regeneration? The answer is yes. Wound healing requires the migration of stem cells to the injury site where they participate in the repair process. A unique model system to study tissue regeneration is the flatworm planarian, which is capable of regenerating new tissue at the sites of injury. Moreover, planarian can also remodel existing tissue to restore a fully functional tissue. The regeneration process involves the migration of specific stem cells called neoblasts. Neoblast migration involves nuclear changes that are associated with DNA damage and repair. Upon their migration to the injury site, the DNA damage is reduced (Reddien, 2018). When DNA damage is induced prior to the injury, migration is delayed, and the sensitivity of the stem cells to further DNA damage is increased compared to stationary neoblasts. Depletion of key components of the DNA repair system revealed that nuclear activation of the DNA repair machinery is vital for proper cell migration in planarian (Sahu et al., 2021). Depletion of Atm during normal regeneration in planarians revealed that Atm is required for the regeneration process; however, activation of Rad51, which is a key player in the repair of DNA through homologous recombination, led to the demise of the planarians. Interestingly, depletion of Atm after sublethal IR hampered the ability of the stem cells to repopulate and to promote the regenerative process. Together, these results suggest that Atm is necessary for tissue regeneration in those cells that were exposed to DNA damage but that it does not play a role in normal migration-induced DNA damage (Sahu et al., 2021).

The Non-Nuclear Function of ATM

ATM has non-nuclear activities in different cellular domains and organelles. In neuronal cells, and especially Purkinje neurons, of mice, ATM is predominantly located in the cytoplasm (Barlow et al., 2000). Dar et al. (2006) examined Atm subcellular localization in mouse cerebellar cells using confocal immunofluorescence microscopy and ex vivo immunofluorescence microscopy and found that Atm localization in the cytoplasm and the cytoplasm (Juran et al., 2012). Atm activation by IR is mediated by the autophosphorylation at S1987 in mice (S1981 in human) (Lee and Paul, 2020). The non-nuclear function of ATM was recently detected in cells of Purkinje neurons as well as in the nuclei of other cerebellar cells upon IR (Dar et al., 2006). If ROS-induced Atm activation is indeed cytoplasmic, phosphorylated Atm should have been detected in the cytoplasm, but it was not. It is possible that in cerebellar cells S1987 autophosphorylation occurs predominantly in the nucleus, whereas other autophosphorylation sites or covalent Atm dimerization can take place in the cytoplasm. It is also possible that only some ROS such as hydrogen peroxide results in cytoplasmic activation of Atm. This notion is supported by the fact that mitochondria-derived hydrogen peroxide promoted Atm dimerization and activation (Zhang et al., 2018).

There is also evidence that cytosolic ATM is involved in the general recycling process. It is known that ATM is important for the regulation of damaged mitochondria and peroxisomes in response to increased oxidative stress [reviewed in (Blignaut et al., 2021)]. Under conditions of oxidative stress, ATM is activated and triggers the activity of the LKB1/AMPK pathway, which in turn activates the transcription factor YAP. However, the covalent dimerization of ATM triggers the formation of Mtorc1 and thereby induces autophagy. ATM can also suppress TORC1-mediated signaling pathways in response to hypoxia through the phosphorylation of Hif1α (Cam et al., 2010). ATM also mediates mitophagy, a mitophagy-mediated degradation process (Li et al., 2018). The non-nuclear function of ATM is also involved in cell metabolism. For example, ATM is involved in the control of the respiratory rate, which encodes the mismatch repair enzyme, leading to increased genomic instability. Increasing oxidative stress exacerbated growth retardation and markedly reduced the mean survival time of mice following IR. In contrast, increasing genomic instability caused a moderate increase in radiation sensitivity but a dramatic increase in aggressive lymphomas compared to Atm+/ mice. These results demonstrate that Atm deficiency-induced oxidative stress and genomic instability are responsible for different phenotypes of A-T.

ATM sensitivity significantly reduces hippocampal long-term potentiation. Using spontaneous synaptic dye release, Li et al. (2009) showed that Atm can form a complex with two presynaptic vesicular proteins VAMP2 (also known as synaptobrevin) and Synapsin-1. VAMP2 is associated with neurotransmitter release and is involved in the process of exocytosis. Synaptobrevin is involved in synaptic transmission and is also involved in synaptic transmission and is also involved in synaptic transmission and synaptic remodeling (Nuszkiewicz et al., 2020). The non-nuclear function of ATM is also involved in cell metabolism. For example, ATM is involved in the control of the respiratory rate, which encodes the mismatch repair enzyme, leading to increased genomic instability. Increasing oxidative stress exacerbated growth retardation and markedly reduced the mean survival time of mice following IR. In contrast, increasing genomic instability caused a moderate increase in radiation sensitivity but a dramatic increase in aggressive lymphomas compared to Atm+/ mice. These results demonstrate that Atm deficiency-induced oxidative stress and genomic instability are responsible for different phenotypes of A-T.

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ATM activation requires the activation of the DNA repair machinery. The DNA damage is repaired by the action of DNA repair enzymes, which are involved in the repair of DNA breaks. Upon their arrival at the injury site, the level of oxidative stress increases, leading to the stimulation of specific pathways responsible for survival and DNA damage repair. Induction of ATM by ROS regulates protein homeostasis, mitochondrial function, and ROS levels and activity. Cell-cycle checkpoint activation of various types of autophagy can be regulated either by an activated monomeric ATM or the covalent dimer (Lee et al., 2018).

ATM, like ATR, is a central player in DNA damage response; ATR mainly functions in the repair of SSBs and resolution of stalled replication forks [reviewed in (Blackford and Jackson, 2017)]. Like ATR, ATM is also detected in the cytoplasm, most notably in neurons. ATM and ATR have both been localized to cytoplasmic vesicular structures, including synaptic vesicles where they associate with the synaptic vesicle proteins VAMP2 and SYNAPSIN-1 (Li et al., 2009). ATM associates with excitatory, glutamatergic vesicles, whereas ATR associates with inhibitory, GABAergic vesicles (Cheng et al., 2018). The complementarity also extends to their pathways of degradation. ATM and ATR are both large proteins (3056 and 2644 amino acids, respectively) with similar three-dimensional structural motifs. ATM is degraded primarily by the proteasome, whereas ATR is degraded primarily by the proteasome (Cheng et al., 2020). The large sizes of ATM and ATR raise the intriguing possibility that these proteins can serve as scaffolds for complex protein associations that provide support for the reorganization of the cytoskeleton as it occurs during autophagy and apoptosis (Deng et al., 2020). These interactions can also be involved in the recruitment of the glycogen synthase kinase GSK3β resulting in the degradation of the nascent peptidyl-phosphate complex of a subunit as well as g-tau, which accumulates due to mitochondrial-dependent cell death in response to endoplasmic reticulum stress.

Reduced mitochondrial functionality was detected in Atm−/− cells as well as in cells treated with small-molecule inhibitors of ATM. ATM is detected in mitochondria and is activated by mitochondrial dysfunction (Lee and Paul, 2020). ATM deficiency in thymocytes leads to increased levels of ROS, to disruption of the regulation of the respiratory capacity, and to abnormal mitophagy (Valentin-Vega et al., 2012; Tal et al., 2018). Lee et al. (2018) have shown that cells expressing an oxidant-dependent form of ATM (C2991I) have reduced mitochondrial membrane potential and mitophagy compared to wild-type cells. ATM is also detected in the redox state of cells, which is important for cell survival in response to oxidative stress. For example, ATM promotes mitochondrial switching from aerobic to anaerobic metabolism, whereas ATR promotes mitochondrial switching from anaerobic to aerobic metabolism (Lee et al., 2018). ATM can also control the cellular redox state by upregulating the pentose phosphate pathway, which plays a key role in the production of the crucial antioxidant NADPH. Activated ATM phosphorylates HSP27, which binds to α-tubulin and reduces the formation of tubulin-linked dimers (Lee et al., 2018). ATM can also control the cellular redox state by upregulating the pentose phosphate pathway, which plays a key role in the production of the crucial antioxidant NADPH. Activated ATM phosphorylates HSP27, which binds to α-tubulin and reduces the formation of tubulin-linked dimers (Lee et al., 2018). ATM can also control the cellular redox state by upregulating the pentose phosphate pathway, which plays a key role in the production of the crucial antioxidant NADPH. Activated ATM phosphorylates HSP27, which binds to α-tubulin and reduces the formation of tubulin-linked dimers (Lee et al., 2018).
phosphorylates the transcription factor NRF1, enhancing its ability to form dimers and its nuclear translocation. In the nucleus, NRF1 upregulates the nuclear-encoded mitochondrial genes thereby increasing the activity of the electron transport chain. In mice, Atm deficiency reduces the ability of mitochondria to synthesize ATP and thus diminishes cellular homeostasis (Chow et al., 2019). These results led Chow et al. (2019) to suggest that Atm functions as a guardian of mitochondrial output. Low levels of cellular NAD⁺ and mitochondrial dysfunction were detected in Atm⁻/⁻ neurons derived from mice and C. elegans (Stern et al., 2002; Fang et al., 2016). Replication of NAD⁺ levels stimulated neuronal DNA repair and improved mitochondrial functionality via mitophagy (Fang et al., 2016). This treatment also extended the lifespan and motricity in both worm and mouse models. Fang and Bohr suggested that NAD⁺, as a signaling molecule between nuclear DNA integrity and mitochondrial functionality, two factors that are critically important for aging (Fang et al., 2016).

### Atm Deficiency Results in Impaired Functionality of Neurons and Glial Cells

Levine-Small et al. (2011) recorded the activity of multiple neurons and measured the firing rates of individual neurons, spike signals of individual neurons, inter-spike intervals, and the raw spike signals of individual neurons in cortical neural cultures grown from WT and Atm⁻/⁻ mice on a microelectrode array. The firing of individual neurons and the number of spikes were not affected either by Atm deficiency or by DNA damage. However, none of the differences in cortical neural network activities were detected between wild-type and Atm⁻/⁻ neural-glia cultures. Similar to wild-type cultures, Atm⁻/⁻ cultures had the ability to synchronize. Careful analyses revealed that upon DNA damage the Atm⁻/⁻ networks lost their ability to maintain synchronization, persistence over time, instead losing sustained synchronisation after DNA damage (Levine-Small et al., 2011). Kanner et al. (2018) found that Atm regulates the dynamics of cerebellar networks. The number of global synchronization events, in which most if not all the cultured cells fire together, was significantly reduced in Atm⁻/⁻ networks, and the number of sparse synchronization events, in which a few cells fire most cells still remain silent, were significantly increased in Atm⁻/⁻ cerebellar cultures compared to wild-type cultures. It is important to note that these differences were detected in untreated cerebellar neural-glia cultures. Remarkably, a chimeric culture of wild-type astrocytes and Atm⁻/⁻ neurons had network dynamics and numbers of synapses at wild-type levels. In contrast, no neuronal survival or activity was detected in chimeric cultures of wild-type neurons and Atm⁻/⁻ astrocytes (Kanner et al., 2018). Furthermore, Atm deficiency severely hampers the complexity of the Bergmann glia and vela astrocytes in the whole cerebellum. Surprisingly, Atm deficiency increased the number of excitatory synapses in the cerebellar cultures, whereas in the whole cerebellum the level of inhibitory synapses was upregulated. Astrocytes are known to affect synapse formation, maturation, and elimination (Kanner et al., 2018). Excessive numbers of synapses in Atm⁻/⁻ cultures and cerebella may stem from increased formation or decreased elimination. We found that Atm deficiency led to increased levels of the autophagy markers p70S6K and p62 both in cultures and whole cerebella (Kanner et al., 2018). Atm negatively regulates mTOR thereby enhancing autophagy (Shilooh and Ziv, 2013), and the increased levels of both p70S6K and p62 are correlated with inhibited autophagy. Thus, we concluded that the increased number of synapses in Atm⁻/⁻ cultures and cerebella are likely due to reduced autophagy. Taken together, these findings demonstrate the vital role that astrocytes play in neural homeostasis and in the dynamics of brain networks.

Our group has shown that astrocyte function is impaired in Atm⁻/⁻ mice, which also have severely impaired retinal vascularization and leaky blood vessels (Raz-Prag et al., 2011). These findings highlight the role of Atm in maintaining the integrity and functionality of the nervous system and in regulating innate immune function. Characterization of the retina in young and aged Atm-deficient mice revealed that, at 2 months of age, angiography was slightly impaired in Atm⁻/⁻ vasculature compared to wild-type controls. Astrocytes and endothelial cells were demonstrated to be involved in the regulation of blood vessels in Atm⁻/⁻ retinas. Atm loss also significantly reduced the complexity of the processes of astrocytes, which likely results in the observed atrophied structural organization of the astrocytic network. These results suggest that impaired vascularization in the central nervous system plays an important role in the etiology of A-T, and vascular abnormalities may underlie or aggravate brain degeneration.

Human and rodent neurons are similar (Hodge et al., 2019), and the fundamental difference between the human and the rodent brain lies in the number and complexity of the glial cells (Oberheim et al., 2006). A comparison of the properties of humans (Zhang et al., 2016) and mouse astrocytes reveals that mouse astrocytes have higher tricarboxylic acid cycle activity, reduced lactate production, higher resistance to ROS, and higher expression of catalase (Ye Zhang, University of California, Davis; personal communication). Since astrocytes play such an important role in brain functionality as well as in brain degenerative diseases (Oberheim et al., 2006; Acigolu et al., 2021), it is our notion that highly durable and resistant mouse astrocytes ameliorate the toxic effects of Atm deficiency. This is the reason that Atm deficiency in mice does not completely mimic the effects of Atm loss in humans.

Further evidence that astrocytes play a role in the etiology of A-T was provided by Petersen et al. (2012) who showed that Atm knockout in Drosophila glial cells but not in neuronal cells was sufficient to cause the hallmark symptoms of A-T. Figure 1 depicts the roles of ATM in various neural networks.
Future Directions

A-T is an incurable, multisystem disease that affects neural, glial, vascular, and immune system function in the cerebellum. The most devastating symptom of A-T is cerebellar ataxia, which severely disables young A-T patients, leaving them wheelchair-bound for the rest of their lives. A-T research uses many types of different cells as model systems to understand the role of ATM in cell functionality and homeostasis. These include ATM-deficient human fibroblast and lymphoblast cell lines as well as neuronal-like cell lines isolated from different animal models of A-T. In addition, some studies utilize primary cultures of cerebral cortical neurons or different cerebral primary cultures of neurons and glial cells. The interpretations of the results obtained from the in vitro systems must be validated in vivo.

The heterogeneity observed in the model systems demonstrates the diverse functions of ATM. Extrapolation from one model system to another is highly problematic. Therefore, studying the appropriate model system is critically important for the generation of data relevant to the design of A-T treatments. In particular, understanding the role of each cerebellar cell in the etiology of A-T is critically important. The immediate cure for A-T would be a genetic therapy in which the mutated ATM gene is replaced by a wild-type copy of the gene in all cells. At present, this type of therapy is not available. Thus, more pragmatic treatments should be tested.

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