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The cerebellar degeneration in ataxia-telangiectasia: A case for genome instability

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ABSTRACT

Research on the molecular pathology of genome instability disorders has advanced our understanding of the complex mechanisms that safeguard genome stability and cellular homeostasis at large. Once the culprit genes and their protein products are identified, an ongoing dialogue develops between the research lab and the clinic in an effort to link specific disease symptoms to the functions of the proteins that are missing in the patients. Ataxi A-T elangiectasia (A-T) is a prominent example of this process. A-T's hallmarks are progressive cerebellar degeneration, immunodeficiency, chronic lung disease, cancer predisposition, endocrine abnormalities, segmental premature aging, chromosomal instability and radiation sensitivity. The disease is caused by absence of the powerful protein kinase, ATM, best known as the mobilizer of the broad signaling network induced by double-strand breaks (DSBs) in the DNA. In parallel, ATM also functions in the maintenance of the cellular redox balance, mitochondrial function and turnover and many other metabolic circuits. An ongoing discussion in the A-T field revolves around the question of which ATM function is the one whose absence is responsible for the most debilitating aspect of A-T – the cerebellar degeneration. This review suggests that it is the absence of a comprehensive role of ATM in responding to ongoing DNA damage induced mainly by endogenous agents. It is the ensuing deterioration and eventual loss of cerebellar Purkinje cells, which are very vulnerable to ATM absence due to a unique combination of physiological features, which kindles the cerebellar decay in A-T.

1. Ataxia-telangiectasia (A-T): a pleiotropic genome instability disorder

Understanding the molecular and physiological foundations of genetic disorders is key to developing treatment and should lead to new biomedical insights into these and many other diseases. Genome instability disorders present a special challenge and opportunity in this regard, representing critical homeostatic crossroads of genome dynamics and cellular metabolism. These disorders are caused by deficiencies in coping with DNA damage, and are characterized by combinations of chromosomal instability, progressive degeneration of various tissues, compromised development and/or growth, skeletal malformations, segmental premature aging, cancer predisposition and sensitivity to DNA damaging agents [1]. The human multisystem, autosomal recessive disorder, ataxia-telangiectasia (A-T; OMIM#208900), includes many of these components [2,3]. It is caused by null mutations in the *ATM* (A-T, mutated) gene [4–6], which encodes the homeostatic protein kinase, ATM. A-T is found in many ethnic groups in varying frequencies of 1:40,000 – 1:200,000 live births. Since the identification of the *ATM* gene in our lab in 1995 [4,5,7], a wealth of information has accrued about the roles of the ATM protein in many cellular circuits [8–19], but major questions remain about the links between ATM documented functions and various components of the pleiotropic A-T phenotype.

The presenting and prominent symptom of A-T is progressive cerebellar ataxia (defective movement coordination whose foremost early manifestation is lack of balance when walking), which relentlessly progresses to a multifaceted motor dysfunction that usually includes choreoathetosis, which confines most patients to a wheelchair at the beginning of their second decade. Oculomotor abnormalities, swallowing difficulties and slurred speech are common, and facial expression is limited. The main underlying pathology of these symptoms is progressive cerebellar cortical degeneration that involves mainly Purkinje, granule, and basket cells. The gradual loss of Purkinje cells (PCs) is preceded by their abnormal dendritic arborization, shrinkage of the cell soma and disruption of their axons, and occasional presence of heterotopic PCs in the molecular layer. The progressive degeneration of the entire cerebellum can be followed in most cases during the first and second decade of life by radiologic imaging. Peripheral neuropathy and

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degenerative changes in the basal ganglia and spinal cord may develop during the second decade of life. A-T is also characterized by oculocutaneous telangiectasia (dilated blood vessels), chronic lung disease often leading to pulmonary failure, immunodeficiency that spans B- and T-cell lineages, recurrent sinopulmonary infections in some patients, thymic degeneration, primary gonadal failure, endocrine abnormalities, elevated serum alpha-fetoprotein, marked predisposition to lymphoreticular malignancies, segmental premature aging, chromosomal instability, and acute sensitivity to ionizing radiation (IR). Although the clinical phenotype of 'classical A-T' is well-defined, there is considerable variability in age of onset, progression, and severity of these symptoms. The variability of the A-T phenotype is further expanded by 'mild A-T', which results from combinations of *ATM* mutations that leave residual amounts and/or activity of the ATM protein [20].

The cellular phenotype of 'classical A-T' is represented in primary skin fibroblasts and immortalized cell lines as chromosomal instability [21] and sensitivity to genotoxic agents, particularly those that induce double-strand breaks (DSBs) in the DNA [22,23], premature senescence of primary fibroblasts [24], accelerated telomere shortening [25], and alterations in many metabolic circuits (see below).

2. Genome instability disorders: defective response to the daily wear and tear on the DNA

The cellular response to DNA lesions is usually investigated in the laboratory under non-physiological conditions. Typically, large amounts of DNA damage are induced in cultured cells using high doses of physical or chemical DNA damaging agents. Such experiments are obviously important for studying the biochemistry of DNA repair, but if the primary cause of a disease is thought to be a defective response to DNA damage, one needs to model the actual DNA damage that the body's cells are faced with every single day. This damage is induced mainly by endogenous reactive oxygen species (ROS) produced during metabolism that induce a plethora of lesions in cellular DNA [26] (Table 1). The DNA in body tissues may also be adversely affected by environmental chemicals and radiations, but the amount of that damage in the inner organs is probably far less than the ongoing damage caused by endogenous ROS. Examples of genome instability disorders caused by defective response to environmental agents are syndromes stemming from malfunction of the nucleotide excision repair (NER) pathway, such as xeroderma pigmentosum and related disorders, where most of the symptoms appear in the skin and reflect severely compromised response to DNA lesions induced by environmental UV radiation [27]. However, when multisystem disorders such as A-T are investigated, the DNA lesions presented in Table 1 should be among the first considered.

3. The DNA damage response

The cellular response to DNA damage is much broader and far-ranging than the DNA repair machineries. DNA lesions induce, to various extents, a broad cellular reaction: the DNA damage response (DDR). This vast signaling network simultaneously activates DNA repair mechanisms and modulates chromatin organization as well as many physiological processes (e.g., activation of special cell cycle checkpoints). It temporarily creates the physiological environment required for optimal DNA repair while preventing potential damage from the abrupt standstill of cell cycle dynamics. The full reach of the DDR involves numerous protein post-translational modifications, relocalization and alteration of function or mode of action, that lead to marked modification of the cellular proteome, epigenome and transcriptome – all in service of getting the job of repair done [28]. The DDR network is vigorously activated by DSBs [29], which can be induced by exogenous agents or endogenous ROS, or form normally during physiological processes such as meiotic recombination or the maturation of the antigen receptor genes. The DSB is therefore a major DNA lesion used in the laboratory to investigate the DDR in all its breadth. Notably, prior to discovery of the *ATM* gene, a major component of the DSB response network – activation and stabilization of the p53 protein – was found by M. Kastan and colleagues to be missing in A-T cells [30]. p53 is a major regulator of the DSB-induced cell cycle checkpoints and transcriptome alterations [31]. That seminal observation further corroborated the early notion that cells from A-T patients harbor a major deficiency in the DSB response network.

The DSB response [29,32,33] begins with rapid recruitment of a broad, heterogeneous group of sensor/mediator proteins to the lesion sites where protein conglomerates are formed. These meticulously organized structures are involved in chromatin reorganization and epigenetic alterations in the vicinity of the breaks in preparation for their eventual repair via several possible repair pathways [33]. The common post-translational modifications that are induced in these proteins are phosphorylation, dephosphorylation, and modification by members of the ubiquitin family. In parallel to these complex but finely-tuned processes at the break sites, the DDR modulates multiple cellular circuits throughout the cell. All of these processes are initiated and maintained to a large extent by the action of protein kinase transducers that phosphorylate numerous key players in these circuits, thereby affecting the function, stability, interactions, or subcellular localization of their phosphorylation targets [34]. The major transducer in the DSB response network is the ATM protein kinase.

Table 1
Endogenous DNA Damage in Mammalian Cells.

Damage	Events per cell/day
Single-strand break	55,200
Depurination	12,000
Depyrimidination	600
0^6-methylguanine	3,120
Cytosine deamination	192
Glucose-6-phosphate adduct	2.7
Thymine glycol	270
Thymidine glycol	70
Hydroxymethyluracil	620
8-oxo-guanine	178
Inter-strand cross-link	8
Double-strand break	8

The table was originally compiled by E. Seeberg, based among others on refs [278–282].

4. ATM: a protein kinase for all seasons

A mostly nuclear protein with a distinct cytoplasmic fraction, ATM belongs to a family of PI-3 kinase-like protein kinases (PIKKs) [34–37]. Among others, this family includes the mammalian target of rapamycin (mTOR), the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs) and ataxia-telangiectasia and Rad3-related protein (ATR), which are involved in responding to genotoxic and other stresses and stimuli. ATM, DNA-PK and ATR maintain complex relationships based on various degrees of redundancy in their response to genotoxic stresses, and sometimes mutual, regulatory phosphorylations, as in the case of ATM and DNA-PK [34–38]. Importantly, ATM's kinase activity is markedly enhanced in response to DSBs [39,40]. This vigorous activation, which is dependent on the MRE11-RAD50-NBS1 (MRN) complex [41–43], involves autophosphorylation on several sites and separation of activated ATM monomers that, prior to the DSB stimulus, form quiescent, non-covalent homodimers [44,45] (also reviewed in [12]). The activated monomers phosphorylate numerous players in various branches of the DSB response network [46,47], and this highly structured network robustly modulates numerous physiological circuits in a fine-tuned manner [29,32,33].

Several years ago, I suggested a broader, overarching role for ATM in maintaining genome stability in addition to its cardinal role in mobilizing the DSB response [10]. This model was based on our studies of ATM-mediated damage response pathways as well as a close look at the

raw data in several phosphoproteomic screens for ATM targets [46–48]. According to this conjecture, ATM supports other DNA repair pathways that respond to various genotoxic stresses, takes part in resolving non-canonical DNA structures that arise in DNA metabolism, and contributes to regulating other aspects of genome integrity such as nucleotide metabolism, the response to replication stress, and resolution of the occasional conflicts that arise between DNA damage and the transcription machinery. ATM's involvement in these processes is based on targeting key proteins in these pathways and modulating their activity. ATM is not critical for any of these processes in the same way it is for the DSB response, but rather contributes to their regulation (in most cases, their enhancement) when the need arises, particularly in the face of large amounts of damage or aberrant structures. This ongoing role of ATM is among its routine functions in the constant maintenance of genome stability, while its powerful role in the DSB response is reserved for responding to this harmful lesion, which is in the minority of daily DNA lesions (Table 1). Thus, when ATM is missing, not only is there a markedly reduced response to DSBs, but the ongoing modulation of many pathways in response to occasional stresses becomes suboptimal. This function of ATM may explain the moderate, variable sensitivity of A-T cells to a broad range of DNA damaging agents (reviewed in ref. [10]).

Most of the literature on the physiological roles of ATM revolves around its role in maintaining genome stability. In parallel, work in many laboratories provided evidence for a broader role of ATM in cellular homeostasis [8–19,49–81], that relies in part on its cytoplasmic fraction [49,75–79,81–96]. Some of these observations raised a question our group had asked early on: Is ATM a sensor of oxidative stress? [97,98] Indeed, a regulatory role for ATM in maintenance of the cellular redox balance was repeatedly noted in reports on elevated oxidative stress in ATM-deficient cells and tissues [17,86,97–119], and it was suggested that ATM could be activated by ROS [120]. A mechanistic answer came from the laboratory of T. Paull, who discovered an ATM activation mechanism in response to elevated ROS levels that differed markedly from the DSB-induced activation: ATM monomers responded to elevated ROS by forming active homodimers via covalent disulfide bridges, in a DSB- and MRN-independent manner [121,122]. This mode of activation was reported to direct ATM to specific substrates, some of them cytoplasmic [74–76,92,121–123], and affect specific cellular circuits [74–77,92,123–125]. The distinction between the DSB-induced and oxidative modes of ATM activation was further refined using separation-of-function *ATM* mutations [74,126]. Notably, processes that depended on the oxidative activation of ATM included mitochondrial function and autophagy, and specific loss of this activation route led to substantial protein aggregation [126]. ATM's catalytic activity was recently found to be required also for the cellular response to unfolded proteins, which is necessary for relieving endoplasmic reticulum stress caused by their accumulation [81].

A major component of ATM-mediated modulation of redox balance is ATM's involvement in mitochondrial homeostasis, with the probable involvement of its mitochondrial fraction [127,128]. Indeed, an increasing number of studies have demonstrated that ATM loss affects mitochondrial maintenance, function and turnover [19,74,123,126–145]. Mitochondrial redox sensing by ATM was functionally linked to specific metabolic circuits: the pentose phosphate pathway [62,74], and up-regulation of nuclear genes encoding mitochondrial proteins that enhances mitochondrial function. The responsible transcription factor in the latter pathway was found to be nuclear respiratory factor 1 (NRF1), which is activated via phosphorylation by oxidatively activated ATM [123]. These studies reflect the rising awareness to the functional link between the maintenance of genome stability in the nucleus and mitochondria [143,146]. Another cytoplasmic sub-fraction of ATM was identified in peroxisomes and was functionally linked to peroxisome turnover [79,80,86,147].

Some of the above metabolic functions include the dynamics between the oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD), which impact cellular metabolism, oxidative stress, cell senescence and organismal aging [144,145,148,149]. Joint work between A. Barzilai's lab and our group showed early on a progressive decrease in both NAD^+ and NADH in brains of Atm-deficient mice [150]. This metabolic hallmark of murine Atm-deficient tissues was further substantiated by the Bohr lab and found to be linked to the massive DNA damage-induced poly(ADP-ribosylation) of proteins, a defective mitochondrial turnover in Atm-deficient tissues, and abrogation of the nuclear-mitochondrial axis connecting the DDR to mitochondrial dynamics [142,144–146,151,152]. Notably, replenishment of the reduced NAD^+ reservoirs in atm-1-deficient C. elegans or Atm-deficient mice rescued many phenotypic hallmarks of the protein's deficiency in these organisms [151].

5. Understanding A-T by elucidating ATM functions

Understanding the molecular pathophysiology of a genetic disorder caused by loss or inactivation of a protein is based on answering two interrelated questions: 1. Which cells and tissues are sensitive to the loss of the protein's functions and therefore give rise to corresponding symptoms? 2. What are the protein's functions whose loss is critical in those particular cells? The latter question is especially important, and can be challenging, when the culprit protein is multifunctional and the disease is a multisystem one. Notably, in most A-T patients the ATM protein is missing entirely, since most A-T mutations truncate the protein [4,153,154–168] (https://databases.lovd.nl/shared/genes/ATM) and truncated ATM is unstable. Even if some truncated protein remains, short, carboxy-terminal truncations are sufficient to eliminate the adjacent catalytic domain and render ATM inactive. Clearly, therefore, many cell types and tissues in the human body can proliferate, differentiate and function without ATM and not give rise to marked symptoms throughout the patient's life. This despite the numerous homeostatic functions of ATM described above. On the other hand, several cell types are extremely vulnerable to ATM absence. What are the ATM functions that make its loss so harmful in these cells?

The major role of ATM in the DSB response readily explains the profound radiation sensitivity and variable degrees of chromosomal breakage observed in A-T patients. The immunodeficiency, the clonal lymphoid translocations with the recurrent breakpoints, and the predisposition to lymphoreticular malignancies are explained by ATM's involvement in managing DSBs formed during the rearrangement and maturation of the adaptive antigen receptor genes [169]. Likewise, the gonadal degeneration attests to ATM's critical role in processing of DSBs formed during meiotic recombination [170]. Importantly, chromosomal breakage – an A-T hallmark – shows striking differences among tissues, ranging from occasional breaks and translocations observed in cultured peripheral lymphocytes or skin fibroblasts [21], to extensive fragmentation in cells undergoing meiotic division [170]. Thus, the ATM-deficient phenotype may differ markedly among tissues due to how critical a specific role of ATM is in that tissue.

The segmental, accelerated aging in A-T [14] probably contributes to various tissue-specific, progressive symptoms in an overarching manner. The enhanced aging pace is probably fueled by the combination of genome and epigenome instability, perturbation of redox balance, defective telomere maintenance, and the disturbance of mitochondrial homeostasis, peroxisomal function and NAD balance, all of which echo ATM's involvement in relevant metabolic circuits. However, the acute cerebellar atrophy – a cardinal symptom of A-T with the most severe impact on the quality of patients' life – remains unexplained and a subject of intense discussion [2,49,54–60,70–73,88,94,143,150,151,171–178]. The lively exchange of hypotheses described below revolves around the above questions: Which cerebellar-specific cells are vulnerable to ATM loss? Which of the many ATM functions is critical in these cells?

6. The cerebellar atrophy in A-T: premises

An important, underlying question in this discussion is whether the cerebellar demise in A-T stems from a defect in a specific developmental pathway or is the result of an ongoing, stochastic process leading to deterioration of this tissue throughout the cerebellar lengthy development (much of which occurs after birth) and later. Many mutations that undermine genome stability severely abrogate the development of the nervous system already during fetal development, and some of them subsequently lead to microcephaly [175,179,180]. The neurological phenotype of A-T usually culminates towards the beginning of the second decade of life. We assume the slow, relentless development of this phenotype reflects an ongoing, stochastic detrimental process, most of which is postnatal, that gradually leads to random malfunction or loss of cells, rather than the loss of a critical developmental pathway; the latter would have led to an earlier, more acute and more rapid degenerative process.

Another premise for understanding the neurological aspect of A-T is realizing the cerebellar specificity in the early stage of the disease [2]. As noted above, the effect of ATM loss on different tissues and cell types is highly differential, as evidenced by the progression of symptoms over time and the highly variable functionality of different organs and tissues during the patient's life. The neurological symptoms of A-T are usually evident at the beginning of the second year of life, progress relentlessly, and in the early years reflect primarily cerebellar decline. The cerebellar symptoms are a fundamental component of 'classical A-T'. They may exhibit later age of onset and slower progression in 'mild A-T', which is characterized by residual ATM activity, but they are a consistent, prominent part of the broad phenotypic variability of A-T [2,3,20,181].

ATM has been implicated directly in synaptic functions [88,94,95], presumably via its cytoplasmic fraction. Can this general function in the nervous system explain the striking cerebellar specificity? The cerebrum of A-T patients was observed to exhibit occasional vascular changes, scarring and white matter lesions, but their clinical significance was unclear [182,183]. In fact, the cerebrum appears to remain largely functional in the patients, in stark contrast to the relentlessly decaying cerebellum. Accordingly, the cognitive deficits of A-T patients are largely those directly associated with the cerebellum [2,184–186]. On a personal note, my own acquaintance with A-T patients from various ethnic groups during more than four decades indicated to me that, despite the physical limitations imposed on them by declining cerebellar capabilities, frequent social isolation and sometimes deprivation of scholarly resources, the mental and intellectual capacities and even social skills of many of them were similar to those of healthy peers. Some exhibited a bright intellect, often coupled with surprising communication and social skills even during the advanced stages of the disease. It should be remembered that these individuals face considerable difficulties expressing these capabilities while coping with severe physical limitations, and often social stigmata. A broad variety of nervous system cells and circuits are presumably required to function at their best to maintain these sophisticated skills in the face of such difficulties, all the while the patient's cerebellum is on persistent downhill course. This marked difference between the structural and functional dynamics of the cerebellum and cerebrum further underscores the extreme vulnerability of the cerebellum, out of the entire nervous system, to ATM absence. Indeed, a broad diversity of cerebellar ataxias highlights the special sensitivity of the cerebellum to defects in gene regulation, DNA repair, mitochondrial life cycle and protein turnover [187–189]. A key to understanding the cerebellar sensitivity to ATM absence is identifying the specific cell type in which the cerebellar demise begins in A-T patients.

The cerebellar deterioration in A-T patients eventually affects all cell types in this organ, but histopathological studies of ATM-deficient cerebella have consistently noted the early loss of PCs, which preceded that of other cell types [2,3,20,181]. A noteworthy work by Paula-Barbosa et al. [190] meticulously examined cerebellar ultrastructure in an 8-yr-old A-T patient who died of cancer, using electron microscope analysis. They noted that the most striking feature at that stage of the disease was the loss of PCs. Some the remaining PCs showed signs of degeneration, such as shrinkage of cell soma, dwindling dendritic trees, and loss of dendritic spines. The authors concluded that a selective and progressive degeneration of PCs was taking place in the patient's cerebellum. The ultrastructural appearance of the granule cell layer was still normal in this patient, including the structure of the vessel walls of the gliovascular formations, and the number of granule cells, which was still comparable to that of control tissues. However, in other patients reported by that time, decreased granule cell numbers were also noted. Paula-Barbosa et al. therefore asked whether the decrease of granule cells could result from an independent degenerative process or a retrograde trans-synaptic effect following the loss of their targets (i.e., PCs). They resolved in favor of the latter possibility. They noted that in an earlier study of an A-T patient with a long and moderate disease course, marked reduction in PC number was observed in a less advanced stage of the cerebellar decay while the amount of granule cells was still normal [191]. They concluded: "It is likely that the selective degeneration of Purkinje cells is the primary target of the mutant gene".

Of note, the ATM protein was found many years later to be present in notable amounts in the nuclei of human PCs. Several reports had claimed that ATM was mainly cytoplasmic in human [84] and rodent [192,193] PCs. Our lab later showed that ATM was mainly nuclear in human neuron-like cells obtained by induced differentiation in culture [194,195], and similar findings were obtained in mouse PCs in collaboration with the Barzilai lab [196]. The Brooks lab then showed that the majority of cellular ATM was nuclear in human PCs as well [197].

7. Vulnerability of Purkinje cells to ATM absence: a case for genome instability

7.1. Purkinje cells are particularly prone to transcriptional stress

A direct line may be drawn from the insight of Paula-Barbosa et al. [190] in 1983 to Huang and Verbeek's 2018 review [189] that asked, "Why do so many genetic insults lead to Purkinje cell degeneration and spinocerebellar ataxia?" These and other authors noted structural and functional characteristics of PCs that make them highly susceptible to many genetic insults [189,198,199]. Different combinations of these features (listed below) characterize other neuronal cells too. DNA repair is critical in many non-dividing, differentiated long-lived cells, which can be found in all branches of the nervous system, and the impact of oxidative DNA damage is particularly high in neurons, in which enhanced energy consumption and mitochondrial activity generate excessive amounts of ROS. However, *collectively* the unique array of factors found in PCs compared to other neuronal cells creates a special physiological environment that augments the harmful effect of the deficient response to DNA damage and eventually makes it a severe functional impediment.

PCs are the primary output neurons of the cerebellar cortex. They integrate massive excitatory synaptic input, as well as a high firing rate that often reaches very high frequencies, presumably accompanied by exceptionally high metabolic activity, and consequently highly elevated oxidative stress. Given ATM's role in redox balance regulation, its absence probably overfuels oxidative stress in PCs. On the other hand, the intense transcriptional activity in PC nuclei should lead to special vulnerability of their genome to DNA damaging agents. Interestingly, using organotypic cultures derived from mouse cerebellum, we found that most of the chromatin in the very large nuclei of PCs is euchromatic, and only a minute fraction is heterochromatic, in stark contrast to the surrounding cells [200] (Fig. 1). This unique, relaxed chromatin state is likely associated with the high transcription rate in PCs, exposing the cellular genome to the harmful effects of ROS more than in the surrounding cells. Importantly, transcribed DNA is particularly vulnerable to ROS attacks [201]. This is probably why the genomic distribution of

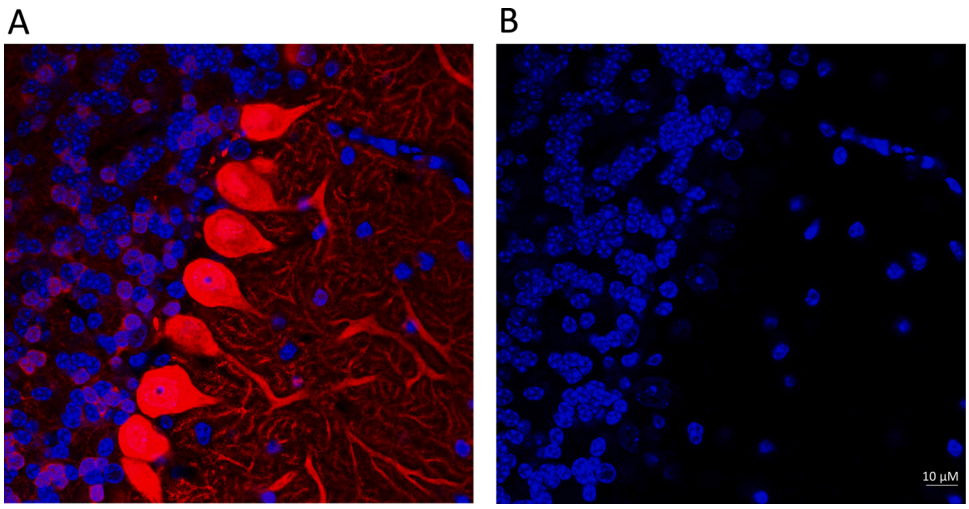

Fig. 1. A section of the cerebellar cortex of a wild-type, 14-month-old male C57BL mouse. A: Co-staining with DAPI (DNA dye, blue) and anti-calbindin D-28k (PC marker). B: The DAPI layer of Fig. 1A. Note the stark difference between the extremely low DAPI staining of the large PC nuclei (which occupy most of the cellular volume in PCs) and the bright nuclear staining of the cells surrounding them. (Contributed by Sharone Naor).

ongoing SSBs was found to be non-random: this lesion is enriched in regulatory elements, exons, introns, and specific types of repeats and exhibits differential preference for the template strand between exons and introns [202].

7.2. The ATM-deficient Purkinje cell: a relentless downhill course

Given the broad role we attribute to ATM in responding to endogenous genotoxic stress [10], the above background should herald disaster when ATM is absent. The intensive transcriptional activity in PCs probably means that many genes (other than housekeeping ones) are important for the function and survival of these cells, and while being transcribed become easy targets for DNA damaging agents. Increasing evidence suggests that the collision of the transcription machinery with DNA lesions contributes markedly to genome instability [203]. Interestingly, evidence for noncanonical ATM activation as a result of RNA polymerase II stalling was obtained, involving a cross-talk between ATM and the splicing machinery [204–206]. Ongoing genotoxic stress may also lead to increased formation of RNA:DNA hybrid R-loops in transcribed regions, which in turn exacerbate genome instability and interfere with transcription dynamics [207]. Notably, R-loops formed in transcribed regions following elevated oxidative stress attract homologous recombination (HR) factors [208], and it was suggested that an RNA-driven HR-based repair pathway may function in terminally differentiated neurons [209]. It will be interesting to examine whether ATM might be involved in such pathway. Other consequences of DNA damage in transcribed regions is abnormal mRNA splicing, early transcription termination or transcriptional readthrough, and formation of abnormal secondary RNA structures, all generating anomalous mRNAs, some of which do not leave the nucleus and therefore are never translated [210]. All of these lead to the same end: functional inactivation of transcription units (Fig. 2).

Thus, in the absence of ATM, random inactivation of transcription units probably occurs throughout the long life of these cells. Stochastically, PCs in which combinations of critical genes are inactivated functionally deteriorate and eventually die. The lack of regeneration and the small finite number of PCs, together with their critical role in the cerebellar output, make the loss of each individual cell a stepping-stone in

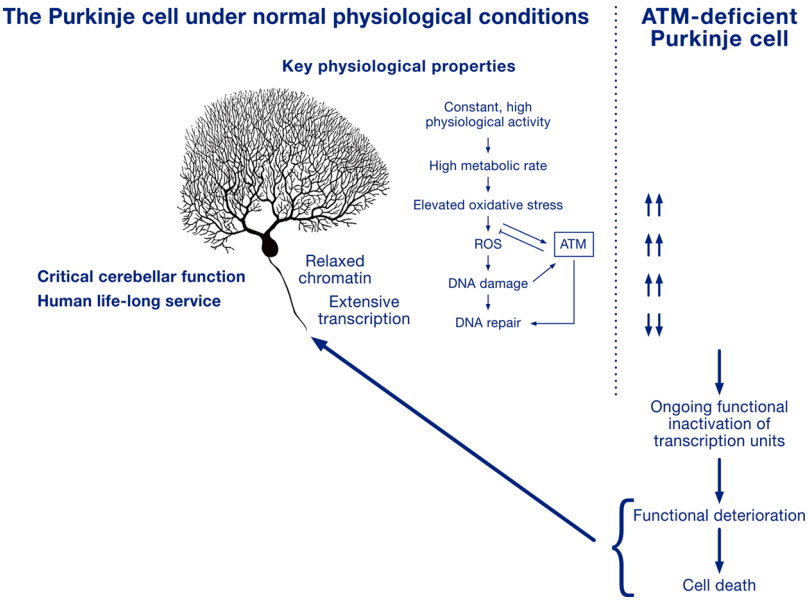

Fig. 2. A model depicting the unique internal physiological environment of Purkinje cells that makes ATM absence critical for their function and survival.

the cerebellar deterioration in A-T patients. Notably, long before their actual disappearance, abnormally functioning PCs can interfere with cerebellar function more than their loss altogether, accounting for the ataxia and other cerebellar symptoms noticed in A-T patients prior to marked loss of PCs and the ensuing collapse of the entire tissue.

7.3. Supportive evidence for the model from ataxias associated with DNA repair deficiencies

Several other postnatal, progressive cerebellar ataxias demonstrate that these conditions can be caused merely by defective response to endogenous DNA damage. Among the DNA lesions induced by endogenous ROS, single-strand breaks (SSBs) take the lion's share (Table 1). Their ongoing repair depends primarily on efficient processing of their damaged or modified termini that regenerates 'legitimate' ends for further processing by DNA metabolizing enzymes [211–213]. Several deficiencies of such end-modifying enzymes lead to autosomal-recessive cerebellar ataxias often accompanied by peripheral neuropathy (thoroughly reviewed in refs. [179,180,214,215]; see also refs. [216,217]. Briefly, this group includes the following disorders: ataxia with oculomotor apraxia type 1 (AOA1) caused by deficiency of aprataxin (APTX), which removes 5'-adenylates at DNA nicks or breaks that result from abortive DNA ligation reactions [218]; spinocerebellar ataxia with axonal neuropathy 1 (SCAN1) resulting from a mutation that inactivates the enzyme tyrosyl-DNA-phosphodiesterase 1 (TDP1), which hydrolyzes phosphotyrosyl peptides remaining at DNA 3' strand ends from trapped topoisomerase I (Top I) [219–222]; AOA4, attributed to specific mutations in the gene for polynucleotide kinase/phosphatase (PNKP), which either removes 3'-phosphates from strand ends or phosphorylates 5'-hydroxyl ends, thereby regenerating legitimate strand termini [214,217]; and ataxia with oculomotor apraxia-XRCC1 (AOA-XRCC1), in which a key player in SSB repair, XRCC1, is mutated. Notably, although PNKP has been implicated in the repair of both DSBs and SSBs, the pathological mutations in the corresponding gene affect primarily SSB repair [217]. Recently, mutations that inactivate tyrosyl-DNA-phosphodiesterase 2 (TDP2) were identified in the rare 'spinocerebellar ataxia autosomal recessive 23' (SCAR23) [223,224] – a more complex syndrome. TDP2 resolves the transient cleavage complex between DNA ends and topoisomerase II (Top2) in Top2-induced DSBs in transcribed DNA [219,225,226]. To this list can be added ataxia with oculomotor apraxia type II (AOA2) [227–229], which is caused by specific mutations in the gene encoding senataxin (SETX); this DNA:RNA helicase with roles in RNA homeostasis, regulation of transcription termination, and R-loop resolution, has recently emerged as a player in the interface between RNA metabolism and DNA damage [230–233]. All of these ataxias, including A-T, exhibit variable ages of onset and rates of progression, but ultimately lead to severe cerebellar demise depicted in strikingly similar MRI images.

A-T is more complex than these disorders, presumably due to ATM's role as a homeostatic hub of many cellular circuits. Also, the segmental premature aging in A-T [14] may contribute to early neuronal aging [2,178,234–237]. Still, a common thread among all of these diseases, including A-T, is postnatal, progressive cerebellar atrophy coupled to defective response to endogenous DNA damage, which most likely affects genome transcription and RNA processing. Notably, topoisomerase-1 (Top1) cleavage complexes – transcription-blocking lesions - were found to activate ATM [238,239] and their resolution was abrogated in ATM-deficient neuronal cells [240,241]. PC nuclei were found to contain particularly high amounts of Top1 [197]. It should also be noted that TDP1 and PNKP were identified as ATM substrates, and their ATM-mediated phosphorylation enhanced and optimized their activity [242–244]. These findings sparked our conjecture that ATM assists many DNA repair pathways by phosphorylating their key players [10]. SSB-induced activation of ATM was observed in dividing cells [245], but whether such activation occurs in terminally differentiated cells remains unclear. Nevertheless, the overarching role that we attribute to ATM in responding to a variety of DNA lesions [10] provides a wide umbrella for the case for defective response to DNA damage as the primary cause of the cerebellar degeneration in A-T.

Another example from the clinic, which deserves a separate discussion in the context of A-T, is A-T like disease (A-TLD) [246,247]. This extremely rare, autosomal-recessive disorder is very similar to A-T, hence its name. Indeed, despite some differences, A-TLD's hallmark is common to both diseases – progressive cerebellar degeneration. Despite the later age of onset and slower progression in A-TLD, the final neurological phenotypes and cerebellar MRI images are roughly similar in the two genetically distinct diseases. A-TLD is molecularly defined by its causative mutations – hypomorphic mutations at the *MRE11* gene encoding the nuclease component of the MRN complex [248]. This three-protein complex is a primary DSB sensor and fulfills several roles in the initial detection and end-processing of this lesion and perhaps others, as well as the initiation of the ensuing signaling network [249–252]. The phenotypic similarity between A-T and A-TLD led us to postulate that the molecular basis for this similarity was a requirement of the MRN complex for ATM activation. According to this hypothesis, defective activation of ATM by DNA damage leads to the A-TLD phenotype. We obtained solid evidence for such a requirement [41], which was subsequently corroborated and mechanistically studied to become the canonical, DSB-induced and MRN-dependent mode of ATM activation (reviewed in ref. [12]. The *MRE11* mutations that lead to A-TLD affect the organization and action of the MRN complex in several ways [253–255]. Importantly, hypomorphic *NBS1* and *RAD50* mutations cause two variants of a different genome instability disorder, the Nijmegen breakage syndrome (NBS), which is less similar to A-T compared to A-TLD [256–258]. Nevertheless, the molecular basis of A-TLD [41] argues for placing the cerebellar aspect of this disease as well as that of A-T in the genome stability domain. Many other aspects of the complex A-T phenotype that are not seen in A-TLD are presumably contributed by the absence of the homeostatic functions of ATM, which are not MRN-dependent. This notion calls for the interesting possibility that low-level, MRN-dependent activation of ATM is stimulated continuously in response to endogenous DNA lesions, not all of which are necessarily DSBs. Compared to the explosive activation of ATM in response to DSBs [44], studying such ongoing, low-level activity of ATM might be experimentally challenging, but its existence is collectively suggested by the above premises and rationale, and by the long and progressive course of A-T.

8. Future perspectives

The discussion on the molecular basis of the cerebellar degeneration in A-T continues and acquires new dimensions as new roles for ATM in cellular homeostasis are uncovered. A central experimental tool for investigating this question and testing emerging hypotheses is the mouse model of A-T. The first *Atm*-knockout mouse was hailed as "a paradigm of ataxia-telangiectasia" [259]. However, since then numerous studies with a variety of *Atm*-manipulated mice have shown that Atm-deficient mice display great variations of the hallmarks of the human disease, ranging from amplification of a human A-T symptom in the case of cancer predisposition to an extremely diminished version of the human cerebellar phenotype [17,259–263]. This phenotype can be discerned in the animals using fine tests that reveal delicate behavioral deficits, and histological analysis that occasionally discloses ectopic PCs or PCs with irregular shapes [109,151,175,262–265]. But, following such a subtle phenotype is challenging. In addition, the expression of the disease features in mouse models may be strongly affected by strain background, as was convincingly shown for the predisposition to lymphomas conferred by the *Atm-/-* genotype [266]. These difficulties raised doubts about the use of mouse models to study the cerebellar aspect of A-T [263].

We assume, however, that the physiological functions of ATM are largely similar in humans and mice, and the different neurological

phenotypes caused in the two organisms by ATM absence stem from structural-physiological differences between their nervous systems. Such differences were recently demonstrated at the level of the PC transcriptome [267]. Nevertheless, since mouse models bearing severe DNA repair deficiencies do show neurological phenotypes [268], we can assume that more than Atm deficiency is required to push the murine cerebellum to a more severe, human-like phenotype. In a useful mouse model, the very mild cerebellar phenotype of the Atm-deficient animal would be augmented by adding genetic alterations that lead to further pressure on the animal's ability to cope with the ongoing DNA damage. Such genetic changes might be moderate, e.g., heterozygosity for mutations in genes encoding proteins that deal with the endogenous DNA damage. Another genetic alteration, one that would make the mouse model amenable to extended follow-up, would be to reduce the predisposition to lymphomas. Alternatively, a combination of tissue-specific, conditional genetic alterations based on the Cre-lox technology could be used.

A direct approach to human PCs can potentially be obtained using in-culture differentiation to PCs of induced pluripotent stem cells (iPSCs). iPSCs that were derived from A-T patients retained the A-T cellular phenotype [269–272], and this phenotype could be corrected when the WT *ATM* allele was restored in these cells using CRISPR/Cas9 technology [273]. Cerebellar progenitors derived from A-T iPSCs resulted in cells with characteristics of post-mitotic neurons, with different gene expression patterns in ATM-deficient cerebellar progenitors compared to controls [274]. Obtaining definitive cerebellar PCs from iPSCs is technically challenging, but continuous progress in this direction is being made [267,275–277]. A recent optimization of the protocol for obtaining human PCs in this way has indicated that the cells exhibit PC-specific gene expression patterns [267].

Understanding the molecular basis of the cerebellar decay in A–T should point the way to new treatment modalities that will target primarily this debilitating symptom. Its successful treatment will likely impact on other aspects of A-T and other cerebellar ataxias.

Declaration of Competing Interest

None.

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