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Review

Ataxia-telangiectasia-like disorder (ATLD)—its clinical presentation and molecular basis

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Abstract

Comparison of the clinical and cellular phenotypes of different genomic instability syndromes provides new insights into functional links in the complex network of the DNA damage response. A prominent example of this principle is provided by examination of three such disorders: ataxia-telangiectasia (A-T) caused by lack or inactivation of the ATM protein kinase, which mobilises the cellular response to double strand breaks in the DNA; ataxia-telangiectasia-like disease (ATLD), a result of deficiency of the human Mre11 protein; and the Nijmegen breakage syndrome (NBS), which represents defective Nbs1 protein. Mre11 and Nbs1 are members of the Mre11/Rad50/Nbs1 (MRN) protein complex. MRN and its individual components are involved in different responses to cellular damage induced by ionising radiation and radiomimetic chemicals, including complexing with chromatin and with other damage response proteins, formation of radiation-induced foci, and the induction of different cell cycle checkpoints. The phosphorylation of Nbs1 by ATM would indicate that ATM acts upstream of the MRN complex. Consistent with this were the suggestions that ATM could be activated in the absence of fully functional Nbs1 protein. In contrast, the regulation of some ATM target proteins, e.g. Smc1 requires the MRN complex as well as ATM. Nbs1 may, therefore, be both a substrate for ATM and a mediator of ATM function. Recent studies that indicate a requirement of the MRN complex for proper ATM activation suggest that the relationship between ATM and the MRN complex in the DNA damage response is yet to be fully determined. Despite the fact that both Mre11 and Nbs1 are part of the same MRN complex, deficiency in either protein in humans does not lead to the same clinical picture. This suggests that components of the complex may also act separately.

Keywords: Ataxia-telangiectasia; Ataxia-telangiectasia-like-disorder; Nijmegen Breakage syndrome

1. Introduction

Ataxia-telangiectasia (A-T) ([1]; Chun and Gatti, this issue) is one of a group of recessively inherited ataxias and a prominent example of a genomic instability syndrome. A-T is caused by loss of function of the ATM protein due to mutations in the corresponding gene, *ATM*. ATM is a nuclear protein kinase that is responsible for the activation of the highly branched cellular response to double strand breaks in the DNA (see Kurz and Lees-Miller in this issue for review). A-T can be diagnosed in very young children at about the time that they start to walk. The presence of both progressive cerebellar ataxia and telangiectasia (dilated blood vessels, usually in the eyes) is seen as a requirement for the clinical diagnosis of A-T [1]. Other major clinical characteristics of A-T are immunodeficiency, genomic instability, predisposition to lymphoreticular malignancies and sensitivity to ionising radiation (Chun and Gatti, this issue).

The disease recognised as classical A-T is usually caused by biallelic truncating mutations that result in the total absence of ATM protein. The proportion of A-T patients with two truncating *ATM* mutations, however, may depend on the population being studied. In some individuals, the presence of other types of *ATM* mutation may result in the onset of ataxia being delayed until teenage or even early adulthood. One can also speculate that some patients may have either a milder form of the disorder or a different presentation that is not recognised as being caused by mutation of the *ATM* gene. The range of clinical presentations may, therefore, be wider than currently appreciated and may depend on the spectrum of *ATM* mutations in a particular population. The corollary of this scenario is that there may be phenocopies of A-T caused by mutations in other genes. Ataxia-telangiectasia-like dis-

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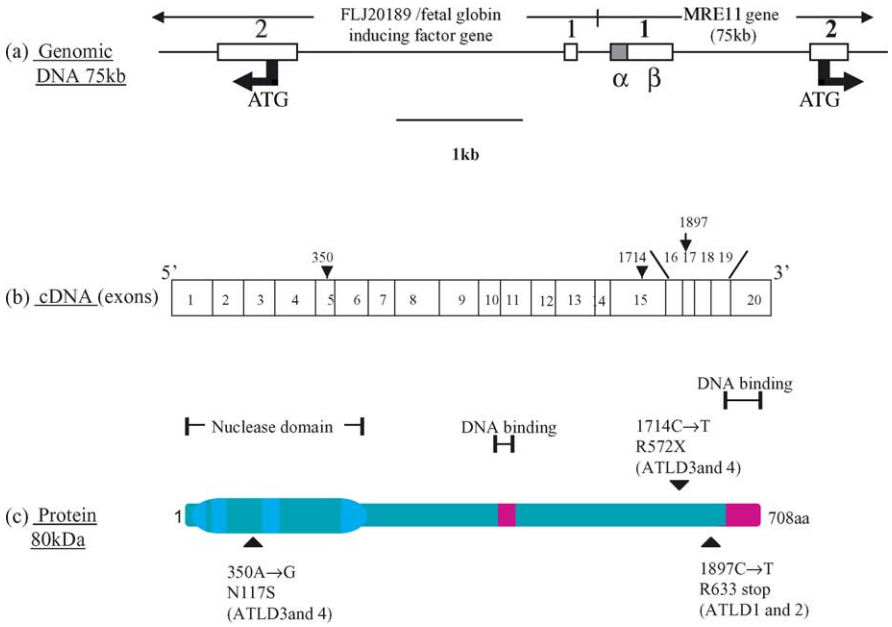

Fig. 1. The gene, cDNA (after Pitts et al. [3]) and protein (after D'Amours and Jackson [63]) for human Mre11 protein. The mutations causing known cases of ATLD are indicated.

order (ATLD) is such a case and is caused by mutation in the *hMRE11* gene (Fig. 1) [2].

2. Clinical features of patients with hMRe11 deficiency—ataxia-telangiectasia-like disorder

Ataxia-telangiectasia-like disorder is very rare with, at present, only six known cases, four in the UK and two in Italy [3]. The clinical features of patients with A-T-like disorder are very similar to those of A-T (Table 1); the clearest similarity being with the progressive cerebellar ataxia that the ATLD patients show. In contrast to A-T, however, ATLD patients show no telangiectasia [4,5]. Compared with A-T, ATLD is characterised by a later onset of the neurological features, and slower progression of the disorder to give the overall appearance of a milder condition than A-T in the early years. ATLD patients also show normal levels of total IgG, IgA and IgM although there may be reduced levels of specific functional antibodies. Since hMre11 is a component of a complex with Nbs1 and Rad50 [6], it is perhaps surprising that a deficiency in Mre11 gives rise to an A-T-like disorder rather than a more Nijmegen breakage syndrome (NBS)-like disorder. NBS is caused by hypomorphic mutations in the gene encoding the Nbs1 protein (Digweed and Sperling, this issue). NBS is characterised by microcephaly, growth retardation, immunodeficiency and predisposition to tumours. At the clinical level A-T, NBS and ATLD are quite distinct although there are some similarities (Table 1). Patients from each disorder show a measurable immunodeficiency and both A-T and NBS patients have an increased risk of developing lymphoid tumours. B and T cell tumours are seen although the proportions do not appear to be the same for these two disorders. It is not known whether ATLD patients have a predisposition to cancer as too few patients have been described so far. It is of note, however, that $Mre11^{ATLD1/ATLD1}$ mice do not develop lymphomas [7].

All three disorders show an increased level of chromosome translocations in the peripheral blood involving chromosomes 7 and 14. At the cellular level, all three disorders exhibit hypersensitivity to ionising radiation, measured chromosomally and by cell survival, radioresistant DNA synthesis (RDS) and failure to induce stress activated protein kinases following exposure to IR [2,8,9].

Table 1
Comparison of the clinical features of ataxia-telangeictasia (A-T), ataxia-telangiectasia-like disorder (ATLD) and Nijmegen breakage syndrome (NBS)

Clinical feature	Classical ataxia-telangiectasia	Ataxia-telangiectasia-like disorder	Nijmegen breakage syndrome
Ataxia	+	+	−
Telangiectasia	+	−	−
Dysarthria	+	+	−
Abnormal eye movements	+	+	−
Raised serum AFP level	+	−	−
Reduced immunoglobulin levels	+	−*	+
Pulmonary infections	+	NK	+
7/14 chromosome Translocations	+	+	+
Lymphoid tumours	+	NK	+
Other tumour types	+	NK	+
Skin abnormalities	+	NK	+
Microcephaly	−	−	+
Craniofacial abnormalities	−	−	+
Normal intelligence	+	+	+/−
Congenital malformations	−	−	+

+: presence of feature; −: absence; NK: not known.
* Deficiency in specific functional antibodies.

3. *hMRE*11 mutations and hMre11 expression in ATLD

Attempts to make null mutants of *hMRE11* and *hRAD50* resulted in lethality [10,11], suggesting that the MRN complex is essential. Consistent with this, the ATLD patients each express, from a hypomorphic *hMRE11* allele, some hMre11 protein which is either truncated or full-length and mutant. In one UK family truncated hMre11 protein was expressed in cells homozygous for the mutation 1897C > T (633R > stop) and in the second family full length mutant hMre11 was expressed from an allele 350A > G (117N > S), but not from the second allele 1714C > T (572R > stop) (Fig. 1). In the latter family nonsense mediated mRNA decay appeared to operate very effectively on the transcripts from the 1714C > T allele [3]. Although hMre11 protein is expressed in ATLD cells, the level of expression of hMre11 between patients is not normal but varies according to genotype. Also notable is a considerable reduction in levels of Nbs1 and hRad50 proteins in hMre11-deficient cells.

The N-terminal region of hMre11 is required for interaction with the C-terminal 101 aa of Nbs1 [12–14] and required for localisation to the nucleus (Fig. 1). In lymphoblastoid cells derived from NBS patients homozygous for the 657del5 mutation the predicted truncated protein of 26 kDa resulting from this deletion is present. Also found in NBS LCLs, is a 70 kDa Nbs1 protein lacking the N-terminal domain but carrying the ser 343 site. Interestingly, the p26 protein was not associated with hMre11 but the 70 kDa protein was, as might be predicted. In NBS LCLs, the p70 protein was localised to the nucleus with approximately 50% of hMre11 suggesting that the p70 protein facilitates retention of the Mre11 complex in the nucleus of NBS cells [15]. Like *hMRE11*, *NBS1* is an essential gene and NBS patients carry hypomorphic *NBS1* mutations.

The strength of interaction of mutant hMre11 with both the Nbs1 and hRad50 components of the MRN complex in cells from ATLD patients is dependent on the *hMRE11* mutations present. Protein from cells homozygous for 633R > stop or heterozygous for 117N > S and 572R > stop both interact with Nbs1 and hRad50 although at lower than normal levels. However, a stronger interaction between hMre11 and hRad50 was observed in cells from patients with 117N > S and 572R > stop compared with the interaction of hMre11 and hRad50 in cells homozygous for *hMRE11* mutation 633R > stop [2].

While ATLD patients biallelic for mutations resulting in total loss of protein are not expected in the population there is potential for patients homozygous or heterozygous for missense mutations but it is not known what clinical phenotype might be associated with such genotypes. The existence of different clinical phenotypes for ATLD and NBS suggests that despite its presence in the same complex, Mre11 has a function(s) quite distinct from Nbs1 (and possibly hRad50).

4. The MRN complex and the DNA damage response

There is evidence for the MRN complex or its components being involved in different responses to cellular damage induced by ionising radiation.

4.1. Localisation of the MRN complex

The MRN complex has been shown to rapidly associate with sites of DNA damage in response to agents that induce DNA DSBs [16]. There are at least three known types of MRN foci [17,18]. In untreated cells, type I foci are seen where Mre11 co-localizes with PML bodies [17,19,20]. Within 10 min of DNA damage and up to 8 h post-treatment type II foci are observed which are small and numerous. From 4 h post treatment and lasting up to 24 h type III foci, also referred to as irradiation-induced foci (IRIF), are observed which are large irregularly shaped aggregates with unknown function. ATLD cells (with hMre11 mutated) [2] and NBS cells [6,17] or cells expressing Nbs1 protein with targeted mutations in the FHA or BRCT domains [13,21] fail to form type III foci.

4.2. Interaction of the MRN complex with γH2AX—sites of DNA DSB repair

In response to double strand breaks the histone H2A isoform H2AX is rapidly phosphorylated (γH2AX) at ser 139 and forms foci at the sites of DNA damage [22–24]). This phosphorylation is ATM dependent with DNA-PK possibly playing a secondary role [25]. The phosphorylation of H2AX in response to IR does not require Nbs1 as H2AX phosphorylation is normal in NBS cells [26,27]. γH2AX interacts with 53BP1 [28] and the FHA/BRCT domains of Nbs1 [26] and co-localizes with 53BP1, the MRN complex and Brca1 at sites of DNA damage induced by ionising radiation [29–31]. Phosphorylation of Nbs1 ser 343 was not required for the interaction of Nbs1 with γH2AX; neither was Mre11 a requirement for the interaction of Nbs1 with γ-H2AX. For A-T cells, with reduction in γH2AX a defect in Nbs1/γH2AX interaction might be expected but, curiously, the effect of this, appears to be small [26].

The assembly of the DNA damage response proteins at the sites of DNA damage does not solely depend on γH2AX but also requires MDC1/NFBD1 [32–36]. In response to DNA damage induced by IR, UV and HU, MDC1/NFBD1 is phosphorylated in an ATM and Nbs1 dependent manner. Irrespective of treatment with DNA damaging agents MDC1 interacts with the proteins of the MRN complex, 53BP1, Smc1 and to a lesser extent with ATM and FANCD2 [32,34]. MDC1 is involved in the formation of MRN and BRCAI IRIF [32–36]). Similar to cells with deficiency of the MRN complex, cells deficient in MDC1/NFBD1 were unusually sensitive to ionising radiation, exhibited RDS, failed to induce the intra S-phase checkpoint, and were unable to activate the G2/M cell cycle checkpoint [32,34,37].

4.3. Cell cycle checkpoints—phosphorylation of p53 ser 15 in ATLD and NBS cells

Following IR damage the p53 protein is activated and stabilized in the cell [8]. This leads to transactivation of several p53 target genes, among them the gene encoding $p21^{WAF1/CIP1}$, which inhibits the progress from G1 to S. The stabilisation of p53 in ATLD cells after exposure of cells to ionising radiation is normal [2]. NBS cells have also been reported to stabilise and activate p53 normally [38–40] although other reports have disputed this [41,42]. If NBS cells stabilise and activate p53 normally, this would suggest a normal G1/S checkpoint in NBS cells. p53 ser 15 phosphorylation may modulate the transactivation of genes by p53. Lim et al. [39] and Gatei et al. [43] suggested that there were normal levels of p53 ser 15 phosphorylation in NBS cells following exposure of cells to 5 Gy γ-rays. Girard et al. [27] reported that although Nbs1 was not required for the phosphorylation of ser 15 at high radiation doses (5 Gy), it was required for efficient phosphorylation at low doses (1 Gy).

4.4. Cell cycle checkpoints—the ATM-Nbs1-Smc1 S-phase checkpoint

Interestingly, A-T, ATLD and NBS cells are all defective in the S-phase checkpoint. In response to exposure to IR ATM phosphorylates Nbs1 at ser 343 in vivo, the primary Nbs1 residue phosphorylated. This phosphorylation is required for the S-phase checkpoint [20,39,44]. In vitro studies also showed that ATM phosphorylates ser 278 [44], and ser 397 and ser 615 [20] of Nbs1 which may also be involved in the radiation-induced mobility shift of Nbs1. IR-induced Nbs1 phosphorylation demonstrated by mobility shift was observed in lymphoblastoid cell lines derived from ATLD patient 3 but nearly absent in ATLD2 cells [18]. In contrast Falck et al. [45] observed a normal Nbs1 mobility shift in ATLD2. Curiously, the phosphorylation of full length Nbs1 as seen in ATLD3, however, is not sufficient to prevent a deficient S-phase checkpoint in these cell lines. It is not clear whether this is due to the level of Nbs1 required or the requirement for other factors for the S-phase checkpoint in order to avoid RDS. Clearly there are additional S-phase checkpoint pathways.

ATM has been shown to phosphorylate the structural maintenance of chromosome Smc1 protein at serine 957 and serine 966 in response to ionising radiation and this pathway is thought to be involved in the S-phase cell cycle checkpoint. ATM phosphorylation of Smc1 was dependent on both Nbs1 and BRCA1 according to Kim et al. [46], although Yazdi et al. [47] reported only a dependence on Nbs1 and an apparent independence of BRCA1.

Using laser 'scissors' Kim et al. [46] showed that cohesin as measured with Smc1 antibody was recruited to the sites of damage in normal cells. Smc1 was also recruited to damage sites in A-T and NBS cells but not in *hMRE11* mutated cells suggesting a role for hMre11 in recruitment of cohesin to these damage sites. It appears that there is separation of function for recruitment of Smc1 to damage and its phosphorylation. Recruitment is ATM and Nbs1 independent but phosphorylation is both ATM and Nbs1 dependent.

4.5. Cell cycle checkpoints—ATM-Chk2-Cdc25A-Cdk2 S-phase checkpoint

The precise role of Chk2 in the DNA damage response is still to be determined. [48–53]). Chk2 is reportedly involved in an S-phase checkpoint pathway (ATM-Chk2-Cdc25A-Cdk2) in parallel with the ATM-Nbs1-Mre11 pathway ([45]; Lukas et al., this issue). The defect in the S-phase checkpoint is greater in A-T compared with NBS and ATLD cells [2] and it is suggested that residual RDS in both ATLD and NBS cells is due to the presence of normal activation of Chk2 and the ATM-Chk2-Cdc25A-Cdk2 pathway and a deficiency in the ATM-Nbs1-Mre11 pathway. Falck et al. [45] suggested that Chk2 phosphorylation was normal in both NBS and ATLD2 cells, at least in terms of the Chk2 mobility shift.

NBS cells are reported elsewhere, however, to be impaired in Chk2 phosphorylation after treatment with 5, 10 and 20 Gy ionising radiation [27,40]. Expression of an Nbs1 mutant lacking the Mre11 binding domain did not restore Chk2 phosphorylation, suggesting that formation of the MRN complex was required for ATM dependent activation of Chk2 in response to IR [40].

4.6. Cell cycle checkpoints—the ATM/Nbs1-Mre11/FANCD2 component of the S-phase checkpoint

Increasing evidence suggests that Fanconi anaemia (FA), NBS and ATLD are syndromes with related cellular defects. NBS and ATLD cells are also hypersensitive to mitomycin C, a DNA cross linking agent, that induces chromosomal breakage and quadriradial chromosome formation [14], a phenotype used to diagnose FA. Overlapping clinical features have also been observed between NBS and FA patients [54,55].

FANC A, C, E, F and G proteins assemble into a multisubunit nuclear complex involved in the monoubiquitination of FANCD2 at lysine 561 in response to DNA damage and during S-phase. Monoubiquitinated FANCD2 colocalises with Brca1 [56] and Rad51 [57] in subnuclear foci, implying a role for the FA pathway in homologous recombination. Interestingly, $Brca1^{-/-}$ cells are unable to form FANCD2 foci.

$FANCD2^{-/-}$ cells are hypersensitive to ionising radiation and are defective in their ability to activate the S-phase cell cycle checkpoint [57], which are characteristics of A-T, NBS and ATLD cells. In response to ionising radiation ATM phosphorylates FANCD2 on ser 222, which is required for the activation of the S-phase cell cycle checkpoint [57]. How ATM dependent phosphorylation affects the check-

point is unknown. IR failed to activate phosphorylation of ser 222 of FANCD2 in the ATLD1 cell line as well as NBS cells suggesting that Nbs1 and Mre11 facilitate or activate ATM-dependent phosphorylation of FANCD2 ser 222 following exposure to IR [14]. The phosphorylation of Nbs1 on ser 343 appears to be required for FANCD2 ser 222 phosphorylation.

4.7. Requirement of the Mre11 complex for activation of ATM

Recent work has suggested that the function of the Mre11 complex may be required for full ATM activation. This conclusion came partly from using the 633R > stop ATLD cells representing a more severe form of ATLD and cells compound heterozygous for 117N > S and 572R > stop [58]. Findings include the observation that ATM autophosphorylation at ser 1981 [59], used as a marker for ATM activation, was reduced in ATLD and NBS cells suggesting that a functional MRN complex is required for autoactivation of ATM. Phosphorylation of Chk2 (Nbs1 dependent at low dose), p53 ser 15 (variably dependent on Nbs1) and Hdm2 (Nbs1 independent) were all reduced in ATLD3 cells but more so in ATLD2 [58]. Using an entirely different system, infection with an adenovirus lacking the E4 region that results in a cellular DNA damage response, activation of ATM was also shown to be Mre11 dependent [60].

4.8. One DNA damage response protein complex (MRN) but different clinical phenotypes

It is clear that at the cellular level, the responses to DNA damage are very similar between NBS and ATLD cells although quantitatively not as severe as similar responses (IR induced cell survival, RDS, chromosome aberrations, etc.) in A-T cells. Quantitative differences in cell survival, RDS, and chromosome damage following IR exist between these two cell types [2] but qualitative differences (different types of response) have been difficult to identify. Presumably the interplay of damage response pathways, whether involving damage sensors, transducers or mediator/effectors, intimately involves the MRN complex at more than one of these stages, thereby conferring a similar cellular phenotype in ATLD and NBS cells.

The wider clinical response is a different matter and the clinical differences between ATLD and NBS patients expose our incomplete knowledge both of the role of the MRN complex and possible additional separate roles of its components. Interestingly, the clinical differences are quite widespread involving, the central nervous system, some congenital defects and possibly the predisposition to cancer, particularly lymphoid tumours (Table 1). The progressive cerebellar defect in ATLD is similar to that seen in A-T, although age at presentation and rate of progression of ATLD would suggest that it is not as severe as classical A-T. The reason for the different clinical phenotypes between NBS and ATLD is unknown although there are several possible explanations. It was suggested that since most NBS patients are homozygous for one *NBS1* mutation, and there is a founder effect, the inference was that they might also be homozygous at other loci, which, if linked, might influence the common phenotype of NBS patients (Petrini, 2000). However, other NBS patients homozygous for different NBS1 mutations are now known who have a similar phenotype. Animal models of NBS and ATLD are a potential means of indicating whether *NBS1* and *hMRE11* mutations result in similar phenotypes (Petrini, 2000). However, although animal models exist, they do not completely recapitulate patient phenotypes, especially the neurological aspects, few mutations are modeled and so comparisons are not straightforward [7,61]. If mutation of different components of a complex does not result in a similar clinical outcome then a possible alternative is that the components have roles in addition to being part of a complex. What is the evidence that Nbs1 and hMre11 have roles independent of the MRN complex? The components of the complex may act separately as is the case for the interaction of Nbs1 and γH2AX [26] or of Nbs1 with TRF2 [62]. There may also be tissue specific interactions, involving the lymphoid system or parts of the central nervous system. Nbs1 and hMre11 (so far) appear not to have overlapping roles in lymphoid cells at least concerning lymphoid tumour development. Investigation of the role of hMre11, hRad50 and Nbs1 in different cell types, including neuronal cells, may reveal them to have hitherto unknown functions.

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