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Review

The two edges of the ATM sword: Co-operation between repair and checkpoint functions

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Abstract

ATM is a central component of a signal transduction process that responds to DNA double strand breaks (DSBs) ultimately effecting cell cycle checkpoint arrest and/or apoptosis. Recent studies have shown that ATM also regulates a mechanism of processing a subset of DNA ends that appear to be difficult to ligate, since they are rejoined with slow kinetics in control cells. In the absence of this process, which involves the nuclease, Artemis, the DSBs either remain unrejoined or potentially undergo misrejoining. Thus, ATM's checkpoint function specifically facilitates its repair function. Here, we discuss the contribution of this novel function of ATM to survival after ionising irradiation and to cancer avoidance. We suggest that ATM's strength as a damage response protein lies in the co-ordination of its repair and checkpoint functions making a razor sharp knife out of two blunter edges.
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It was thirty years ago, in 1975, that ataxia telangiectasia (A-T) was first recognised as representing a damage response disorder with the observation that skin fibroblasts from A-T patients display radiosensitivity, a feature that correlated with A-T radiosensitivity observed in the clinic. Just a few years later, chromosome analysis led to the conclusion that A-T cells display unrepaired strand breaks, which was proposed to be causal of A-T radiosensitivity [1]. Analysis of chromosome aberrations in A-T cells carried out over the ensuing years provided remarkable insight into the basis underlying the DNA damage response defect in A-T cells, leading to the conclusion that A-T cells fail to repair or misrepair chromosome breaks. Failure to carry out the repair of potentially lethal damage (RPLD) represented another feature of A-T characterised during the 1980s [2–4]. RPLD represents the enhanced recovery of cells held under non-growing conditions for a period after exposure to ionising radiation (IR). Failure to display RPLD was deemed to represent an inability to carry out a repair process that operated during the holding conditions, with evidence strongly suggesting that this represented a form of double strand break (DSB) repair. Collectively, these data strongly suggested that A-T is a repair defective syndrome. However, the phenomenon of radioresistant DNA synthesis (RDS), namely a failure to arrest DNA synthesis in the face of DNA damage, was another described characteristic of A-T cells. This raised the alternative possibility that the defect in A-T is a failure to respond to DNA damage rather than an inability to repair the damage [5,6]. This was firmly consolidated in 1992 with a report that A-T cells, in addition to failing to arrest DNA synthesis in S phase, also fail to undergo p53-dependent G1/S arrest after exposure to IR [7] providing strong evidence that A-T is a checkpoint defective disorder. This conclusion was received with some criticism, however, since, whilst suitably explaining some features of A-T cells, it did not adequately explain their dramatic radiosensitivity, the RPLD phenotype or aspects of the A-T phenotype based on the chromosome analysis [8–11]. The notion that A-T represents a checkpoint defective condition gained credence when the gene defective in A-T, ataxia telangiectasia mutated (ATM), was identified as a protein kinase [12,13] with structural similarity to PI-3 kinases. Subsequently, a vast literature demonstrating the role of ATM in regulating a damage response pathway that ultimately leads to cell cycle checkpoint arrest and apoptosis has been described and few would argue that ATM is not a central player in checkpoint signalling, and thus that A-T represents a checkpoint defective disorder. However, recently, ATM has also been shown to regulate a component of DNA repair demonstrating that A-T also represents a repair defective disorder [14]. Here, we aim to review these two aspects of ATM function and discuss how they influence the A-T phenotype.

ATM's role in cell cycle checkpoint control

The role of ATM in the regulation of cell cycle checkpoint arrest has been reviewed previously [15,16] and only a brief

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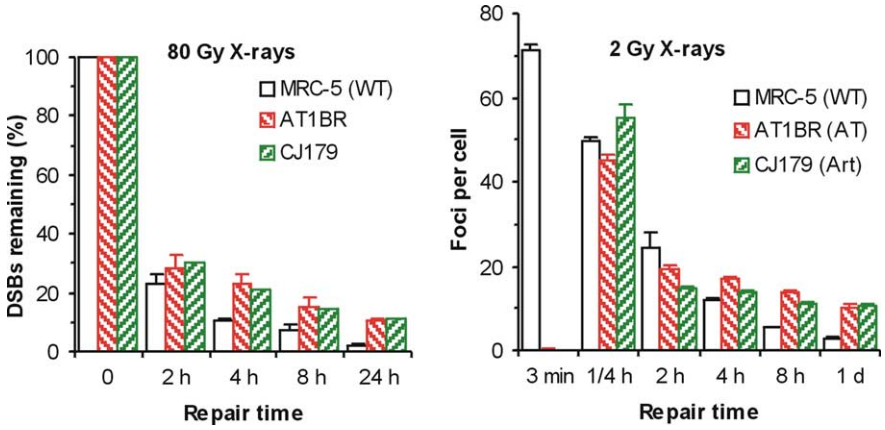

Fig. 1. Kinetics of DSB repair in ATM and Artemis deficient cells. The left panel shows DSB repair monitored by PFGE. DSB repair monitored indirectly by the rate of loss of γ-H2AX foci is shown in the right panel.

overview will be given here. Recent studies have provided strong evidence that the primary sensor of a DSB is the Mre11-Rad50-Nbs1 (MRN) complex, which is required for ATM activation [17,18]. A major difficulty at reaching this conclusion is the fact that the MRN complex is essential for growth, potentially due to a distinct role of MRN in ataxia telangiectasia and Rad3-related (ATR) signalling [19]. Thus, the mutations identified in Mre11 and Nbs1 in ataxia telangiectasia like disorder (ATLD) and Nijmegen Breakage Syndrome (NBS), respectively are hypomorphic, a feature making it difficult to assess results obtained with them. Notwithstanding this limitation, the analysis of ATLD cell lines has demonstrated that Mre11 is required for ATM activation [17]. NBS cells are only mildly reduced in ATM activation but, in distinction, ATM fails to accumulate in foci in NBS cells [17,20]. Important insight was recently gained by the finding that Nbs1 directly interacts with ATM and, whilst being dispensable for ATM activation, is required to localise ATM to the site of damage [20]. However, the different components of the MRN complex are required for each other's activities; for example, Nbs1 is required to localise Mre11 to the nucleus and Mre11 enhances Nbs1 stability. Collectively, these combined activities of the MRN complex result in its requirement for ATM-dependent phosphorylation events, consistent with diminished cell cycle checkpoint arrest in the ATLD and NBS cell lines [18,21].

Multiple ATM-dependent substrates have been identified but here we will focus on those known to be required for ATM-regulated cell cycle checkpoint arrest and repair. The first of these are involved in the G1/S checkpoint, which in addition to requiring ATM has also been shown to be dependent on p53 [22]. ATM both directly and indirectly, via Chk2, phosphorylates p53 [7,23,24]. In addition, Mdm2, the ubiquitin ligase that regulates p53, is also a target of ATM phosphorylation [25,26]. Together, IR-induced modifications of p53 and Mdm2 stabilize p53 and enhance transcription of p53 regulated proteins. p21(WAF1/Cip1), an inhibitor of cyclin-dependent kinases (Cdks), is the principal p53 regulated target effecting G1/S arrest, which it promotes by inhibiting the ability of cyclinE/Cdk2 to phosphorylate the retinoblastoma (RB) protein (see [27] for a review). ATM also regulates an intra-S phase checkpoint, which underlies the classical RDS phenotype. For this checkpoint, the prevailing evidence suggests that ATM-activated Chk2/Chk1 phosphorylates Cdc25A leading to enhanced Cdc25A degradation as well as increased binding of 14-3-3 protein [28,29]. These two events prevent Cdc25A phosphatase from activating Cdk2, an event required for origin firing. There is, additionally, evidence for a parallel pathway regulated by ATM that is distinct from the Chk2/Cdc25A pathway and requires the MRN complex [30]. Since, Brca1 and Smc1 are also required for efficient intra-S phase arrest after IR, it is possible that these proteins also function in the MRN-dependent pathway. The existence of two overlapping pathways is consistent with the finding that Chk2 deficient mouse embryo fibroblasts show only a partial intra-S phase checkpoint defect [31]. Finally, ATM also regulates a G2/M checkpoint which is required to arrest cells which are in G2 at the time of irradiation. The likely steps in this process depend on activation of Chk1/Chk2, leading to the inhibition of the Cdc25C phosphatase and hence a delay in the activation of cyclinB1/Cdk1. However, via feedback regulation, there is also evidence that the other two Cdc25 phosphatases, Cdc25A and Cdc25B also participate in this process, and that they may also be regulated by the Chk1/Chk2 kinases [27].

An early step in the DNA damage response is phosphorylation of the histone H2A variant, H2AX, a step which can be carried out redundantly by ATM and DNA-PK, and is thus observed in A-T cell lines [32,33]. Phosphorylated H2AX extends to megabase regions of DNA from the site of the DSB and can be visualised as discrete foci using immunofluorescence with antibodies directed against phosphorylated H2AX, designated γ-H2AX [34]. H2AX phosphorylation is required for the retention rather than the recruitment of several damage response proteins at the site of damage [35–37]. These proteins, termed mediators, include MDC1, 53BP1, BRCA1 and the MRN complex and are characterised by the presence of BRCT motifs. The presence of these proteins facilitates to some degree the phosphorylation of certain ATM substrates as well as cell cycle checkpoint arrest but their precise involvement in checkpoint signalling is still unclear.