Radiotherapy and Oncology 76 (2005) 119–122
www.thegreenjournal.com

Review

ATM signaling and 53BP1

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Abstract

The ATM (mutated in Ataxia-Telangiectasia) protein kinase is an important player in signaling the presence of DNA double strand breaks (DSBs) in higher eukaryotes. Recent studies suggest that ATM monitors the presence of DNA DSBs indirectly, through DNA DSB-induced changes in chromatin structure. One of the proteins that sense these chromatin structure changes is 53BP1, a DNA damage checkpoint protein conserved in all eukaryotes and the putative ortholog of the S. cerevisiae RAD9 protein. We review here the mechanisms by which ATM is activated in response to DNA DSBs, as well as key ATM substrates that control cell cycle progression, apoptosis and DNA repair.

Keywords: ATM; 53BP1; NBS1; MRE11; RAD50; DNA damage checkpoint

Overview of checkpoint responses to DNA damage

Of all the types of DNA damage, DNA double strand breaks (DSBs) represent the greatest threat to the integrity of the genome. To respond to this threat, eukaryotic cells have developed mechanisms that sense the presence of DNA DSBs and initiate a DNA damage response that is appropriate for the extent of the damage. This response includes DNA repair, but also a so-called 'checkpoint' response that leads to cell cycle delay or, in multicellular organisms, to programmed cell death or senescence [16,24,31]. The checkpoint response is critical for maintaining genomic integrity. The cell cycle delay provides time for DNA repair, as evidenced by rescue of the radiosensitive phenotype of yeast checkpoint mutants when cell cycle progression is experimentally slowed down [36]. In multicellular organisms genomic integrity is also maintained by programmed cell death or senescence, both of which prevent cells that might accumulate mutations from replicating and possibly developing into cancer [3,13].

Significant progress has been made in elucidating the DNA DSB checkpoint pathway. This pathway consists of sensors that sense the presence of DNA DSBs, signal transducers that generate and amplify the DNA damage signal and effectors that induce cell cycle delay, programmed cell death or senescence. The signal transducers and effectors have been characterized the most, although recently progress has been made in characterizing DNA DSB sensors. The signal transducers for DNA DSBs are the kinase ATM and its downstream kinase Chk2 [16,24,31]. These two kinases constitute a kinase cascade that amplifies the DNA damage signal and phosphorylates multiple targets, which are substrates of either ATM or Chk2 or both. Most of these substrates are effectors of the DNA DSB checkpoint pathway. In what follows, we briefly describe the function and regulation of ATM, Chk2 and some of their best characterized substrates. We then conclude with recent insights regarding 53BP1, which turns out to be one of the sensors of DNA DSBs upstream of ATM.

ATM activation

ATM belongs to a family of kinases that have sequence homology to phosphoinositide 3-kinase (PI3K) [31]. It is activated rapidly after formation of DNA DSBs in every phase of the cell cycle and phosphorylates serine or threonine residues that are followed by a glutamine. The mechanism by which ATM is activated in response to DNA DSBs is being elucidated (Fig. 1). In non-irradiated cells ATM exists as a dimer, is not phosphorylated and is present throughout the nucleus. After irradiation, which leads to formation of DNA DSBs, ATM becomes a monomer, it is phosphorylated on Ser1981 and some pool of it is present at sites of DNA DSBs [1]. It is well-established that ATM phosphorylation on Ser1981 represents autophosphorylation; however, precisely how ATM switches from its inactive (dimeric non-phosphorylated) to its active (monomeric phosphorylated) form is unclear. Studies in yeast suggest that recruitment of Tel1, the yeast ATM ortholog, to sites of DNA DSBs requires Nbs1, a protein that is rapidly recruited to sites of DNA DSBs

0167-8140/$ - see front matter doi:10.1016/j.radonc.2005.06.026

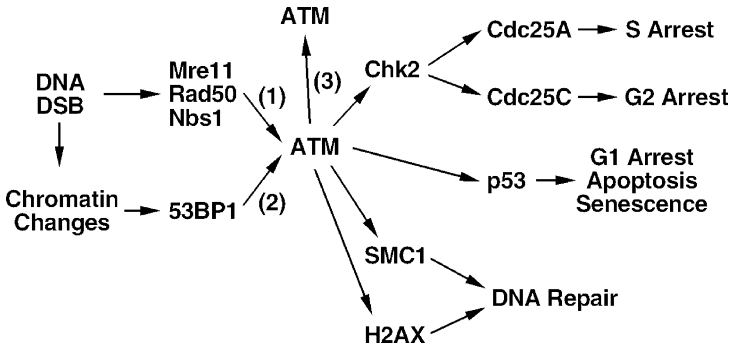

Fig. 1. Current model of the ATM signaling pathway. ATM is activated by the Mre11–Rad50–Nbs1 complex (1) or 53BP1 (2). The former is thought to be recruited at the DNA double strand break (DSB), whereas the latter is recruited at chromatin regions flanking the DNA DSB and extending up to a few megabases from the DSB. ATM activated at sites of DNA DSBs may also phosphorylate and activate ATM in the nucleoplasm (3). A subset of ATM substrates (Chk2, p53, SMC1 and histone H2AX) and their function are also indicated.

and which exists in cells in complex with Rad50 and Mre11 [27]. Studies in yeast and human cells further indicate that suppressing Nbs1 or Mre11 function compromises significantly the conversion of ATM from its non-phosphorylated to phosphorylated form [7,25,32,33]. In addition, in vitro the ability of ATM to phosphorylate various substrates is enhanced in the presence of the Mre11–Rad50–Nbs1 (MRN) complex [20]. These observations suggest a model for ATM activation that involves recruitment of MRN to sites of DNA DSBs and subsequent recruitment and activation of ATM by MRN. However, other observations suggest that ATM activation may not occur exclusively at sites of DNA DSBs. Changes in chromatin structure can also activate ATM, even in the absence of DNA DSBs, suggesting a model whereby altered chromatin structures lead to phosphorylation of Ser1981 of ATM in trans and dissociation of inactive ATM dimers into active monomers [1]. A variation of this latter mechanism may also activate ATM molecules that are far away from the sites of DNA DSBs: an activated ATM molecule that has diffussed away from the sites of DNA DSBs may phosphorylate a dormant ATM dimer dissociating it into two active ATM monomers. Recent results suggest that 53BP1 is also involved in ATM activation, since suppression of 53BP1 leads to reduced ATM autophosphorylation [25]. A model on how 53BP1 could be involved in ATM activation is discussed below.

ATM substrates and role in the checkpoint response

Once ATM is activated it phosphorylates multiple substrates [31]. Two of these, Chk2 and p53, mediate many of the cell cycle effects of ATM, while two others, SMC1 (structural maintainance of chromosomes 1) and histone H2AX, are important for cell survival after irradiation (Fig. 1). Chk2, is a protein kinase, that once activated amplifies the DNA damage signal of ATM. Two of the key substrates of Chk2 are Cdc25A and Cdc25C [5,12,23,38]. Cdc25A and Cdc25C are protein phosphatases; Cdc25A activates Cdk2 and promotes progression through S phase, while Cdc25C activates Cdc2 and promotes progression from G2 into mitosis. When Cdc25A and Cdc25C become phosphorylated by Chk2, their function is inhibited and cells delay progression through S phase or arrest in G2. Cell cycle arrest in G1 is mediated by p53, which is a substrate of both Chk2 and ATM [2,6,9,14,30]. p53 is a transcription factor that induces expression of p21/waf1, a Cdk2 inhibitor. p53 can also induce expression of genes that induce apoptosis; and in certain tissues, such as for example, the hematopoietic system, induction of p53 leads to apoptosis, rather than cell cycle arrest.

It is not clear where in the cell Chk2 and p53 are phosphorylated by ATM. It is possible that activated ATM at sites of DNA DSBs phosphorylates pools of Chk2 and p53 that transiently associate with activated ATM. Alternatively, active ATM that is released from the sites of DNA DSBs or that is activated far away from the sites of DNA DSBs may phosphorylate Chk2 and p53 throughout the nucleus of the cell. What is clear, is that neither phosphorylated Chk2, nor p53, accumulate at sites of DNA DSBs [10,22]. Instead, activated Chk2 and p53 diffuse throughout the nucleus, so that Chk2 can phosphorylate its various substrates, while p53 can be targeted to the promoters of genes, whose expression is induced by p53.

Unlike, Chk2 and p53, phosphorylated SMC1 and histone H2AX are found exclusively at sites of DNA DSBs [18,28,37]. Histone H2AX is part of chromatin and SMC1 is a chromatin-associated protein; thus, these two proteins do not diffuse freely in the cell, which may explain why their phosphorylated forms are found only at sites of DNA DSBs. SMC1 phosphorylation is critical for cells to survive after irradiation, as indicated by the observation that cells harboring a mutant form of SMC1 that cannot be phosphorylated by ATM are as radiosensitive as cells that lack ATM [19]. SMC1 phosphorylation is not required for cell cycle arrest, implying that it favors survival after irradiation by facilitating DNA repair. Similar to SMC1 phosphorylation, histone H2AX phosphorylation also appears to be important for DNA repair and is not required for cell cycle arrest [4,8]. The mechanisms by which SMC1 and histone H2AX phosphorylation facilitate DNA repair are not known, but recent evidence suggests that phosphorylated histone H2AX recruits chromatin remodeling complexes to sites of DNA DSBs [26,34].