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GENOTOXIC STRESS

Signals from the Nucleus: Activation of NF-κB by Cytosolic ATM in the DNA Damage Response

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In response to genotoxic stress induced by DNA double-stranded breaks (DSBs), the inhibitor of κB kinase (IKK) to nuclear factor κB (NF-κB) pathway is activated, which can promote cancer progression and increase the resistance of cancer cells to ionizing radiation or chemotherapeutic drugs. The kinase ataxia telangiectasia mutated (ATM) has a critical role in the activation of NF-κB in response to genotoxic stress. Two reports reveal key cytoplasmic functions of ATM in triggering IKK activation upon DNA damage. After induction of DSBs, ATM is exported from the nucleus and stimulates the ubiquitin ligase activity of tumor necrosis factor receptor–associated factor 6 (TRAF6) or X-linked inhibitor of apoptosis protein, which catalyze the auto-polyubiquitylation of TRAF6 and the polyubiquitylation of the IKK adaptor ELKS, respectively. Ubiquitylation promotes the assembly of signalosomes containing the kinase TAK1 (transforming growth factor β–activated kinase 1). These signalosomes are the site of activation of the cytosolic IKK complex, which stimulates NF-κB–dependent induction of a proliferative and antiapoptotic gene program. These studies show that ATM executes essential functions outside the nucleus in response to DSBs.

The transcription factor nuclear factor κB (NF-κB) is involved in the regulation of many cellular processes, including immune cell responses, proliferation, apoptosis, and cellular transformation leading to carcinogenesis (*1*, *2*). There are two pathways, dubbed the canonical and noncanonical pathway, by which NF-κB can be activated in response to ligand binding to either cell surface or cytosolic receptors. DNA damage, which occurs in the nucleus, also leads to NF-κB activation and has become a paradigm to study how nuclear signals can initiate NF-κB signaling. The kinase ataxia telangiectasia mutated (ATM), mutations in which cause severe defects including immunodeficiency and high incidence of lymphoma, was identified as a key player of NF-κB activation in response to genotoxic stress (*3*–*5*). DNA double-stranded breaks (DSBs) are a form of genotoxic stress and can be caused by ionizing radiation (IR) or chemotherapeutic drugs, such as topoisomerase I inhibitor camptothecin (CT), topoisomerase II inhibitor VP16 (also termed etoposide), or the DNA-intercalating agent doxorubicin (DOX). After DSBs, a small fraction of the NF-κB essential modulator (NEMO, also known as IKKγ) that is not bound to the catalytic inhibitor of κB (IκB) kinase (IKK) complex subunits IKKα and IKKβ translocates to the nucleus. Nuclear NEMO is consecutively sumoylated by the E3 ligase PIASy (protein inhibitor of activated STAT Y), phosphorylated by ATM, and mono-ubiquitylated by a yet unidentified E3 ligase (*6*, *7*) (Fig. 1). In addition, auto-ADP (adenosine 5′-diphosphate)–ribosylation of the DNA damage sensor poly(ADP-ribose) polymerase-1 (PARP-1) facilitates these nuclear posttranslational reactions by transiently recruiting activated ATM, NEMO, and PIASy into a complex (*8*). NEMO and activated ATM are then exported from the nucleus, where they mediate activation of the cytosolic IKK complex through an unknown mechanism.

Two studies provide new insights into the cytosolic role of ATM in response to genotoxic stress (*9*, *10*). Both groups found that, as for the canonical IKK to NF-κB pathway that is activated in response to various stimuli (*11*–*13*), after DNA damage transforming growth factor β (TGFβ)–activated kinase1 (TAK1) phosphorylates the activation loop of IKKβ and thereby acts as an essential regulator downstream of ATM in the NF-κB pathway activated by genotoxic stress (Fig. 1). In response to DSBs, ATM did not directly phosphorylate TAK1, but rather acted indirectly by enhancing the formation of a TAK1-containing signaling module (*9*, *10*). Both groups also found that the TAK1-binding proteins TAB2 and TAB3 are also required for NF-κB activation in response to DSBs (*9*, *10*). In canonical NF-κB signaling, TAB2 associates selectively with Lys^{63}-linked ubiquitin chains to mediate TAK1 activation in response to stimulation of cell surface receptors (*14*, *15*). The involvement of TAB2 in the response to DSBs suggested that ATM may also modulate cytoplasmic ubiquitylation events to trigger TAK1-mediated IKK activation. Consistent with this model, the ubiquitin-conjugating enzyme UBC13, which catalyzes the attachment of Lys^{63}-linked ubiquitin chains to target proteins, was also identified as an essential component of ATM-mediated IKK activation in response to DSBs (*9*, *10*). Thus, there appears to be a common evolutionary origin of the cytoplasmic events that govern canonical and DSB-induced activation of the IKK to NF-κB pathway.

Although both groups provide evidence that attachment of a Lys^{63}-linked ubiquitin chain bridges ATM to TAK1 activation in response to DSBs (*9*, *10*), the details of the mechanisms of IKK activation differ. The differences may reflect variations in the experimental settings: The study by Hinz *et al*. relied on stimulation of IR-induced DNA damage (*9*), whereas Wu *et al*. predominantly studied DNA damage caused by chemotherapeutic drugs (*10*). Although both of these DNA damage paradigms produce DSBs, they induce damage over different time courses. IR-induced DNA damage is initiated by a short pulse within a few seconds, whereas genotoxic drugs are present for several hours. These differences in the experimental setup may result in the activation of distinct effector pathways.

In response to IR, Scheidereit and colleagues demonstrate ATM-dependent activation of the E3 ligase TRAF6 (*9*). TRAF6 also functions in NF-κB signaling in response to stimuli that activate the innate and adaptive immune systems (*11*, *16*, *17*). Upon nuclear export, ATM directly associates with TRAF6 to stimulate its autoubiquitylation. A short TRAF6-interacting fragment of ATM is sufficient to stimulate the E3 ligase activity of TRAF6, suggesting that the ATM adaptor function, rather

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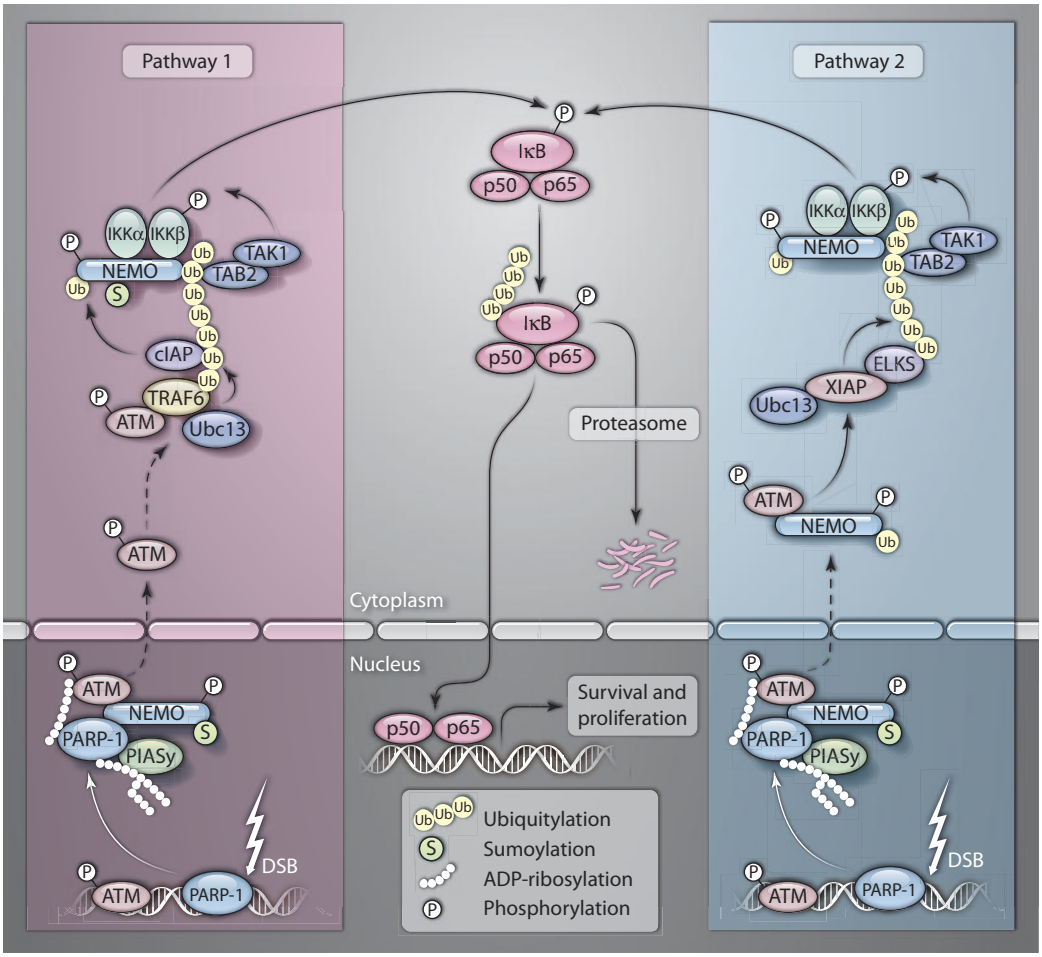

Fig. 1. Signals from the nucleus initiate cytoplasmic NF-κB activation in response to DNA damage. NF-κB activation can occur through two pathways: Pathway 1 shows the response to double-stranded breaks (DSBs) caused by ionizing radiation (IR), and Pathway 2 shows the response to DSBs caused by chemotherapeutic drugs. DNA lesion sensor poly(ADP-ribose) polymerase 1 (PARP-1) and the kinase ataxia telangiectasia mutated (ATM) are recruited to DNA DSBs. IR-induced DNA damage triggers the calcium-mediated export of ATM and cytosolic assembly of the TRAF6-containing signalosome for IKK activation. DNA-damaging agents, such as camptothecin or VP16, trigger the coupled export of ATM and sumoylated NEMO and cytosolic activation of a XIAP- and ELKS-containing signalosome for IKK activation. The activated IKK complex phosphorylates the NF-κB inhibitor IκB to initiate its degradation by the 26*S* proteasome and release of the NF-κB heterodimer (p65-p50), which translocate to the nucleus to activate transcription of proliferative and antiapoptotic target genes.

than the kinase activity of ATM, is involved in this process (*9*). Thus, the cytoplasmic function of ATM in the DNA damage response appears similar to that of signaling adaptors that promote TRAF6 auto-ubiquitylation in the innate immune response, such as IRAK1 (interleukin-1 receptor-associated kinase 1) in the response to lipopolysaccharide and interleukin-1β or MAVS (mitochondrial antiviral signaling) in the response to intracellular viruses (*18*). Furthermore, similar to auto-ADP-ribosylated PARP-1 that acts as a signaling scaffold in the nucleus, auto-ubiquitylated TRAF6 recruits a cytoplasmic complex that contains TAB2, TAK1, NEMO, and cellular inhibitor of apoptosis protein 1 (cIAP1) (*9*). By induced proximity, this ATM-induced and TRAF6-dependent complex may facilitate TAK1 autophosphorylation and IKKβ trans-phosphorylation. Although cIAP1 is dispensable for TAK1 activation, cIAP1 can catalyze mono-ubiquitylation of NEMO at Lys^{285}, which contributes to the activation of the IKK complex in response to DSBs (*9*, *19*).

Wu *et al*. identify X-linked inhibitor of apoptosis protein (XIAP) as the E3 ubiquitin ligase that mediates ATM-dependent TAK1-IKK-NF-κB activation upon treatment of cells with the chemotherapeutic DNA-damaging agents VP16, CT, and DOX (*10*). XIAP is required for TAK1 and NF-κB activation after DNA damage (*19*), and Wu *et al*. show that XIAP functions as an E3 ligase that ubiquitylates ELKS (protein rich in glutamate, leucine, lysine, and serine), which also functions in DNA damage–dependent NF-κB activation (*7*, *10*). As suggested for auto-ubiquitylated TRAF6, covalent attachment of K63-linked ubiquitin chains to ELKS may promote clustering and thereby enhance proximity of the TAB2-TAK1 and NEMO-IKKα/β complexes to allow efficient NF-κB activation (*10*) (Fig. 1). However, it remains to be seen whether the ATM adaptor function or kinase activity is required for triggering ELKS ubiquitylation.

Future analyses should determine whether TRAF6- and XIAP-mediated processes regulate two separate and independent modes for activating the TAK1-IKK-NF-κB pathway in response to DSBs or whether TRAF6 and XIAP cooperate to catalyze substrate ubiquitylation. Even though TRAF6 or ELKS ubiquitylation correlates with TAK1-IKK activation, mapping and mutagenesis of ubiquitin acceptor sites, as has been done for Lys^{285} in NEMO (*9*), will establish the functional relevance of specific modifications.

In addition to having different E3 ligases, the two models also differ in the putative requirement of NEMO for cytosolic ATM function. Nuclear ATM is exported after VP16 treatment in a NEMO-dependent manner to the cytoplasm, suggesting that both are exported by a piggyback mechanism (*7*). Consistent with this model, NEMO deficiency abrogates ATM-dependent TAK1 activation in response to VP16 or CT (*10*). In contrast, ATM export initiated by IR relies on cellular calcium but is independent of NEMO (*9*). In this scenario, exported NEMO is still sumoylated and sumoylation might serve as a signal for its mono-ubiquitylation at Lys^{285}. It remains to be seen whether the different DSB-inducing

agents are responsible for the distinct effects or if further experiments can reconcile the differences in ATM export mechanisms.

Both studies also report that exported ATM is phosphorylated, reflecting that the cytoplasmic ATM pool is catalytically active (*9*, *10*). However, the causes and consequences of cytosolic ATM kinase activity remain unresolved. Two separate pathways control the kinase activity of ATM in response to oxidative stress or DNA damage (*20*), and it is possible that oxidative stress plays a role in the regulation of ATM kinase activity outside the nucleus. Mutants that are defective in oxidative stress–induced activation of the kinase activity should reveal whether oxidative stress is involved in activation of cytosolic ATM kinase activity and if it has a role for the cytosolic functions of ATM in DNA damage response.

Uncovering the exact mechanism of DNA damage–induced NF-κB signaling is of clinical relevance, because the antiapoptotic effects of NF-κB are thought to be a major obstacle to effective antitumor chemotherapy or radiotherapy. Combining agents that effectively antagonize the activity of IAPs (*21*, *22*) with genotoxic agents may overcome drug resistance of cancer cells. A more detailed understanding of the cytosolic ATM adaptor function for NF-κB activation may suggest novel therapeutic strategies.

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