Cell Death and Differentiation (2011) 18, 2–4
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Editorial

c-Abl tyrosine kinase in the DNA damage response: cell death and more

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Cell Death and Differentiation (2011) **18,** 2–4; doi:10.1038/cdd.2010.132

The robust cellular response to DNA damage (DNA damage response or DDR) comprises multiple intertwined signaling networks that function in concert to optimize the manner in which a cell reacts to genotoxic stress. Downstream DDR signaling depends on two main upstream activators—the PI3K-related protein kinases ATM and ATR, whose respective activation by distinct types of DNA damage (DD) leads to a rapid phosphorylation of multiple proteins involved in processes such as DNA repair, cell-cycle arrest and programmed cell death (reviewed in Jackson and Bartek[1], Lavin[2], Cimprich and Cortez[3]).

The ubiquitously expressed c-Abl tyrosine kinase has been widely associated with various aspects of the DDR (reviewed in Shaul and Ben-Yehoyada[4]). The tightly regulated kinase activity of c-Abl undergoes robust activation in response to ionizing radiation (IR) and other types of genotoxic stress.[5,6] A well-defined function of c-Abl in the context of the DDR is the induction of p73-dependent cell death[7–9] whereby c-Abl phosphorylates p73[7] and Yap1[10] to induce cell death in response to DD. A recent study has also characterized c-Abl involvement in the p63-dependent cell death response of oocytes to DD.[11]

During the past decade extensive evidence has accumulated linking c-Abl with proteins intimately involved in the signaling and execution of DNA repair and cell-cycle arrest as well as with key upstream DDR regulators. c-Abl activation and phosphorylation following DD were reported to depend on the presence of ATM[12,13] tentatively positioning c-Abl downstream of ATM. Other c-Abl interactions in this response, many of which result in phosphorylation of the relevant proteins by c-Abl, include DNA-PKcs, Rad51 and Rad52—central components of the NHEJ and HR repair mechanisms, respectively,[14,15] WRN—a helicase implicated in DNA repair,[16] BRCA1—a tumor suppressor crucial for cell-cycle checkpoint control and DNA repair,[17] Rad9—a mediator of DD sensing and cell-cycle arrest,[18] and TopBP1—an important regulator of DD-induced S/G_2-M checkpoints.[19] It is evident therefore that c-Abl may occupy a more central position in the DDR than initially appreciated and may possibly be implicated in the regulation of double strand break (DSB) repair and cell-cycle arrest. However, the true significance of these c-Abl interactions remains rather elusive and its ensuing function in the DDR is poorly understood.

In this issue of *Cell Death and Differentiation*, Wang *et al.*[20] revisit the question of c-Abl involvement in the DDR. The authors use primary MEFs, avoiding the potentially confounding effects of cell immortalization/transformation, to address important questions regarding the involvement of c-Abl in the primary stages of the DDR and specifically in the activation of the key transducer kinases ATM and ATR. The authors find that activation of the ATM and ATR kinases following treatment with doxorubicin (Dox), a DSB-inducing agent, and that of their immediate respective substrates Chk2 and Chk1, was compromised in the absence of c-Abl, suggesting strong dependence of the respective pathways on c-Abl. c-Abl-deficient cells also failed to exhibit effective DD-induced phosphorylation of p53 Ser18, usually performed by ATM, ATR or DNA-PK. The same appeared to be true for treatment with single strand breaks (SSBs)-inducing agents such as HU, which was able to activate c-Abl, supporting parallel c-Abl involvement in both the ATR- and the ATM-dependent pathways. Examining the DDR-relevant physiological properties of these c-Abl$^{-/-}$ MEFs, the authors find that these exhibited lower cell death, higher percentages of S/G_2/M cells, elevated DNA synthesis and higher levels of DSBs as well as SSBs 8–24 h following DD by IR. Notably, c-Abl$^{-/-}$ MEFs were resistant to HU-induced cell death, further supporting a role for c-Abl in the response to SSBs. Proper autophosphorylation of both ATM and ATR as well as their tyrosine phosphorylation was markedly reduced in the absence of c-Abl, suggesting a significant effect of c-Abl on the activation state of both kinases and implying that these may be subject to direct phosphorylation by c-Abl, a connection further validated by detection of their physical interaction with c-Abl. The authors venture further to show that c-Abl-dependent tyrosine phosphorylation of ATR occurred in the N-terminal region and identify two phosphorylation sites of potential importance for ATR activation. When expressed in ATR-deficient cells, ATR mutants in either or both residues were inefficient in restoring the DDR signaling defects of these cells, indicating that their phosphorylation is likely to be of importance for the activation of ATR-dependent downstream DDR events.

Taken together, these observations by Wang *et al.* suggest that c-Abl is significantly involved in the early stages of the

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DDR and is necessary for proper activation of the ATM and ATR kinases and their respective downstream signaling pathways. Their findings also suggest that c-Abl is required for the proper execution of DNA repair of both DSBs and SSBs and is involved in DD-induced cell-cycle arrest. It is of note, however, that in contrast to the molecular events, most of which were tested following Dox or HU treatment, the functional assays were performed mainly following IR and are thus somewhat difficult to evaluate in the same context. It is also of note that an early study has reported that DNA synthesis in c-Abl$^{-/-}$ MEFs at early times following IR was similar or slightly lower compared with wt.[12]

Interestingly, examining the levels of Dox-induced repair foci, Wang *et al*. find that foci levels of early DDR regulators such as ATM, ATR and Mre11 were refractory to the presence of c-Abl, whereas foci levels of later DDR mediators such as BRCA1, 53BP1, Rad51 and TopBP1 were higher in c-Abl$^{-/-}$ cells. This observation seems to imply that c-Abl is not required for the recruitment of ATM and ATR to DD sites or for the initial assembly of repair foci and attributes to c-Abl a role in the late rather than the immediate stage of the response.

Combining these observations, the authors propose a model according to which primary ATM activation and recruitment to DD sites occur independently of c-Abl and are required for the activation of c-Abl by ATM. In turn, c-Abl phosphorylates ATM, amplifying ATM activation. c-Abl also phosphorylates and activates ATR-dependent signaling, which is induced in response to ssDNA generated by the processing of DSBs, possibly functioning thus to relay signals from the DSB-activated ATM-dependent response to the SSB-activated ATR-dependent response (Figure 1, arrow (a)).

Notably, previous findings—both biochemical and physiological—have strongly suggested that c-Abl functions as a negative rather than a positive regulator of DDR-related processes. Several of the documented interactions between c-Abl and DDR components were reported to result in inhibition of the activities of the relevant proteins. Thus, phosphorylation of DNA-PKcs (by c-Abl) leads to dissociation of the DNA–PK/Ku complex,[14,21] phosphorylation of WRN inhibits its exonuclease and helicase activities[16] and phosphorylation of Rad51 inhibits its binding to DNA as well as its function in DNA strand exchange reactions.[15] Furthermore, the recent finding that inhibition of c-Abl in mice may prolong reproductive outcome following chemotherapy[11] seems to suggest that c-Abl involvement in activation of the immediate and critical stages of the DDR is unlikely to be crucial. Notably, a recent study that has addressed the physiological implications of c-Abl in DSB repair has found that inhibition of c-Abl kinase activity results in markedly lower post-repair genomic fragmentation levels, indicating higher DSB repair in the absence of c-Abl activity.[22] This study also showed the effect to be time dependent, concomitant with slow activation of c-Abl following IR, suggesting that c-Abl specifically downregulates late-stage DSB repair. According to this model, c-Abl functions as a negative regulator of late DSB repair (Figure 1, blunt-ended line (b)), possibly responsible for the downregulation and termination of repair, in addition to its function in cell death at late DDR stages (Figure 1, arrow (c)). Interestingly, in the mentioned study[22] levels of γ-H2AX foci

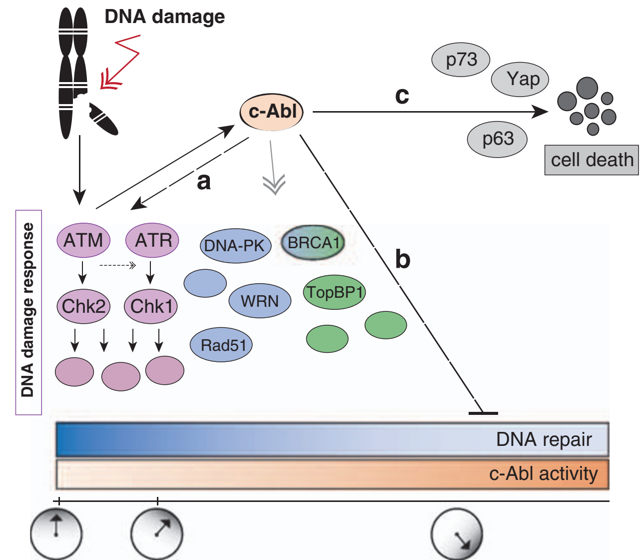

Figure 1 The primary steps of the DNA damage response constitute immediate activation of DNA repair and induction of cell-cycle arrest. At later stages of the response DSB repair becomes significantly slower and is terminated while the cells recover and resume progression through the cell cycle. In response to DNA damage c-Abl is activated, potentially also by the ATM kinase. (**a**) Wang *et al*[20] demonstrate that c-Abl may be necessary for the full activation of ATM and ATR and that of their respective signaling pathways. Accumulating evidence implicates c-Abl in interactions with many key DDR proteins involved in DNA repair (blue) and cell-cycle arrest (green). Some of the known c-Abl targets in this context are shown. (**b**) The outcome of many of these interactions suggests that c-Abl has an inhibitory role in the response. In addition, a recent study[22] has demonstrated that c-Abl may functionally downregulate late-stage DSB repair concomitant with late activation of c-Abl by DNA damage. (**c**) c-Abl is best known in the DDR for its involvement in DNA-damage-induced p73-dependent cell death. A recent study reported a similar function for c-Abl in p63-dependent cell death in oocytes.[11] Downregulation of late DSB repair by c-Abl could potentially constitute a prerequisite for later engagement of cells in DNA-damage-induced programmed cell death

were found to be markedly elevated on inhibition of c-Abl activity at late times following IR, analogous to the current study by Wang *et al*.[20] describing a similar effect with respect to TopBP1, BRCA1, 53BP1 and Rad51 in c-Abl$^{-/-}$ cells. Jointly, these findings imply that c-Abl may negatively affect the recruitment of proteins to DSB repair foci or, alternatively, induce DSB foci disassembly at the late stages of the DDR, possibly as part of its inhibitory effect on DSB repair.

The difference in the described findings and the ensuing models could potentially result from differences in the experimental systems applied and in the methods used for DD induction. Alternatively, the kinetics of c-Abl activation by DD could constitute an important factor underlying this discrepancy. c-Abl activation kinetics in response to DD constitute a major unresolved question with respect to c-Abl involvement in the DDR. Although some early studies have detected c-Abl activation at relatively short times following DD (∼1–3 h),[6,9,13] others have reported a rather delayed and gradual mode of c-Abl activation.[8,22] None however has meticulously tested c-Abl activation on the order of minutes up to single hours following DD—the critical time frame within which most immediate DDR events occur. Neither do we have

a collective picture of c-Abl activation kinetics at early and late times following DD. Our understanding of the above-described findings can undoubtedly be markedly improved by better and detailed characterization of c-Abl activation kinetics in the context of the DDR.

Altogether, the current work by Wang *et al*. introduces a new twist into the story of c-Abl involvement in the DDR, suggesting that c-Abl may have a significant role in the activation of the key upstream molecular events governing the initiation and propagation of the response. Although the study advances our understanding of the involvement of c-Abl in the molecular events that occur in response to DNA damage, additional work is required to obtain a comprehensive understanding of the physiological outcomes of this involvement. However, this study constitutes an important step in the effort to characterize c-Abl function in the DDR and may serve to facilitate new studies toward a better understanding of this function.

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