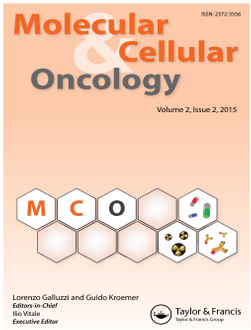

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AUTHOR'S VIEWS

It takes three to the DNA damage response tango

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ABSTRACT

The DNA damage response is robustly activated by DNA double-strand breaks and controlled by three apical protein kinases of the PI3-kinase-related protein kinase (PIKK) family: ataxia-telangiectasia, mutated (ATM), ataxia-telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK). Phosphoproteomic analysis reveals the relative share of these PIKKs in coordinating this network, and compensation by ATR and DNA-PK for ATM absence in the genetic disorder, ataxia-telangiectasia (A-T).

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Cells respond to genotoxic stress by activating an elaborate signal transduction network termed the DNA damage response (DDR).[1] The DNA double-strand break (DSB) – a critical DNA lesion – robustly activates this network. Three PI3-kinase-related protein kinases (PIKKs) – ataxia-telangiectasia, mutated (ATM), ataxia-telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK) – are apical regulators of this signaling cascade.[2] ATM is best known for its role as the primary transducer of the DSB response, DNA-PK – for its prominent part in the nonhomologous end joining pathway of DSB repair, and ATR – for coordinating the replication stress response.[2] Evidence suggests that the three PIKKs maintain complex relationships in the DSB response. Their central roles in maintaining genome integrity have made them attractive targets for cancer therapy.[3,4] Germline null alleles of the *ATM* gene cause in humans the genome instability syndrome, ataxia-telangiectasia (A-T), and hypomorphic mutations in the genes encoding ATR and the catalytic subunit of DNA-PK lead to ATR-Seckel syndrome and severe combined immunodeficiency (SCID), respectively.[5]

We carried out a high-throughput, phosphoproteomic analysis to determine the relative shares of the three PIKKs in the phosphoproteome dynamics that follow DSB induction.[6] We dissected this response using chemical inhibitors of them, and the analysis was carried out in cell lines derived from healthy donors and A-T patients. A-T cells, which are devoid of ATM, were used to unravel possible compensatory responses of ATR and/or DNA-PK to ATM's absence. The results of the high-throughput screen were extensively validated using a targeted proteomic technique, selected reaction monitoring (SRM). We also matched our results against a panel of 34 previous screens for DDR factors in an effort to highlight strong candidates for new DDR factors and thereby create a useful resource for their future, detailed investigations.

Crosstalk between the PIKKs was reflected in the data by a group of ATM substrates that showed sustained phosphorylation upon DNA-PK inhibition. We tied this phenomenon to the previously reported DNA-PK-mediated attenuation of ATM that occurs following direct phosphorylation of ATM by DNA-PK.[7,8] An important question concerning the functional relationships between the PIKKs is whether they can take over each other's role under certain circumstances. This question is particularly relevant to genetic disorders caused by loss or impaired function of these proteins, and to tumor cells in which their genes are mutated. The total absence of ATM in most A-T patients led us to ask whether ATR and DNA-PK operate differently when the third member of the trio is lacking. We found that while most ATM-dependent substrates in the DSB response in control cells indeed did not respond to DSB induction in A-T cells, some did respond in these cells in an ATR- or DNA-PK-dependent manner. The substrate group that is ATR-dependent in A-T cells is the bigger one and follows a slower kinetics compared to that in control cells. The portion that is 'compensated' by DNA-PK is smaller and follows a more rapid kinetics (Figure 1).

The ability of ATR and DNA-PK to act in A-T cells on substrates that they normally do not target suggests that different PIKK family members can potentially recognize the same targets. There is, in fact, ample evidence that different PIKKs can phosphorylate the same substrates *in vitro*. However, phosphorylation in cells is determined and modulated by the physiological context, and is not promiscuous. Notably, the group of ATM substrates that were 'compensated' for by ATR in A-T cells after DSB induction was enriched for substrates that ATR usually targets in control cells in response to replication stress. We surmise that ATR's ability to partially compensate for ATM absence might also contribute to the selective sensitivity of some ATM-null tumors to ATR inhibitors.[3]

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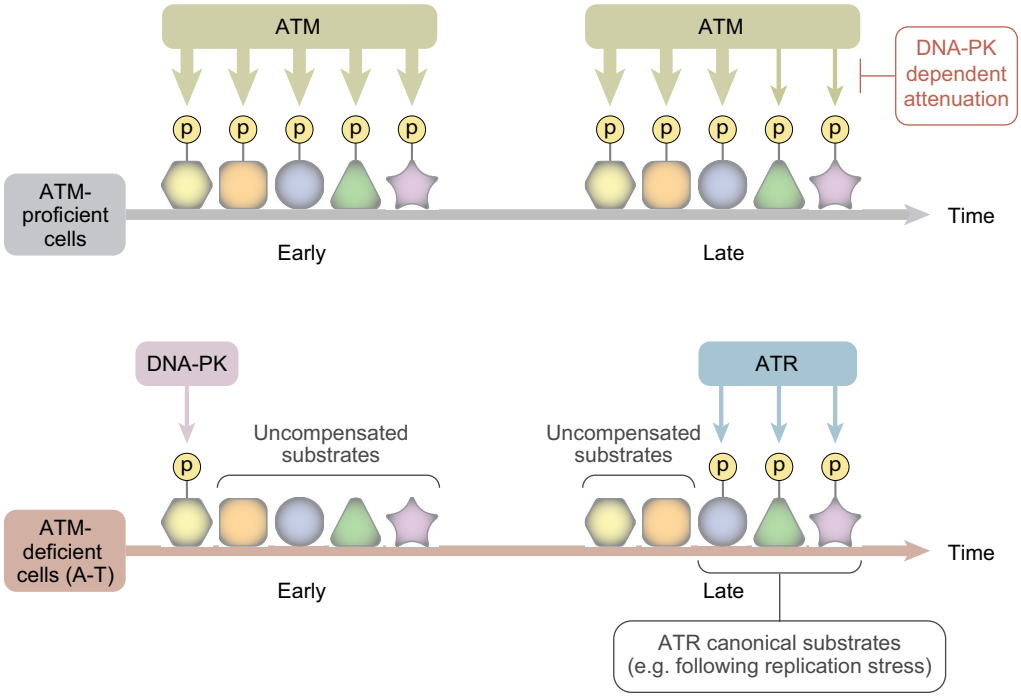

Figure 1. Potential mechanism for PI3-kinase-related protein kinase (PIKK) collaboration in the cellular response to genotoxic stress. In cells proficient for ataxia-telangiectasia, mutated (ATM), ATM responds rapidly to DNA double-strand breaks (DSBs) by phosphorylating numerous substrates. As the response progresses, ATM's activity is attenuated in a manner dependent on the DNA-dependent protein kinase (DNA-PK). In ATM-deficient cells, many ATM-dependent branches of the DDR are missing, but some ATM substrates are targeted by ataxia-telangiectasia and Rad3-related (ATR) or DNA-PK. The compensation by DNA-PK occurs rapidly and encompasses fewer substrates, while the ATR-mediated compensation occurs later and involves a bigger subset of targets, many of which are normally targeted by ATR following replication stress. A fraction of the ATR-dependent compensation observed in A-T cells also occurs when ATM is chemically inhibited. Figure taken from Schlam-Babayov et al.[6]

Interestingly, we observed certain ATR-dependent compensation also after inducing DSBs while ATM is chemically inhibited. However, in this situation, ATR's compensatory activity spanned fewer targets than in A-T cells. It is important to note that the physiological situations conferred by ATM absence and ATM inhibition are markedly different. Striking evidence for this is the difference between the moderate phenotype of Atm-deficient mice and the embryonic lethality conferred in this organism by expression of catalytically inactive Atm.[9,10] Studies have shown that inactive ATM is recruited to DSB sites similarly to active ATM. Plausibly, the presence of catalytically inactive ATM in these sites attenuates the compensatory activity of ATR and DNA-PK that is observed in A-T cells, and this could contribute to a harsher cellular and organismal phenotype when ATM is present but catalytically inactive.

Our study sheds light on the fine-tuned relationships between the three PIKKs in health and disease (Figure 1). These findings raise a question whether drugs that would enhance the ATR/DNA-PK-dependent compensation for ATM loss could be used to ameliorate A-T symptoms. The crosstalk between the PIKKs also has implications for the development of cancer therapy drugs that target these protein kinases. Further understanding of these relationships will affect both fields.

Abbreviations

A-T	Ataxia-telangiectasia
ATM	Ataxia-telangiectasia, mutated
ATR	Ataxia-telangiectasia and Rad3-related
DDR	DNA damage response
DNA-PK	DNA-dependent protein kinase
DSB	Double-strand break
PIKK	PI3-kinase-related protein kinase.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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