A LARGe surprise links ATR and Rho

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Cells are continuously faced with endogenous and exogenous threats to their genomic integrity. Elaborate signaling pathways collectively known as the DNA damage response (DDR) detect and respond to DNA lesions by activating cell-cycle checkpoints, by stabilizing and restarting stalled replication forks and by promoting the repair of DNA lesions. Highlighting the importance of this response for human health, defects in DDR proteins lead to a wide variety of developmental and cancer-predisposition syndromes.¹ The phosphatidylinositol 3-kinase-related protein kinases (PIKKs) ATM, ATR and DNA-PK are master regulators of the DDR and collaboratively phosphorylate hundreds of substrates to help cells cope with genomic stress. The stability and proper accumulation of these giant kinases was previously shown to require the Hsp90 co-chaperone Tel2/hClk2 which acts as a key player of the DDR.² In an effort to uncover novel interactors and potentially additional functions of Tel2/hClk2, the Collis laboratory performed a yeast 2-hybrid screen which identified the Leukemia-Associated Rho Guanine exchange factor 12 (LARG) as a new interacting partner of Tel2.³ LARG is a specific guanine exchange factor (GEF) for the RhoA GTPase which plays important roles in cytoskeleton reorganization and cellular adhesion in response to extracellular cues. In support for its function in cytoskeletal dynamics, LARG also associates with pericentrin (PCNT) at the centrosome, the main microtubule organizing center of the cell.^{3,4} Interestingly, both Tel2 and PCNT deficiencies negatively impact the ATR-dependent arm of the DDR,^{2,5} which prompted the authors to investigate a role for

LARG in ATR activation. Indeed, depletion of LARG attenuated the response to replication stress as illustrated by decreased γ -H2AX, RPA and CHK1 phosphorylation in response to hydroxyurea. Furthermore, LARG-depleted cells were more sensitive to replication stress-inducing agents and contained increased numbers of centrosomes, both phenotypes associated with impaired ATR signaling. These observations are quite intriguing, however, at this point, a mechanistic understanding of the role of LARG in ATR activation is still lacking. For example, it remains unclear whether LARG acts on ATR activity through its interaction with pericentrin, Tel2 or by itself. In this vein, it would be interesting to determine whether LARG deficiency results in a decreased stability of PIKKs such as ATR similar to what is observed upon Tel2 downregulation.²

More surprisingly, in addition to a role for LARG in the activation of the DDR and in cellular resiliency toward replication stress, the authors also found that knockdown of ATR or Tel2 decreased the efficiency of RhoA-mediated signaling. Recent data has hinted at potential crosstalk between the DDR and Rho signaling pathways. Notably, DNA damage can activate RhoA signaling in the nucleus through the Net1 GEF, however a role for Net1 in the maintenance of genomic stability has not been described thus far.⁶ Aside from the nucleus, another potential site where ATR and Rho signaling may intersect could be at the primary cilium which originates from the centrosome. In fact, both RhoA and ATR are required for optimal ciliogenesis and mutations in many other DDR proteins were found to cause ciliopathies.⁷ In at least one instance,

the defects in cilium biogenesis can be traced back to increased replication stress thereby connecting genomic stability to ciliopathies.⁷

This new body of work from the Collis group adds the LARG GEF to a growing number of centrosomal components that also play roles in the DDR, and starts to dissect the DDR components involved in Rho signaling. How LARG and ATR are connected and how they influence cilium homeostasis should be the focus of future studies. Although previous work has suggested that phosphorylation of LARG and other centrosomal proteins by ATM and ATR occurs during genotoxic stress, the specific consequences of these post-translational modifications remain to be explored. Understanding how the DNA damage and Rho signaling pathways crosstalk with each other in different contexts might be key to pinpoint the molecular origins of many ciliopathies and to fully understand the extensive cellular reprogramming that occurs in response to DNA damage.

References

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