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Endosome traffic machinery meets the p53–p21 axis

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Abbreviations: CDKN1A, cyclin-dependent kinase inhibitor 1A (p21, CIP1); DDR, DNA damage response; IR, ionizing radiation; MAM, mitochondria-associated membrane; NLS, nuclear localization signal; WB, western blot.

SIRT1 regulates p53 transcriptional activation in response to genotoxic insult by deacetylating key lysine residues. We recently identified the multifunctional protein PACS-2 as a SIRT1 inhibitor. After DNA damage, PACS-2 binds and inhibits SIRT1 to increase p53-dependent transactivation of the CDK inhibitor p21 (CDKN1A) and induce cell cycle arrest.

Maintenance of genome stability requires mobilization of the DNA damage response (DDR), both to repair the day-to-day chemical nicks and bruises and resolve acute damage following exposure to ionizing radiation (IR) or chemotherapeutics. Cells respond to these chemical mishaps and overtly acute genotoxic insults by inducing the tumor suppressor protein p53 (TP53, known as p53), whose role in the regulation of the DDR is complex, involving changes in the expression of more than 3,000 genes and activation of cell cycle checkpoints to allow DNA repair.[1,2] The most intensively studied p53 target genes include cyclin-dependent kinase inhibitor 1A (CDKN1A, known as p21), which promotes cell-cycle arrest or senescence, and p53 upregulated modulator of apoptosis (PUMA, also known as BCL2-binding component 3 [BBC3]) and BCL2-associated X protein (BAX, also known as BCL-2-like protein-4), which are activated to induce apoptosis when cellular damage is irreparable.

The transcriptional activity of p53 requires post-translational modifications, including phosphorylation and acetylation, to stabilize p53 and enhance its transactivation functions, respectively. The class III histone deacetylase sirtuin 1 (SIRT1) represses p53 transcriptional activation by deacetylating p53 following DNA damage. SIRT1 can be regulated transcriptionally and post-transcriptionally and through interactions with other cellular proteins in diverse pathways.[2] The complexity of these functions suggests that regulation of SIRT1 activity probably requires additional cellular factors.

We recently identified the multifunctional sorting protein phosphofurin acidic cluster sorting protein-2 (PACS-2) as a novel mediator of p53 action in response to genotoxic insult (**Fig. 1**, panel A).[3] PACS-2 was initially identified by its cytoplasmic roles in mediating mitochondria-associated membrane (MAM) formation, autophagy, and protein traffic in the secretory and endocytic pathways (**Fig. 1**, panel B).[4-8] In response to Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL), PACS-2 switches to a proapoptotic effector that promotes membrane permeabilization of mitochondria and lysosomes, which is required for the activation of executioner caspases and cell death (**Fig. 1**, panel C).[9,10] Our finding that PACS-2 is required for Apo2L/TRAIL-induced apoptosis led us to ask whether PACS-2 would similarly be required for apoptosis induced by DNA damage. Surprisingly, we found that whereas siRNA knockdown of PACS-2 in HCT116 human colon carcinoma cells reduced Apo2L/TRAIL-induced apoptosis, it sensitized cells to doxorubicin-induced apoptosis and this effect was dependent on p53. Thus, PACS-2 has a proapoptotic role in Apo2L/TRAIL action but an anti-apoptotic role in response to genotoxic insult (**Fig. 1**).

To characterize this previously undescribed role for PACS-2 in DDR, we used cell cycle analyses combined with western blotting (WB) and *in vivo* enterocyte migration studies in the gastrointestinal track of *Pacs-2*$^{-/-}$ mice. We observed that PACS-2 knockdown reduced p53 acetylation in response to DNA damage, thereby blunting the induction of p21 and cell cycle progression. This p21 repression increased apoptosis as previously described, which suggests a p21-dependent cytoprotective role for PACS-2 in DDR.[1] Consistent with the above data, we found that the migration of bromodeoxyuridine-positive (BrdU^{+}) enterocytes along the crypt-villus following IR was restricted in wild-type mice but not in

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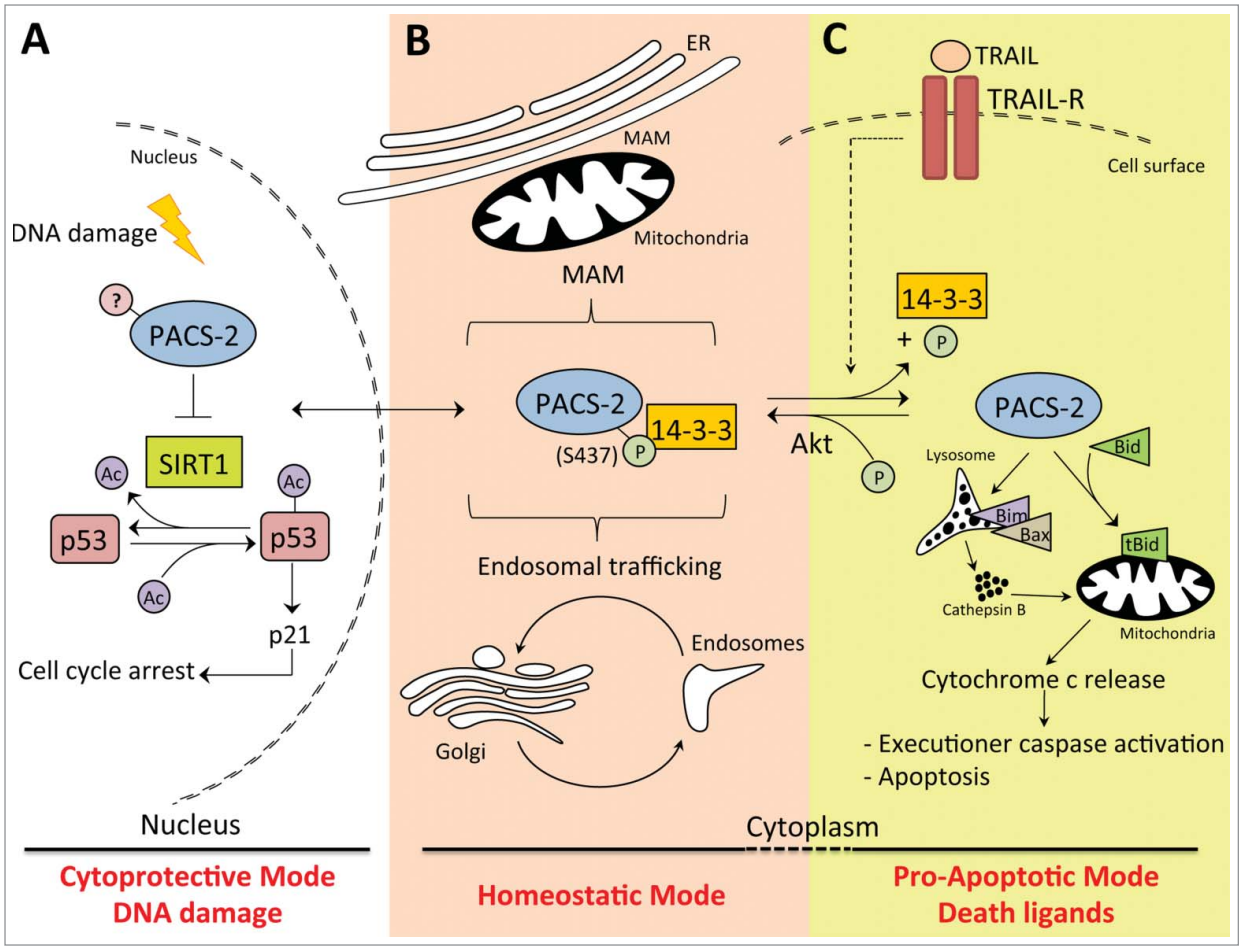

Figure 1. Modes of PACS-2 action. (**A**) DNA damage induces nuclear PACS-2-mediated cytoprotection through inhibition of SIRT1-mediated p53 deacetylation to promote p21-dependent cell cycle arrest. Post-translational modifications that regulate PACS-2 nuclear function have not been identified. (**B**) Phosphorylation state-dependent homeostatic function of PACS-2 to regulate either MAM integrity or cargo trafficking in secretory and endocytic pathways. (**C**) Signaling by death ligands triggers PACS-2 dephosphorylation to induce mitochondria and lysosome membrane permeabilization, leading to caspase-3 activation and cell death. Ac, acetylated lysine; P, Akt-phosphorylated Ser_{437} on PACS-2; ER, endoplasmic reticulum; MAM, mitochondria-associated membrane; TRAIL-R, TRAIL receptor; tBid, truncated Bid.

Pacs-2$^{-/-}$ mice. Together, these data describe the functional importance of PACS-2 as a specific *in vivo* regulator of the p53-p21 axis in DDR.

We confirmed that p53 acetylation and p21 induction levels in PACS-2 depleted cells or tissues were SIRT1 dependent, as SIRT1 knockdown or addition of the SIRT1 inhibitor EX-527 restored p53 acetylation and p21 induction, as observed by WB, as well as p21-dependent cell cycle arrest, in PACS-2 knockdown cells. The inhibition of SIRT1 by PACS-2 was also confirmed by *in vitro* assays showing that PACS-2 directly binds to and inhibits SIRT1-catalyzed p53 deacetylation. Together, these data support a previously undescribed role of PACS-2—the inhibition of SIRT1 following DNA damage—as a cytoprotective component of the p53 signaling pathway, although the specific protein motifs or residues involved and the molecular mechanism by which PACS-2 inhibits SIRT1 catalytic activity are still not fully understood.

The promotion of p53-dependent p21 expression by PACS-2 depended on its ability to traffic to the cell nucleus. Sequence analysis of human PACS-2 identified one nuclear localization signal (NLS) and at least 2 putative nuclear export signals. Correspondingly, mutation of the PACS-2 NLS blocked its nuclear trafficking. Moreover, PACS-2 responded to DNA damage by increased translocation into the cell nucleus, where it repressed SIRT1-mediated p53 deacetylation. Curiously, sequence alignment of the NLS sequences in PACS proteins from different species reveals that PACS-2 underwent a late evolutionary adaptation to acquire nuclear trafficking motifs. This new, and surprising, function of PACS-2 parallels the role of p53 in directing cell cycle arrest found in higher metazoans but not in worms or flies, where p53 function is limited to apoptosis induction. Thus, these findings described a new compartment-specific function of PACS-2 following DNA damage in response to the heightened need for p53 to resolve the

damage. However, the mechanism by which PACS-2 is mobilized from the cytoplasm to the nucleus in response to DNA damage remains unclear.

Overall, our work characterizes PACS-2 as a new inhibitor of SIRT1 to specifically regulate the p53–p21 axis in response to DNA damage. Interestingly, the role of the nuclear pool of PACS-2 in promoting p21-dependent cell cycle arrest could correlate with the role of the cytoplasmic pool of PACS-2 in promoting autophagy.[6] Such coordinated roles would allow arrested cells to survive while DNA damage is repaired. Future work will determine how the ratio of PACS-2 pools is regulated in response to different stimuli and the crosstalk between them.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Haupt S, Louria-Hayon I, Haupt Y. P53 licensed to kill? Operating the assassin. J Cell Biochem 2003; 88:76-82; PMID:12461776; http://dx.doi.org/10.1002/jcb.10311
2. Kenzelmann Broz D, Spano Mello S, Bieging KT, Jiang D, Dusek RL, Brady CA, Sidow A, Attardi LD. Global genomic profiling reveals an extensive p53-regulated autophagy program contributing to key p53 responses. Genes Dev 2013; 27:1016-31; PMID:23651856; http://dx.doi.org/10.1101/gad.212282.112
3. Atkins KM, Thomas LL, Barroso-Gonzalez J, Thomas L, Auclair S, Yin J, Kang H, Chung JH, Dikeakos JD, Thomas G. The Multifunctional Sorting Protein PACS-2 Regulates SIRT1-Mediated Deacetylation of p53 to Modulate p21-Dependent Cell-Cycle Arrest. Cell Rep 2014; PMID:25159152; http://dx.doi.org/10.1016/j.celrep.2014.07.049
4. Atkins KM, Thomas L, Youker RT, Harriff MJ, Pissani F, You H, Thomas G. HIV-1 Nef binds PACS-2 to assemble a multikinase cascade that triggers major histocompatibility complex class I (MHC-I) down-regulation: analysis using short interfering RNA and knock-out mice. J Biol Chem 2008; 283:11772-84; PMID:18296443; http://dx.doi.org/10.1074/jbc.M707572200
5. Betz C, Stracka D, Prescianotto-Baschong C, Frieden M, Demaurex N, Hall MN. Feature Article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. Proc Natl Acad Sci USA 2013; 110:12526-34; PMID:23852728; http://dx.doi.org/10.1073/pnas.1302455110
6. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, et al. Autophagosomes form at ER-mitochondria contact sites. Nature 2013; 495:389-93; PMID:23455425; http://dx.doi.org/10.1038/nature11910
7. Kottgen M, Benzing T, Simmen T, Tauber R, Buchholz B, Feliciangeli S, Huber TB, Schermer B, Kramer-Zucker A, Hopker K, et al. Trafficking of TRPP2 by PACS proteins represents a novel mechanism of ion channel regulation. EMBO J 2005; 24:705-16; PMID:15692563; http://dx.doi.org/10.1038/sj.emboj.7600566
8. Simmen T, Aslan JE, Blagoveshchenskaya AD, Thomas L, Wan L, Xiang Y, Feliciangeli SF, Hung CH, Crump CM, Thomas G. PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. EMBO J 2005; 24:717-29; PMID:15692567; http://dx.doi.org/10.1038/sj.emboj.7600559
9. Aslan JE, You H, Williamson DM, Endig J, Youker RT, Thomas L, Shu H, Du Y, Milewski RL, Brush MH, et al. Akt and 14-3-3 control a PACS-2 homeostatic switch that integrates membrane traffic with TRAIL-induced apoptosis. Mol Cell 2009; 34:497-509; PMID:19481529; http://dx.doi.org/10.1016/j.molcel.2009.04.011
10. Werneburg NW, Bronk SF, Guicciardi ME, Thomas L, Dikeakos JD, Thomas G, Gores GJ. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein-induced lysosomal translocation of proapoptotic effectors is mediated by phosphofurin acidic cluster sorting protein-2 (PACS-2). J Biol Chem 2012; 287:24427-37; PMID:22645134; http://dx.doi.org/10.1074/jbc.M112.342238