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Making short work of p53

A truncated phosphatase shuts down genome guardian in tumor cells.

Kleiblova et al. have uncovered mutations in the phosphatase Wip1 that enable cancer cells to foil the tumor suppressor p53 (1).

Like a battlefield surgeon who has to decide which casualties can be saved, p53 performs triage on cells with injured DNA. If the damage is grievous, p53 spurs the cells to die or senesce. But after milder hits, p53 flips on the DNA damage response (DDR), which instigates repairs, and indirectly activates the G1 checkpoint that prevents cells from advancing any farther in the cell cycle. Once cells have mended their DNA, the phosphatase Wip1 enables them to re-enter the cell cycle by shutting down p53 and DDR proteins such as ATM and Chk1/2 (2, 3). p53 and the DDR stymie cancer cells, so it's no surprise that the rogue cells find ways to circumvent this protection. More than half of all cancers accrue mutations in the p53 gene, for example (4). Kleiblova et al. tested whether some cells instead carry mutations in the *PPM1D* gene, which encodes Wip1.

The team started by analyzing seven human tumor cell lines that are commonly used in research and that harbor functional p53. Two of the lines, U2OS and HCT116, displayed mutations in exon 6 of the *PPM1D* gene, resulting in short proteins missing their C-terminal regions. After a dose of radiation that causes DNA breaks, full-length Wip1 can prevent the formation of ionizing radiation-induced foci where repair enzymes are at work. Truncated Wip1 inhibited the foci as well and thwarted certain key steps of the DDR, such as phosphorylation of the H2AX histone, suggesting it retains many of the functions of full-length Wip1.

To determine how shortened Wip1 causes trouble, the researchers tested its ability to remove phosphate groups. Truncated Wip1 wasn't more active. Instead, Kleiblova et al. discovered, loss of the C-terminal region increases the mutant protein's stability, allowing it to hang around longer in the cell. U2OS and HCT116 cells harbored 10 to 20 times more truncated Wip1 than wild-type protein, even though mRNA levels for each version were about the same. After a dose of cycloheximide to block protein synthesis, the level of normal Wip1 declined about three times faster than did the level of mutant protein, suggesting that cells recycle the abridged version of Wip1 more slowly.

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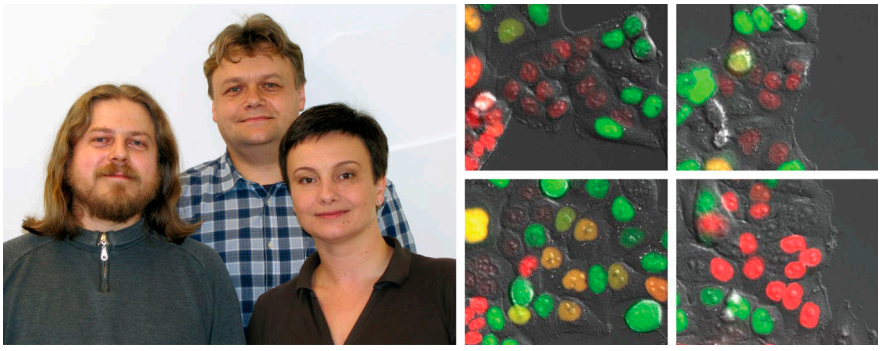
The longer-lasting Wip1 protein allowed cells to speed through the G1 checkpoint and enter S phase. The researchers showed that an siRNA targeting truncated Wip1—but not an siRNA that suppresses only the full-length protein—restored the checkpoint in U2OS cells.

Kleiblova et al. then looked for *PPM1D* mutations in 1,000 patients who had colorectal or breast and ovarian cancer. Four of the patients carried mutations, whereas none of the 450 cancer-free controls did. All of these DNA alterations fell in exon 6 and caused production of shortened Wip1. To the researchers' surprise, the mutations occurred in the cancer patients' non-tumor cells as well. That result suggests that the patients were born with *PPM1D* mutations, which set them up for cancer later in life but apparently caused no other illnesses.

"We've identified a new mechanism that could lead to inactivation of p53 in cells and inactivation of the DNA damage response," says senior author Libor Macurek. Why the mutations cluster in exon 6 of *PPM1D* remains a mystery. The researchers suspect that *PPM1D* mutations could turn up in a variety of tumors. If so, targeting the short but overactive form of Wip1 could provide a new way to treat these cancers.

Focal Point



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(Left to right) Libor Macurek, Zdeněk Kleibl, Petra Kleiblova, and colleagues (not pictured) tracked down mutations in the phosphatase Wip1 in cancer cells that enhance the protein's ability to shut down p53 and switch off the DNA damage checkpoint. The panel shows how cells respond to a dose of radiation that normally activates the G1 checkpoint. The top row shows the cells one hour after the radiation, and the bottom row shows them 17 hours afterward. Cells with truncated Wip1 (left column) pass through the checkpoint and enter S phase (orange nuclei), but most cells treated with an siRNA that targets truncated Wip1 (right column) halt at the checkpoint in G1 (red nuclei).

1. Kleiblova, P., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201210031.
2. Le Guezennec, X., and D.V. Bulavin. 2010. *Trends Biochem. Sci.* 35:109–114.
3. Lu, X., et al. 2005. *Genes Dev.* 19:1162–1174.
4. Hollstein, M., et al. 1991. *Science*. 253:49–53.