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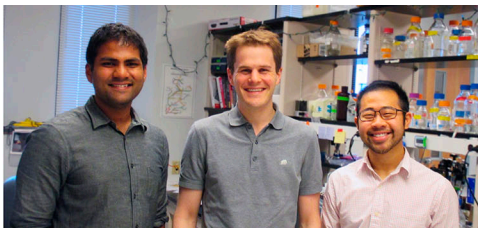
Mitotic cells get a stress test

Two studies identify a signaling pathway that arrests cells that are having difficulty dividing.

FOCAL POINT



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Two teams of researchers, including (left photo) Franz Meitinger (left) and Karen Oegema (right) and (right photo) Vikas Daggubati (left), Andrew Holland (center), and Bramwell Lambrus (right), investigate how cells stabilize p53 and arrest their cell cycle when they lose their centrosomes and are at risk of segregating their chromosomes incorrectly. The researchers find that p53 is stabilized by the p53-interacting protein 53BP1 and the deubiquitinase USP28, independently of the proteins' role in the DNA damage response. The USP28-53BP1-p53 signaling pathway also arrests cells that spend an extended time in mitosis, though whether this is how centrosome loss activates the pathway remains unclear.

Before they enter mitosis, cells duplicate their centrosomes so that they can form a proper, bipolar mitotic spindle. Cells with only one centrosome—or even no centrosomes at all—can still divide, but they tend to make errors in chromosome segregation. Healthy, nontransformed cells therefore apply the brakes when they lose their centrosomes, and arrest by up-regulating the tumor suppressor p53. Two groups now identify some of the proteins required for the p53 response to centrosome loss, and find that these signaling components are also involved in arresting cells that, for one reason or another, are taking too long to divide (1, 2).

Centrosome duplication is triggered by the kinase Plk4. In 2015, Karen Oegema, Andy Shiau, and their colleagues at the Ludwig Institute for Cancer Research in San Diego developed a Plk4 inhibitor called centrinone, and found that nontransformed cells treated with this compound gradually lost their centrosomes and arrested in a p53-dependent manner (3). At the same time, Bramwell Lambrus, Andrew Holland, and colleagues at Johns Hopkins University School of Medicine saw a similar effect when they induced Plk4's rapid degradation (4). "We knew the arrest depended on p53's up-regulation," says Lambrus. "But we didn't know what was up- or downstream of p53 signaling."

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Both groups decided to perform a CRISPR/Cas9 screen to define the pathway required to arrest cells after Plk4 inhibition and centrosome loss (1, 2). In both cases, the researchers found that the p53-binding protein 53BP1 and the deubiquitinase USP28 were required to stabilize p53 after centrosome removal. p53, in turn, arrests the cells by activating the cell cycle inhibitor p21. Bryan Tsou's lab at Memorial Sloan Kettering have also just reported similar findings (5).

Oegema and colleagues, led by postdoc Franz Meitinger, also found that knocking out the ubiquitin ligase TRIM37 suppressed the p53-mediated arrest of centrosome-deficient cells. Cells lacking TRIM37 formed numerous cytoplasmic foci that contained many key centrosomal proteins and were capable of nucleating microtubules. These "centrosome-like structures" were able to compensate for the absence of genuine centrosomes, largely avoiding compromised mitosis and preventing the up-regulation of p53.

In addition to arresting centrosome-deficient cells, p53, 53BP1, and USP28 are all involved in the DNA damage response (DDR), raising the possibility that centrosome loss causes a cell cycle arrest because it somehow induces DNA damage. But neither group has observed signs of DNA damage in Plk4-inhibited cells, and knocking out or chemically inhibiting the DNA damage response kinases ATM, ATR, Chk1, and DNA-PK had no effect on the proliferation arrest induced by centrosome loss (1, 3).

In fact, 53BP1- and USP28-deficient cells still up-regulated p53 and arrested in response to the DNA-damaging agent doxorubicin, indicating that the DDR is at least partially functional in these cells. p53 is also up-regulated when cytokinesis fails (6) or cells take an abnormally long time to divide (7). Meitinger et al. found that 53BP1 and USP28 weren't required to arrest cells after cytokinesis failure, but both sets of researchers found that the two proteins stabilize p53 when mitosis lasts more than ~90 minutes.

Cells treated with Plk4 inhibitors manage 2–3 divisions before they arrest. These divisions take longer than normal, but they don't exceed 90 minutes, suggesting that centrosome loss doesn't induce arrest by prolonging an individual round of cell division. "But we're not convinced that these are completely separate pathways," says Holland. "There may be a cumulative effect where a succession of prolonged divisions builds up a threshold level of stress that causes cell cycle arrest."

"This is a key question going forward," agrees Oegema. "Is there a multigenerational mitotic duration sensor that elevates p53 levels, or is centrosome loss per se an independent stimulus?"

Another key question is how USP28 and 53BP1 stabilize p53. Because 53BP1 is known to bind USP28 as well as p53, a possible scenario is that it bridges the two proteins so that USP28 can deubiquitinate p53, preventing the tumor suppressor from being targeted to the proteasome for degradation.

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