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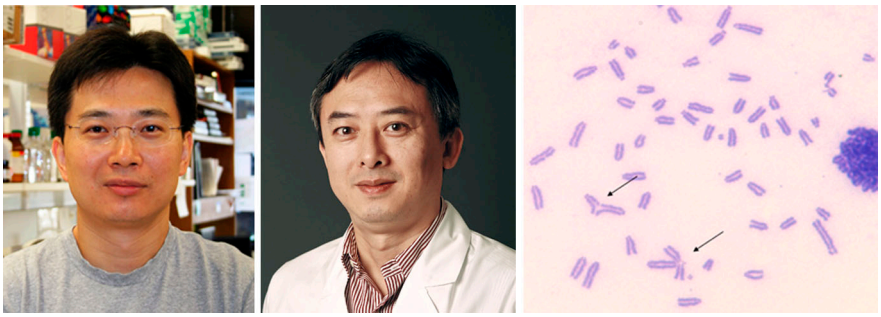
Repair defects put stem cells in a fix

Study reveals that mutations in DNA-dependent protein kinase block multiple DNA repair pathways.

Focal Point



ZHANG PHOTO COURTESY OF MENGXIA LI; CHEN PHOTO COURTESY OF UT SOUTHWESTERN BIOMEDICAL ART SERVICES

Shichuan Zhang (left, who won the 2010 Marie Curie Award from the Radiation Research Society for his work), Benjamin Chen (right), and colleagues (not shown) generated knockin mice expressing a mutant version of the DNA repair kinase DNA-PKcs that can't be phosphorylated by the upstream kinases ATM and ATR and is therefore unable to activate the nonhomologous end-joining repair pathway. Surprisingly, the mice were also defective in the homologous recombination and Fanconi anemia repair pathways, resulting in chromosome aberrations (far right, arrows) and the death of hematopoietic stem cells.

If you can't help out during an emergency, it's better to get out of the way and let someone else take over. The same principle could apply to DNA repair; as Zhang et al. reveal, mutating DNA-dependent protein kinase (DNA-PK) blocks several repair pathways, resulting in the loss of hematopoietic stem cells (1).

DNA-PK consists of a catalytic subunit (DNA-PKcs) and the DNA-binding proteins Ku70/80, which recruit the complex to double-stranded breaks (DSBs). The complex helps repair these breaks by initiating the nonhomologous end-joining (NHEJ) pathway. NHEJ is best known for repairing DSBs caused by ionizing radiation and completing the process of V(D)J recombination in developing lymphocytes. Mice lacking DNA-PKcs are therefore hypersensitive to radiation and are immunodeficient. But NHEJ may also repair DSBs generated during DNA replication, alongside another repair pathway called homologous recombination (2).

Benjamin Chen from UT Southwestern Medical Center in Dallas, TX, is interested in how DNA-PKcs is regulated by the two kinases ATM and ATR, which phosphorylate DNA-PKcs on a cluster of threonine residues (3, 4). Blocking this phosphorylation event by mutating the threonine residues to alanines inhibits DSB repair and increases cells' sensitivity to UV and ionizing radiation (3, 4). "We wanted to understand the physiological significance of this phosphorylation," explains Chen. "So we made a knockin mouse expressing this mutant DNA-PKcs."

> **"[The DNA-PK mutant] occupies DNA ends for longer, blocking other proteins from coming in."**

Apart from being immunodeficient and radiosensitive, mice completely lacking DNA-PKcs are fairly healthy and live a normal lifespan. Yet mice expressing the non-phosphorylatable DNA-PKcs mutant (DNA-PKcs$^{3A/3A}$) failed to thrive and died a few weeks after birth (1). "We were surprised by this phenotype," Chen admits. Similar to DNA-PKcs–null animals, DNA-PKcs$^{3A/3A}$ mice had reduced numbers of B and T lymphocytes, indicating that V(D)J recombination was inhibited in the absence of DNA-PKcs phosphorylation. But the knockin mice lacked other blood-cell lineages too, due to a loss of hematopoietic stem cells (HSCs) from their bone marrow. Transplanting in wild-type bone marrow restored blood cell development and extended the lives of DNA-PKcs$^{3A/3A}$ mice.

Defects in HSCs first appeared in fetal liver, where blood progenitors normally undergo rapid expansion during embryogenesis. HSCs from mutant mice failed to proliferate, appearing instead to accumulate DSBs and die by apoptosis. Blocking cell death by crossing DNA-PKcs$^{3A/3A}$ animals to p53-null mice rescued hematopoiesis and prolonged the survival of DNA-PKcs$^{3A/3A}$ mutants. "We also see genotoxic stress in intestinal crypts and the skin," says Chen, referring to two other sites of highly proliferative stem cells, "so we think this phenotype is related to DNA replication."

But why are DNA-PKcs$^{3A/3A}$ mice susceptible to replication stress, when DNA-PKcs–null mice aren't? Zhang et al. found that NHEJ wasn't the only DNA repair mechanism inhibited by the threonine-to-alanine mutations. Cells from DNA-PKcs$^{3A/3A}$ mice were also defective in the homologous recombination and Fanconi anemia (FA) DNA damage pathways. Indeed, the phenotype of DNA-PKcs$^{3A/3A}$ mice is reminiscent of FA, including bone marrow failure and increased sensitivity to DNA cross-linking agents. On the other hand, the homologous recombination repair pathway was enhanced in cells from DNA-PKcs–knockout mice.

"We think that when the threonine cluster is mutated, DNA-PKcs is defective in NHEJ, but it occupies DNA ends for longer, blocking other proteins from coming in to repair the break," Chen explains. "In a DNA-PKcs knockout cell, other proteins can come in easily." Phosphorylation of the DNA-PKcs threonine cluster may therefore be crucial for switching between the different DSB repair pathways.

Chen plans to test his hypothesis by crossing the DNA-PKcs$^{3A/3A}$ mice to Ku70/80 knockout animals, which don't recruit DNA-PKcs to DSBs and should therefore rescue the mutant phenotype. In the longer term, Chen wants to investigate how DNA-PK and NHEJ cooperate with other DNA damage pathways to repair the DSBs that accumulate during replication.

1. Zhang, S., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201009074.
2. Sonoda, E., et al. 2006. *DNA Repair (Amst.).* 5:1021–1029.
3. Yajima, H., et al. 2006. *Mol. Cell. Biol.* 26:7520–7528.
4. Chen, B.P., et al. 2007. *J. Biol. Chem.* 282:6582–6587.