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## Researchers Identify New Cause of Genomic Instability

Researchers sifting through the indispensable machinery that senses and fixes broken DNA have discovered a new culprit that can induce instability in the genome and thereby set the stage for cancer to develop.

Studies in mice have shown that loss of *H2AX*, a gene that produces a protein called a histone that is part of the chromosomal structure, can tip the delicate balance of proteins that are curators of the human genome. When H2AX ceases to function properly, lymphomas and solid tumors can arise because errors in the genetic code are not always repaired correctly, according to the new research.

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The finding may have important implications for understanding the origin of human cancers because a large number of human tumors are known to contain alterations in the region of chromosome 11 where the *H2AX* gene is located.

The research was reported in an article published in the August 8, 2003, issue of the journal *Cell* by Howard Hughes Medical Institute investigator Frederick Alt and colleagues at Children's Hospital in Boston and Harvard Medical School. Other co-authors are from the Tufts University School of Veterinary Medicine and Brigham and Women's Hospital.

According to Alt, previous studies by other researchers had shown that H2AX was activated when DNA breaks occur. DNA repair proteins fix genetic damage, but they are also called to action during the normal gene rearrangement that occurs in immune cells when they are readying to battle viruses and other threats.

To explore the implications of knocking out the *H2AX* gene, lead author Craig Bassing created a line of mice lacking both copies of the *H2AX* gene.

“Both Craig in our lab and Andre Nussenzweig at the National Cancer Institute produced knockout strains that showed an increased level of genomic instability,” said Alt. Nussenzweig and his colleagues have published an article in the same issue of *Cell* on studies of their *H2AX*-knockout mice, and have found similar increases in genomic instability and cancer.

According to Alt, the mice lacking only *H2AX* genes had only a modest increase in cancer, “which is often the case for many genes that produce cancer, because they operate within a system of cellular checks and balances,” he said. “But when you eliminate two genes that may work in concert to maintain good genomic order, you see things happen that are much more dramatic.”

Thus, the researchers created a double-knockout mouse that lacked both *H2AX* and *p53* —a gene that produces a molecular sentinel protein that suppresses proliferation of cells with damaged DNA. In previous studies, Alt and his colleagues had shown that loss of *p53* in cells that lacked the DNA-repair process known as non-homologous end-joining (NHEJ) resulted in a dramatic increase in cancers.

“When we deleted both copies of *H2AX* and both copies of *p53*, we found a dramatically increased rate of tumors appearing beyond what would be seen with *H2AX* deficiency alone, and far, far beyond *p53* deficiency alone,” said Alt. These cancers developed so rapidly that within a few months all the mice had died, he said. The resulting tumors included both lymphomas arising from aberrant immune cells—which would be common in the loss of NHEJ DNA repair function—and solid tumors, which are not normally seen when NHEJ is compromised.

The researchers also saw cancers arising from malfunction of DNA repair in mature immune cells called B cells. “These kinds of tumors are much more relevant to what goes on in a very large percentage of lymphomas in humans, and adults, in particular,” said Alt.

Alt said that the biggest surprise came when the researchers produced mice missing only one of the two copies of the *H2AX* gene. “Both surprising and potentially very significant for human cancers was that *p53*-deficient mice with deletion of one of their two copies of the *H2AX* gene came down with cancer much earlier,” he said. “They showed a very broad spectrum of tumors that was somewhat different than *p53*-deficient animals missing both *H2AX* genes.”

Furthermore, the studies showed that otherwise normal cells that were missing just one *H2AX* gene had only half the levels of H2AX and also showed genomic instability.

The possibility that only half the levels of the H2AX protein—called “haploinsufficiency”—triggers genomic instability and cancers could be highly significant, said Alt. Such a class of mutation would not have been readily detected in most searches for genes that suppress tumors in humans.

Indeed, Bassing discovered that tumors in these mice still had a functioning *H2AX* gene.

“A major question is why haploinsufficiency of the H2AX protein can cause genomic instability and cancer,” said Alt. “One might explain that quite easily because the protein is not an enzyme, it's a structural protein. So, if there's half as much present, it could cause problems in monitoring breaks in the DNA and in recruiting components of the repair machinery.”

What may be especially relevant to human cancers, said Alt, is that the *H2AX* gene is located in a region of chromosome 11 known to be altered in many human tumors. While there are other potential cancer-causing genes in that region, he said, the current evidence from the mouse model indicates that *H2AX* will very likely prove a major player in cancer-causing genomic instability.

Alt and his colleagues are now collaborating with scientists at the Dana-Farber Cancer Institute to analyze the status of *H2AX* genes in a wide range of human cancers. “We believe that the loss of *H2AX* could prove a major source of the rampant instability associated with the progression of a variety of different tumors,” he said.