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## Better Model of Deadly Brain Cancer

Researchers have created a mouse model that closely mimics human medulloblastoma, the most common type of childhood brain tumor. The new model, which was created by knocking out a key component of the DNA repair machinery, will aid in exploring the genetic roots of this deadly brain cancer.

The researchers, led by Howard Hughes Medical Institute investigator Frederick W. Alt, published their findings the week of April 24, 2006, in the early online edition of the *Proceedings of the National Academy of Sciences*. Catherine Yan, who is in Alt's laboratory at Children's Hospital Boston, was lead author of the article. Other co-authors were from Brigham & Women's Hospital, CBR Institute of Biomedical Research, Children's Hospital and Dana-Farber Cancer Institute, all of Harvard Medical School.

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— Frederick W. Alt

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Although childhood cancers are rare, brain tumors are among the most common. About one out of five childhood brain tumors is medulloblastoma, an aggressive cancer of the cerebellum. Alt and his colleagues produced the mouse model of medulloblastoma by knocking out a gene called *XRCC4*, which produces a protein that plays an important role in stitching together the ends of broken DNA. These breaks which can occur in all cell types from exposure to radiation, chemicals, or other insults, occur specifically in the immune system when genes are snipped and rearranged to produce a vast array of antibodies. The abnormal swapping of chromosomal regions that ensues when such repair goes awry—known as chromosomal translocations—is sometimes harmless, but can contribute to cancer and other diseases.

In earlier studies, Alt and his colleagues discovered that *XRCC4* is a component of nonhomologous end-joining, a process that is essential for the repair of chromosome breaks. They found that knocking out this gene in mice led to widespread death of newly generated neurons and death late in embryonic development. The researchers then combined these experiments with the elimination of a gene for a sentinel protein called p53, which triggers the death of malfunctioning cells. With both *p53* and *XRCC4* missing,

neurons survive and the mice live into early adulthood, but then die of lymphomas caused by translocations of antibody genes. The researchers noted that by this time, the mice were also beginning to develop medulloblastomas.

After they made that observation, Alt's team wanted to zero in on the possible role of *XRCC4* deficiency in medulloblastomas. While their earlier studies involved knocking out the *XRCC4* gene throughout the animals' bodies, now the major goal was to eliminate this protein only in the developing nervous system, so we could specifically determine whether there was a role for nonhomologous end-joining in suppressing cancers of cells besides those of the immune system, he said. We also wanted to know whether getting rid of both *XRCC4* and *p53* in the nervous system would predispose the animals to neuronal tumors, and whether or not those tumors would also be associated with particular chromosomal translocations.

So Yan and her colleagues engineered two strains of mice in which *XRCC4* was knocked out only in neural progenitor cells in the developing nervous system. One strain had only the *XRCC4* knockout, and the other also had a deficiency in both *XRCC4* and *p53*. These mice appeared to develop normally without *XRCC4*, they found. But every mouse lacking both *XRCC4* and *p53* died very early of medulloblastomas. Furthermore, those tumors strongly resembled human medulloblastomas, said Alt.

Analyzing the tumors for genetic abnormalities, Alt and his colleagues found that specific genes were frequently altered in association with recurrent chromosomal translocations—and that affected genes often were those activated or inactivated in human medulloblastomas. Thus, the tumors often showed amplifications of two genes called *N-myc* and *Cyclin D2*, which are characteristic of many human neural tumors, including medulloblastomas. The animals also showed the loss of one copy of a gene called *patched*, which is also characteristic of some human medulloblastomas.

Only in our wildest dreams had we hoped to see these kinds of recurrent translocations, said Alt. It's quite exciting to us that we'll be able to explore mechanistically why they happen when the basic process of end-joining is compromised.

Other mouse models of medulloblastoma have been created by knocking out *patched* or other individual genes that have been implicated in the development of medulloblastoma. However, said Alt, what we did was different. We created an environment in which end-joining was defective and let the biology of the cell sort out the consequences. And while in most other models not every mouse develops medulloblastomas, in our case every animal very reproducibly develops these tumors at a very young age. It's really quite intriguing, too, how this general genomic instability very specifically leads to the selection of tumor cells that have deregulated particular genes such as *N-myc*, *patched* and *Cyclin D2*.

According to Alt, the new mouse model will prove valuable in understanding why *N-myc* is so frequently amplified in human tumors, including

neuroblastomas and medulloblastomas, and the consequences of that amplification. The model also will enable the researchers to better explore causes of the chromosomal translocations, deletions, and amplifications in neuronal cells.

The mouse model should also be useful in testing potential treatments for medulloblastoma. Alt said that other laboratories have consulted them on the possibility of using the model for drug testing. This model could be very useful for such a purpose because every mouse gets tumors with an early onset and the tumors show activation or inactivation of a set of genes that is implicated in human tumors, he said. So, if one wants to test therapies that interfere with pathways involved in human tumors, this should be a good model.