

DECEMBER 10, 1999

Researchers Develop Mouse Models of Neurofibromatosis

Two strains of mice genetically engineered to develop neurofibromatosis type 1 (NF1), a tumor-producing hereditary disease of the peripheral nerves, are likely to improve understanding of the progression of NF1 and provide much needed models in which to evaluate therapies for the disease.

"When we showed those microscopy images to neuropathologists, they were very impressed that we had tumors composed of cells characteristic of human tumors."

— Tyler Jacks

NF1, also called von Recklinghausen's disease, occurs in about one in every 3,500 births. The disorder can cause thousands of painful, disfiguring skin lesions and benign peripheral nerve tumors, called neurofibromas. In some cases, those neurofibromas can turn malignant, rendering the disease potentially deadly.

The research team, which was led by HHMI investigator Tyler Jacks and postdoctoral fellow Karen Cichowski of the Massachusetts Institute of Technology, announced in the December 10, 1999, issue of the journal *Science* that it had produced one set of mice with benign NF1-related tumors and a second set of mice that developed the malignant tumors associated with the disease.

In developing the mouse models, the researchers began with a previously produced strain of *NF1* "heterozygous" mice that were missing one functioning copy of the gene. The *NF1* gene codes for the protein neurofibromin, which is normally a tumor suppressor, preventing cells from proliferating. "Since this first heterozygous animal did not prove to be cancer-prone, we knew we had a connection to the human disease," said Jacks. "However, those animals did not develop the hallmark lesions of NF1." Mice lacking both copies of the *NF1* gene died *in utero*. In order to create mutant mice that would survive, Jacks and his colleagues produced

chimeric mice animals containing a mix of cells that had either both functioning *NF1* genes or no *NF1* genes at all.

When the scientists produced the chimeric mice, the animals showed benign neurofibromas, in particular, tumors in deep nerves that are more likely to turn malignant. Importantly, said Jacks, the chimeric mice with higher percentages of *NF1* -deficient cells showed higher degrees of NF1 pathology.

The researchers also followed the fate of cells lacking *NF1* by using a "reporter gene" to track cells missing the *NF1* genes. Those experiments revealed that neurofibroma tumors invariably included such *NF1* -deficient cells.

"This finding was important because these tumors include many types of cells, and it has been unclear whether the hyperproliferative cells those that comprise the tumor were *NF1* -deficient. Our work suggests that the tumor cells are, indeed, *NF1* -deficient."

Also, said Jacks, electron microscopy of the tumors revealed that they closely resemble human tumors.

"When we showed those microscopy images to neuropathologists, they were very impressed that we had tumors composed of cells characteristic of human tumors," said Jacks. Thus, he said, the mouse model will likely be useful not only in understanding NF1, but in testing gene therapies or drugs to treat the benign tumors.

In creating the mouse model of the malignant tumors, the scientists crossed their heterozygous mice with a strain of mice lacking another tumor-suppressor gene, called *p53* .

"We found that mice defective in only *NF1* or *p53* did not produce this type of malignant tumor, but we did see these tumors at high frequency in mice carrying the two mutations together," said Jacks. "Given this high frequency and the rapid development of tumors, we believe we have a robust model of this malignant tumor that will likely be useful in evaluating therapies."

According to Jacks, a number of drug companies are developing drugs to treat cancers by blocking a key cancer gene called *Ras* , which is also controlled by *NF1* . Thus, such anti- *Ras* drugs might be also prove useful in treating NF1.

Of particular interest to Jacks and his colleagues is the observation that the mutant mice showed differences in pathology when the *NF1* and *p53*

mutations were on the same or opposite chromosomes.

"Animals with both mutations on the same chromosome developed tumors much more quickly," he said. "So, we can argue quite strongly that linkage of two genes is important in cancer modeling in mice and perhaps in humans." Such linkage means that damage to or loss of a portion of one chromosome that knocks out both genes may be a significant general cancer-causing mechanism, said Jacks.

In future studies, the researchers plan to test anti-tumor drugs on their mouse models, as well as produce mice in which *NF1* is deleted in specific types of nerve cells. Targeting such cells will enable more detailed study of the mechanism of tumor production, said Jacks. The scientists also plan to produce *NF1* and *p53* mutations in different strains of mice to determine whether other genetic factors might subtly modify the progress of the disease.

"If we find such modifiers, we might better understand the functions of these genes and also why different patients develop different symptoms of the disease," said Jacks.