

THE MOUSE THAT ROARED

THE STORY OF A MOUSE THAT
COULDN'T GET CANCER

As researchers watched in surprise — even disbelief — one mouse defied all odds of getting cancer, leading Wake Forest scientists down new paths of understanding cancer resistance.

THE SURPRISES BEGAN with the mouse that couldn't get cancer.

At first, Zheng Cui, M.D., Ph.D., thought it must be a mistake — that his lab assistant had somehow forgotten to inject the cancer cells into the abdomen of this one white mouse. After all, other mice inevitably develop cancer following similar injections of mouse sarcoma cells.

So Cui, an associate professor of pathology at Wake Forest University School of Medicine, asked the lab assistant to inject the mouse again. Still no cancer. He doubled the dose. Still no cancer. He kept increasing the dose of the mouse sarcoma cells — one of the most malignant tumors known for mice — to huge levels (10 percent of body weight) and still nothing.

Questions began to haunt Cui:

> "Could this be an extraordinarily cancer-resistant mouse?"

> "What makes this mouse cancer-resistant?"

Cui recalled that he could find no explanations or other cases in the scientific literature of resistance to mouse sarcoma cells, an extremely aggressive cancer. "This kind of mouse had not been described before," he said.

BY ROBERT CONN

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Mark Willingham, M.D., professor of pathology, an experienced cancer researcher and Cui's close colleague in the Department of Pathology, also was intrigued and excited by the mouse. With his encouragement, Cui decided to breed the mouse, a male, to determine if the resistance was genetic. Roughly half the offspring also were resistant to cancer.

"There was a genetic component that could be passed on to offspring," Cui said.

Since then, 1999, nearly 700 cancer-resistant mice have been bred from the original mouse in more than 10 generations and in several different strains.



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— Zheng Cui, M.D., Ph.D.

"This mouse and this colony is probably the first case in which mice can fight off essentially any number of tumor cells," said Cui. "The mice are healthy, cancer-free and have no sign of a shortened lifespan."

The serendipitous discovery produced growing excitement in the pathology laboratories at the Medical Center, and, as word spread, more investigators joined the project.

When the discovery was announced in *Proceedings of the National Academy of Sciences* in April 2003, it brought instant world-wide attention to the Wake Forest scientists. The story was carried by the three major wire services, the BBC, CNN, MSNBC, ABC, NBC, CBS, CBC, scores of television stations, dozens of newspapers, and such prestigious specialty publications as *Scientific American*, *New Scientist*, *The Lancet* and *Nature*.

"This project is unique to Wake Forest," said Willingham. "Everything in this paper was done at Wake Forest by Wake Forest scientists."

But discovery of the mouse was only the first of many surprises, surprises that may challenge conventional thinking on cancer progression and the body's defenses against cancer.

The second surprise came when the team delayed the injection of cancer cells into some of the colony's mice until they were approaching mouse middle-age, at 22 weeks. This time, the injections resulted in huge tumors — but suddenly, often overnight, the tumors disappeared. No cancer cells were left behind. "The mice became healthy and immediately resumed normal activities including mating," Cui said.

It turned out that whether a colony mouse displayed complete resistance to cancer or developed a cancer first, then experienced spontaneous regression, was determined by the age of the mouse at its first exposure to cancer cells. Older mice experienced regression, while younger ones never developed cancer.

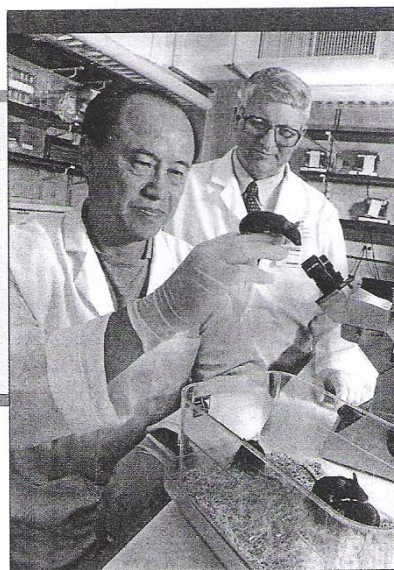
For the first time, scientists at Wake Forest had an animal model of spontaneous regression of advanced cancer.

"Regression of human cancers without treatment is well docu-

mented for many types of cancer, but occurs infrequently," Cui said.

Because the cancer has disappeared in these people, there was no way to study what happened to their tumors.

Cui and Willingham found that once these older mice had been through spontaneous regression, they were



Drs. Zheng Cui (left) and Mark Willingham examine one of the tumor-resistant mice.

immune from future attacks from any cancer cells, the same as their younger cousins.

The third surprise occurred when Cui and Willingham found out how selectively and rapidly cancer cells were destroyed by immune cells. They analyzed what was happening under powerful microscopes and it was possible to capture the killing events on camera. [You can watch these video clips at <http://www.wfubmc.edu/pathology/research/srmouse2.htm>.]

"A resistant mouse can destroy up to 2 million cancer cells in an hour, without apparent damage to normal cells," Cui said. "These observations suggest a previously unrecognized mechanism by which the body can fight off cancer."

Willingham noted that this mouse directly demonstrates that cancer resistance can be inherited. "That's a new concept." Standard cancer theory would have predicted some internal control of cancer cells to prevent the cancer from occurring. But these mice demonstrate "the control of the host and its ability to reject tumor cells once they are already created."

The fourth surprise came when they found they could breed mice from the colony with so-called nude mice, and the nude mice were able to reject the injected sarcoma tumor cells.

It is widely believed by immunologists that T cells, the immune cells that lead to transplant rejection, play a critical role in host defense against cancers. Nude mice don't have T cells, yet these cancer-resistant nude mice still were able to kill the cancer cells.

Cui and Willingham said the cancer cells were killed by another type of immune cell, cells that are traditionally thought to play a major role in host defense against infections: natural killer cells, macrophages and neutrophils. These immune cells are key elements of what scientists term innate immunity.

And these mice add credence to the scientific argument for immune surveillance. "For the past 20 years, immune surveillance had been largely dismissed as not being true," said Willingham. "This mouse finally provides a good example of where it really works."

More recently, the scientists found they can even make normal mice resistant to cancer by giving them immune cells taken from the cancer-resistant mice. This result raises a hope that someday, giving cancer patients cancer-fighting immune cells may become an effective therapy.

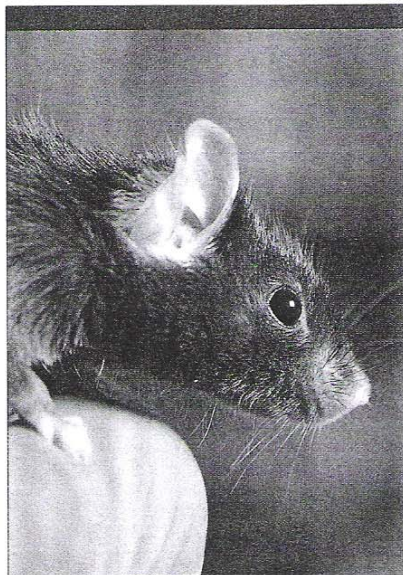
But ultimately what has Cui and Willingham and their colleagues excited is the concept of cancer-resistant genes. "Everybody probably has some awareness that there are genes like BRCA1 that predispose families to cancer. The reverse of that is that families may inherit resistance to cancer," said Willingham.

That is difficult to study because there are no medical cases. "If you've got the good gene, you don't know it because you don't get cancer."

It may help explain why some cigarette smokers never get sick. "The question has always been why not?"

"This mouse shows that there can be a single gene that has a profound effect on the animal's or the person's ability to develop cancer. It is a direct demonstration that such genes exist. And that has not been generally recognized," Willingham continued.

There were other surprises that were also intriguing. Once



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the mice were exposed to cancers, they were protected for life. But if they hadn't been exposed to cancer cells until they were one year old (an old age for mice), the mice would die of cancer despite having the gene. "If they are given cancer cells, they die, even though their offspring are resistant, so we know they carry the gene," said Cui.

That showed, said Willingham, that there is "an age-relatedness to this mechanism. The ability to reject tumor cells declines with age."

Willingham said the explanation may be the steady decline of innate immunity during aging.

"We now have a handle on how to study the age-related decline of anti-cancer immunity. We have always thought of the higher incidence of cancer as we get older as being due to the fact that we accumulate more mutations in cells," he said. These mutant cells are more likely to become cancerous. But another explanation is that "we have clinically detectable cancer because of this loss of the immune mechanism, not just because we have more mutations. And that is what is so striking about this model."

Willingham said, "It might be another way of thinking about cancer. We might actually have a controlling mechanism

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The ongoing research is supported by the Charlotte Geyer Foundation, the National Cancer Institute, the Cancer Research Institute and the Comprehensive Cancer Center of Wake Forest University.

Willingham cautioned that the work is in a mouse. "A mouse is little and 10 percent of a mouse's body weight is still little." Any translation to humans may be five or even 10 years away.

But that doesn't mean that Wake Forest scientists aren't excited.

"There are two questions that people always ask," said Cui:

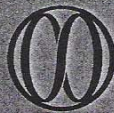
> "Can very aggressive cancer cells be selectively destroyed without harming normal cells?"

> "Can advanced cancer ever regress and life revert to normal?"

"This mouse, perhaps for the first time, gives us a hope for yes answers to both." ①

The Comprehensive Cancer Center of Wake Forest
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the Nation's Best

- *U.S. News & World Report* consistently ranks the Comprehensive Cancer Center of Wake Forest Baptist among the nation's best hospitals for cancer care. (since 1996)
- The Center is one of only 38 cancer centers in the country designated by the National Cancer Institute as *comprehensive*, indicating excellence in research, patient care and education. It is the only center in western North Carolina that carries this prestigious NCI designation.
- Wake Forest Baptist offers more cancer-related clinical trials than any other hospital in western North Carolina. From gene therapy to vitamin and nutrition studies to new surgical, medical and radiological treatments, patients benefit from the leading edge of cancer knowledge and care.
- While clinical trials offer hope for new and better treatments for patients, basic scientists at Wake Forest University Health Scientists are engaged in revolutionary research that is helping to redefine our understanding of cancer.



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