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Researchers Hope New Model Will Help Outmuscle Aggressive Childhood Cancer

The first accurate mouse model of an aggressive childhood muscle cancer has improved researchers' understanding of the cause of the disease and could accelerate the identification of new chemotherapeutics to treat the disorder, which is called alveolar rhabdomyosarcoma.

The researchers, led by Howard Hughes Medical Institute investigator Mario R. Capecchi, described their model of the cancer in two research articles published in the November 1, 2004, issue of the journal *Genes and Development*. Capecchi and his colleagues are at the University of Utah; one co-author was from the Harvard Medical School. The first author on both papers was Charles Keller, who works in Capecchi's laboratory.

"This cancer has an enormous lethality associated with it," said Capecchi. "It progresses extremely rapidly once it appears, such that by the time the child gets treatment, either the tumor is so integrated into the tissue that it is not easily resectable, or it has metastasized." The five-year survival for children with metastatic disease is less than 30 percent, he said.

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According to Capecchi, researchers had known that in the majority of alveolar rhabdomyosarcoma tumors, segments of two specific genes fuse together abnormally. One of these gene segments comes from the *forkhead* gene, and acts as that gene's on-switch. Normally, *forkhead* produces a protein called a transcription factor that controls an array of other genes. The other gene involved in the fusion is either of two *Pax* genes, *Pax3* or *Pax7*, which are both important regulators of muscle development. The fusion of the two gene segments creates a novel transcription factor that somehow

triggers tumor development in affected muscle cells.

Previous attempts to generate mice with the fusion gene did not produce viable animal models, said Capecchi. A major obstacle was that incorporating the fusion gene directly into mouse embryos resulted in immediate lethality.

To overcome this, Capecchi and his colleagues developed what he described as a “conditional knock-in” mouse. They did this by introducing a gene that they had designed to produce only normal protein until the mouse carrying it was cross-bred with a particular mouse strain, which then allowed the fusion gene to be produced specifically in skeletal muscle. This approach eliminated the early lethality due to the fusion, and more accurately recapitulated the behavior of the naturally occurring fusion gene, Capecchi said.

Still, however, alveolar rhabdomyosarcomas occurred only at low frequency in these mice, and the researchers wondered whether they could increase the frequency. They knew that disruption of two other gene pathways had also been implicated in human alveolar rhabdomyosarcomas. These pathways, which normally act as brakes on cell division, involve the genes *Ink4a/ARF* and *Trp53*. The researchers discovered that breeding their first conditional knock-in strain with a conditional inactivation of either *Ink4a/ARF* or *Trp53* genes resulted in mice whose cancers closely mimicked those seen in humans at very high frequencies.

“We find very close resemblance to human tumors, in terms of the pathology, the histology, and the molecular markers,” said Capecchi. “When we give tissues from these mice to pathologists whose specialty is rhabdomyosarcomas, they find them to be remarkably similar to human tumors.”

Importantly for research purposes, said Capecchi, the scientists also incorporated a gene that produces a telltale fluorescent protein when the fusion gene is formed. This feature enables them to isolate actual tumor cells and study their molecular characteristics in detail.

Capecchi and his colleagues see considerable promise that the new mouse model will yield both improved basic understanding of the cancer and a pathway to new treatments. “With this model, we can study the pathology of the tumors in greater detail,” he said. “And more importantly, if we can work out the pathway of the genes involved, then this information could reveal likely targets for drugs that can intervene in the tumor process.”

Studies of the transgenic mice have already provided insight into the cellular origin of the muscle tumors, Capecchi said. Most theories held that the tumors arose from immature muscle stem cells, called “satellite cells,” that were triggered to proliferate abnormally. Satellite cells are the source of cells that repair muscle fibers after injury or overuse.

However, Capecchi and his colleagues found evidence that the tumors actually originated in differentiated muscle cells that the fusion gene caused to “dedifferentiate” into abnormal cells that could then proliferate to form tumors due to the lack of the genetic “brakes.”