

## New Genetic Tactics to Model Alveolar Rhabdomyosarcoma in the Mouse

Charles Keller<sup>1</sup> and Mario R. Capecchi<sup>2</sup>

<sup>1</sup>Department of Cellular and Structural Biology, Children's Cancer Research Institute, The University of Texas Health Science Center, San Antonio, Texas and <sup>2</sup>Howard Hughes Medical Institute and Department of Human Genetics, University of Utah, School of Medicine, Salt Lake City, Utah

### Abstract

**Using conditional knock-in and knock-out techniques, we designed a mouse model of the childhood muscle cancer alveolar rhabdomyosarcoma (ARMS) that is driven by the chromosomal translocation product, *Pax3:Fkhr*. Tumors that closely recapitulate the spectrum of molecular markers and histology seen in human ARMS are exclusively produced in this model. Unexpectedly, expression of *Pax3:Fkhr* in muscle satellite cells did not produce tumors, but it did in differentiating myofibers. Expression of *Pax3:Fkhr* in muscle is necessary but not sufficient to initiate tumorigenesis at high frequency. This model offers new insight into the roots of alveolar rhabdomyosarcoma and illustrates the utility of *Cre-loxP* technology for studying otherwise inaccessible cancers in the mouse.** (Cancer Res 2005; 65(17): 7530-2)

Conditional genetic approaches allow the engineering of mouse models of human cancer that have unparalleled similarity to the human disease. The spatial and temporal control of gene function permitted in these new mouse models can answer questions about tumor cell of origin, the number of mutations required to initiate a tumor, and what secondary changes are required by a cancer for its progression. By controlling the time and place at which tumor initiation occurs, one can bypass nonspecific birth defects associated with the broad expression of oncogenes (or due to the absence of tumor suppressors), thereby controlling the precise moment and cell type in which tumor initiation events occur. In this manner, steps in the progression of a tumor can be studied without having to guess when or where to look.

Alveolar rhabdomyosarcoma is an aggressive childhood muscle cancer. The survival for children with advanced disease is dismal and prognosis has remained unchanged for over two decades (1). Eighty-five percent or more of alveolar rhabdomyosarcomas are associated with a translocation that fuses a *Pax* transcription factor, most often *Pax3*, to a *Fkhr* transcription factor (2). The *Pax3:Fkhr* fusion is believed to act as an oncogene, probably by forcing somatic cells down an aberrant embryonic myogenic developmental pathway. The translocation also destroys one normal copy of the putative tumor suppressor *Fkhr*. Multiple laboratories have attempted to develop a mouse model of alveolar rhabdomyosarcoma by expression of the *Pax3:Fkhr* oncogene in the early embryo, but in each case,

birth defects precluded the somatic formation of muscle cancers.

In designing our mouse model of alveolar rhabdomyosarcoma (Fig. 1), we approached several questions. First, is *Pax3:Fkhr* both necessary and sufficient for tumor initiation? Next, does haploinsufficiency of *Fkhr* play a role in tumor initiation? Finally, what is the cell of origin of this muscle cancer; is it an embryonic or postnatal muscle stem cell, or is it a mature myofiber?

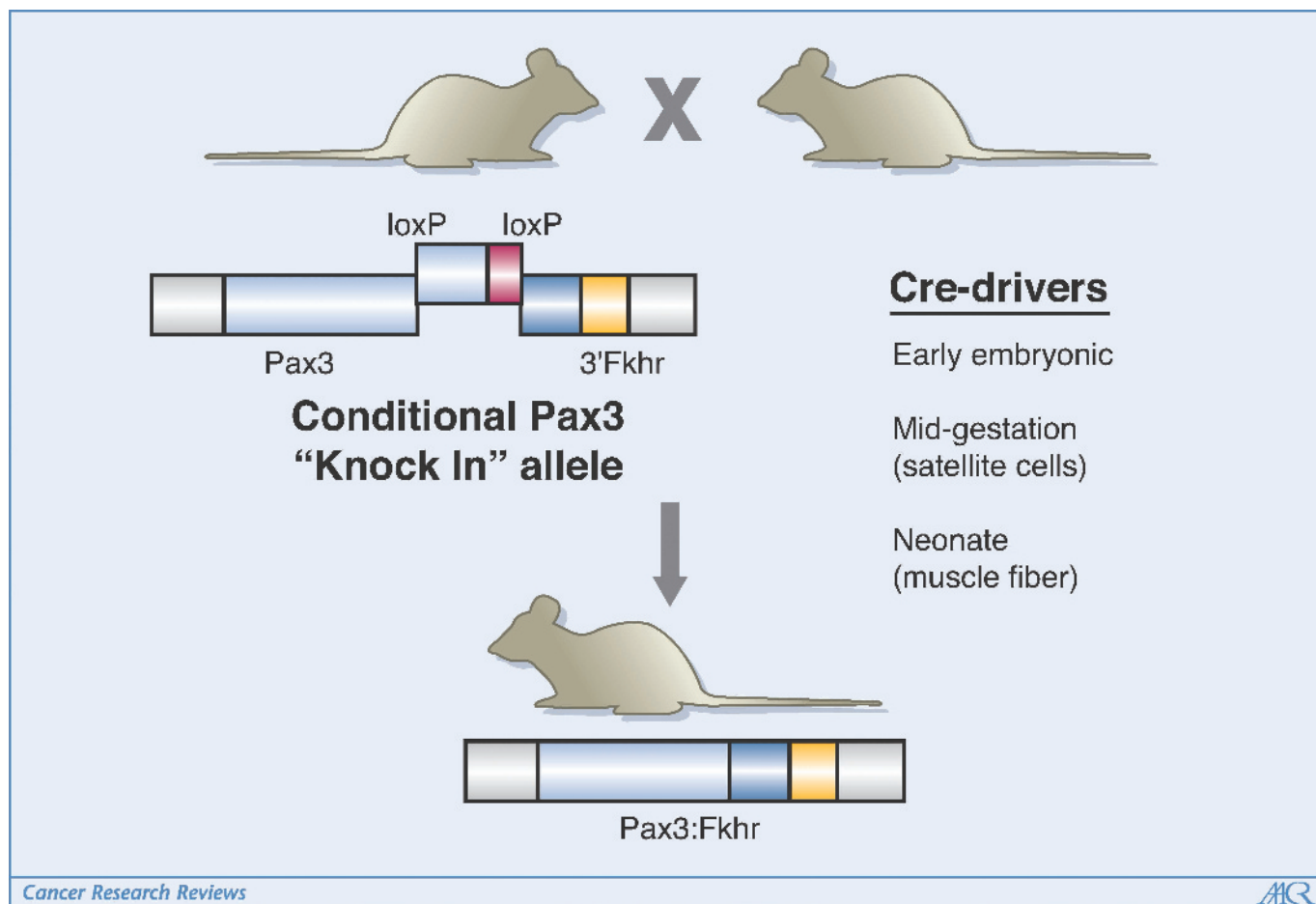
To implement our model, we used gene targeting and conditional genetics to insert a silenced portion of the *Fkhr* gene at the *Pax3* locus whereby *Pax3* could be normally transcribed until the Cre recombinase converted *Pax3* to the fusion gene, *Pax3:Fkhr* (i.e., a conditional knock-in approach). We also constructed a conditional knock-out of *Fkhr*, and Cre drivers that permitted *Pax3:Fkhr* to be expressed at early midgestation, late midgestation, and perinatally in embryonic and postnatal myogenic cells.

When we triggered the switch from *Pax3* to *Pax3:Fkhr* expression at early midgestation in the embryo, resultant muscles were correctly patterned but severely hypotrophic. Neoplasia was not seen. In addition, severe neural tube defects resulted. However, when *Pax3:Fkhr* was triggered later in gestation, in less primitive embryonic myoblasts and eventually in perinatal muscle stem cells (satellite cells), it seemed that the satellite cell pool was depleted and functioning poorly, apparently as a result of *Pax3:Fkhr* impairing the expression of downstream targets of *Pax7*, an important gene in the maintenance of satellite cells. Surprisingly, alveolar rhabdomyosarcomas again were not produced despite expression of the *Pax3:Fkhr* fusion gene in the entire muscle satellite cell pool. In this case, the mice survived to young adulthood. These observations make muscle satellited cells an unlikely candidate as the cell of origin for alveolar rhabdomyosarcoma. Curiously, tumors did form when we triggered *Pax3:Fkhr* expression in mature, differentiating skeletal muscle; however, the frequency of tumor formation was low (1 in 228).

Having our first clue in hand, that maturing skeletal muscle and not embryonic or postnatal muscle stem cells could be the cell of origin of alveolar rhabdomyosarcomas, we considered that *Pax3:Fkhr* was necessary but not sufficient for tumor formation. To better recapitulate the events of the translocation, we used our *Fkhr* conditional knock-out to recreate *Fkhr* haploinsufficiency for maturing skeletal muscle cells expressing *Pax3:Fkhr*. No acceleration of tumorigenesis was observed. We then looked back to observations of the human disease for other cooperative changes that might be responsible for tumor initiation. In many clinical cases, disruption of the pathways for *Trp53* or *CDKN2A* (which encodes *INK4A* and *ARF*) occurred simultaneously with translocation-mediated expression of *Pax3:Fkhr* (3, 4). Using conditional knock-out alleles of either *Trp53* or *CDKN2A* with our *Pax3:Fkhr*

**Requests for reprints:** Howard Hughes Medical Institute and Department of Human Genetics, University of Utah, School of Medicine, Salt Lake City, UT 84112-5331. Phone: 801-581-7096; Fax: 801-585-3425; E-mail: mario.capecchi@genetics.utah.edu.

©2005 American Association for Cancer Research.  
doi:10.1158/0008-5472.CAN-05-0477



**Figure 1.** Modeling alveolar rhabdomyosarcoma in the mouse. The hallmark of human alveolar rhabdomyosarcoma is the presence of the chromosomal translocation fusion gene, *Pax3:Fkhr*. This fusion gene was generated in mice at selected times and in specific tissues using a *Cre/loxP*-mediated conditional "knock-in" approach. One mouse contained an engineered *Pax3* locus that, in the absence of Cre, functioned normally, but in the presence of Cre, converted into the *Pax3:Fkhr* fusion gene. Three mouse strains with separate Cre drivers were crossed with mice carrying the *Pax3* knock-in allele. The first expressed Cre early in embryogenesis in multiple tissues. Breeding this Cre driver mouse to mice with the *Pax3* knock-in allele did not produce alveolar rhabdomyosarcomas because the embryos died at birth. The embryos were nevertheless very informative because they revealed genes that were turned on or turned off by the *Pax3:Fkhr* fusion gene. The second Cre driver, *Pax7:Cre*, generated the fusion gene during midgestation and importantly in muscle satellite cells. These mice survived after birth but surprisingly also did not develop alveolar rhabdomyosarcomas despite having every muscle satellite cell, prebirth and postbirth, express *Pax3:Fkhr*. The third Cre driver, *Myf6-Cre*, expressed Cre in differentiating muscle fibers. These mice did develop alveolar rhabdomyosarcomas that were very similar to human alveolar rhabdomyosarcomas in terms of molecular markers and tissue histology. As anticipated, the frequency of tumor production, ~1 of 200 mice, was low. However, when coupled with conditional disruption of either *Trp53* or *Ink4A/ARF* in the same tissue, nearly every mouse developed alveolar rhabdomyosarcomas exclusively.

knock-in allele, we were able to generate histologically and immunohistochemically authentic alveolar rhabdomyosarcomas at high frequency.

The development of our mouse model underscores the power and complexity of conditional mouse genetics. Through the use of conditional mutagenesis, we were able to circumvent the birth defects associated with expression of our oncogene (*Pax3:Fkhr*) early in embryogenesis and we were able to generate tumors at high frequency but only after addition of secondary mutations required for tumor initiation and the appropriate choice of *Cre* driver to generate the oncogene in the tissue of origin for this disease.

A major implication of this work is that noncarcinomatous (childhood) tumors may not arise primarily from dormant, embryonic precursor cells but instead may arise by dedifferentiation of mature cells. In our model and in children, alveolar rhabdomyosarcomas express markers characteristic of embryonic and postnatal myogenic precursors (5), yet the tumors in our model were triggered in the stages well after the earliest precursors.

It will be interesting to see whether other phenotypically embryonic cancers, such as Wilm's tumor and hepatoblastoma, have an embryonic cell of origin or whether their aberrant gene expression only makes them seem embryonic.

A second implication of this work is that we may finally be able to pinpoint the cell of origin of certain phenotypically vague cancers such as Ewing's sarcoma and synovial sarcoma. Ewing's sarcoma, a soft tissue cancer arising from and destroying bone, has long lacked any evidence for whether it is osteoblast or neuroepithelially derived. Synovial sarcoma is a similarly mysterious case of a tumor that seems to have both epithelial and mesenchymal markers. Fortunately, each of these tumors also has a translocation-mediated oncogene that has been implicated in tumor initiation: *Ews:Flil* for Ewing's sarcoma and *Syt:SSX1* for synovial sarcoma. Using conditional genetic approaches to express the translocation-mediated initiation events across a variety of cell types, we may finally be able to uncover the cell of origin of these tumors.

Finally, a direction of our future work is to use doxycycline- and tamoxifen-inducible Cre drivers so that we can choose with greater precision the exact tissue and time for tumor initiation and be able to follow that tissue from premalignancy to local proliferation, local metastasis, and then, distal metastasis. This focus on progression underlies our conviction that therapeutic target identification and the ability to develop treatments for the cancer will be dependent on identifying the secondary changes that give

the tumors their capability to migrate from and propagate outside their tissue of origin. With the improved understanding of tumor progression, new molecularly targeted, less toxic therapies may be within reach.

## Acknowledgments

Received 2/11/2005; revised 4/22/2005; accepted 6/9/2005.

---

## References

1. Mackall CL, Helman LJ. High-dose chemotherapy for rhabdomyosarcoma: where do we go from here. *J Pediatr Hematol Oncol* 2001;23:266-7.
2. Sorensen PH, Lynch JC, Qualman SJ, et al. PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the children's oncology group. *J Clin Oncol* 2002;20:2672-9.
3. Keller C, Arenkiel BR, Coffin CM, El-Bardeesy N, DePinho RA, Capecchi MR. Alveolar rhabdomyosarcomas in conditional Pax3:Fkhr mice: cooperativity of Ink4a/ARF and Trp53 loss of function. *Genes Dev* 2004;18:2614-26.
4. Keller C, Hansen MS, Coffin CM, Capecchi MR. Pax3:Fkhr interferes with embryonic Pax3 and Pax7 function: implications for alveolar rhabdomyosarcoma cell of origin. *Genes Dev* 2004;18:2608-13.
5. Wijnaendts LC, van der Linden JC, van Unnik AJ, et al. Expression of developmentally regulated muscle proteins in rhabdomyosarcomas. *Am J Pathol* 1994;145:895-901.