Cell of Origin: Mouse Model Offers Insights Into Process of Malignancy

When the final history of cancer research is one day written, retinoblastoma will deserve its own chapter. Studies of this rare childhood tumor have yielded the first widely accepted tumor progression model, the first cloned tumor suppressor gene, and several other firsts in basic cancer research.

Now, another first could be on the way. As recently published in *Cancer Cell*, Canadian scientists reported in mouse studies that they have identified the specific type of cell in the developing retina that produces retinoblastoma, noting when and why the trouble arises. With these data and other recent advances in modeling the tumor in mice, researchers in the field say they may soon be in the enviable position to track retinoblastoma in real time from initiation to metastasis, a long-standing goal of cancer researchers.

Although the finding still must be confirmed in follow-up studies, many say it provides an excellent starting point to explore the notoriously tough to study "cell-of-origin" question that lies at the heart of the cancer process. "It is a meticulous and thoughtful study that for the first time, as far as I am aware, provides a relevant framework for investigating how absence of the retinoblastoma gene product might give rise to a malignant tumor of the retina," said distinguished pathologist Henry Harris, of the University of Oxford in England.

Grappling For Answers

That retinoblastoma research has neared this lofty goal would have been hard to imagine just a few years ago. Scientists in the field have grappled for nearly 20 years to determine the function of the Rb protein within the developing retina, the postage-stamp–sized tissue that lines the back of the eye, where it converts light energy into electrical impulses that the brain translates into vision.

The problem was, in part, the absence of a mouse model that mimics human retinoblastoma, a key tool in studying tumors. Although Rb was the first gene "knocked out," or selectively inactivated, in mice back in the early 1990s, scientists soon discovered that the effect was lethal during embryonic development, meaning no mouse pups survived to full term for further study.

This fact of life presented the field with two technically daunting challenges to create an appropriate mouse model: inactivate the Rb protein only in the developing retina; and time the inactivation to coincide with the replication of retinal progenitor (stem) cells, a step in which previous studies indicated Rb is especially important for normal retinal development.

Dr. Michael Dyer

For much of the 1990s, these technical challenges proved insurmountable. But, as the biological tool box to create mouse models has expanded in recent years, retinoblastoma researchers have found creative ways to peck away at these problems, generating several new and improved mouse models.

In the *Cancer Cell* paper, Rod Bremner, Ph.D., and his colleagues at Toronto Western Research Institute report that they generated the first inheritable retinoblastoma mouse models, in which Rb and/or the related p107 proteins are deleted in peripheral retinal progenitor cells at gestation day 10, or when pluripotent stem cells produce the first retinal precursor cells. Precursor cells are partially differentiated master cells that have the capability to produce the seven major cell types needed to assemble a retina.

Although they knew their mouse models were unique, Bremner said he and his colleagues fully expected that their Rb knockouts would yield results consistent with those of other groups. Namely, as the Rb- or p107-deficient precursor cells divided, the newborn retinal cells would sense a serious flaw in their hardwiring, activate an apoptosis pathway, and commit mass suicide.

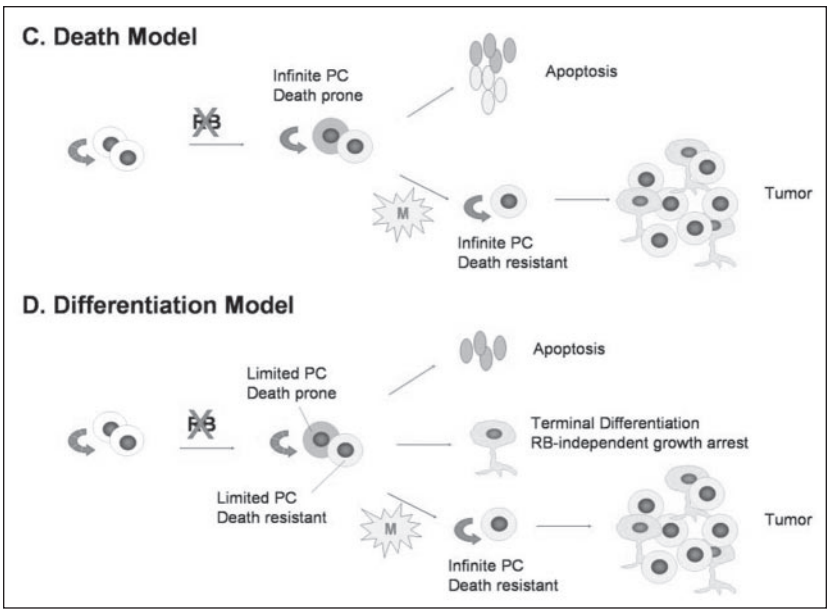
So the group was surprised to find that, although several of the major cell types had predictably committed mass suicide, three—the so-called amacrine, horizontal, and Müller glial cells—continued to divide and form their distinctive retinal layers. "I just about fell off my chair," recalled Bremner of first seeing the data. "We presumed that most of the cells would die. There would be a few stragglers maybe that would still survive, but here we had a complete amacrine layer, which was totally unexpected."

Bremner and colleagues embarked upon a series of experiments that ultimately allowed them to develop the first cell-specific model of Rb and p107 loss in the developing retina. According to this model, the loss of these proteins has no effect on progenitor cells; the changes occur with the precursors. They have a reduced capacity to proliferate, although they still can produce all seven of the major retinal cell types. As Bremner and colleagues went to great lengths to show, all seven cell types divide on their own, suggesting a malfunction in their ability to exit the cell cycle. "They desperately need Rb to exit the cycle, at least initially," said Bremner.

In their desperate state, four of the seven cell types punch the suicide button. But amacrine, horizontal, and Müller glial cells divide on, seemingly oblivious to their flaw, before eventually exiting the cell cycle via another pathway that is wired into the differen-

tiation process. If the model is correct, the research group proposes for the first time that these dividing cells do not have to cheat a death pathway to become a tumor, as the scientific literature has long suggested. Rather, the trick is to acquire additional mutations that keep them stuck in cycle.

"This finding could explain some things," said Alfred Knudson, M.D., Ph.D., a scientist at Fox Chase Cancer Center in Philadelphia and the father of the original "two-hit" hypothesis, or progression model, for retinoblastoma. "I've wondered for some time whether the threshold for apoptosis is altered in embryonic cells. You just don't want them to disappear lightly, because they are building a tissue."



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The "death model" above shows the hypothesis that Rb loss generates cells with infinite division capacity that are death prone. The new model, the "differentiation model" put forth by Rod Bremner, Ph.D., and colleagues, is that Rb loss generates cells that have extended, not infinite, division capacity, and that are death resistant rather than death prone.

Bremner and his colleagues now had come face to face with the cell-of-origin question. Were one or all of these three cell types the source of retinoblastoma tumors? In subsequent work, the scientists identified two proteins (NeuroD and Math3) in dividing retinoblastoma cells that are unique to amacrine cells, suggesting that these small, octopus-like cells that connect one vision-transmitting retinal cell type to another, are the cell of origin.

Michael Dyer, Ph.D., a developmental biologist who studies retinoblastoma at St. Jude's Children's Hospital in Memphis, Tenn., while praising the data of the group, said he has his reservations on the cell-of-origin question. "One of the caveats is that a lot of markers of amacrine cells are also progenitor cell markers," he said. "It becomes kind of confusing when you think about them as amacrine markers. They could be markers of progenitor cells. It's tricky."

Adding to the confusion is the mouse itself. Scientists have known for several years that mice with inactivated Rb do not develop tumors of the retina. Oddly enough, they are prone to pituitary and thyroid tumors instead. This suggests that mouse models may only crudely mimic human retinoblastoma. But, as Bremner noted, the fundamental role of Rb in regulating cell cycle exit should be conserved from mice to man, and his group's data, if nothing else, have helped to elucidate the function of this key protein in retinal development.

Mighty Mouse

Dyer said the differences between mice and men will require a great deal of thought, but these quirks do not invalidate the mouse as a model for human retinoblastoma. "We've been working really hard on the human-versus-mouse question," he said. "I think the mice that we and other groups now have will allow us to do the experiments in the way that you would want to do them. That is, very carefully figure out what's going on, what the cell of origin is, and where the secondary changes are occurring."

How carefully will they be able to look? Dyer said his group has created the first traditional Rb knock-out mouse, which is reported in the July issue of the journal *Cell Cycle*. When coupled with the growing array of gene-inactivating retroviruses now available, the mouse model might allow groups to observe the tumor process from start to finish.

"If you bring these two together [the knock-out mouse and the retroviruses], we believe it's possible to inactivate the gene in one progenitor cell *in vivo* during development and follow its clonal expansion over time," said Dyer, who added that the model would be a valuable tool in confirming Bremner's finding. "Not only ask what happens to different proteins, but look at which genetic changes occur. I don't know of any other *in vivo* genetic model that can follow one cell becoming a full-blown tumor over time."

Dyer said these mouse models are already providing preclinical therapeutic leads. His group recently determined that the frequently used chemotherapeutic agent vincristine is ineffective at killing retinoblastoma cells in the mouse. "Although it works great in culture, we found that vincristine has to be exposed to tumor cells for at least 4 hours to work," said Dyer. "In the mice, vincristine is cleared away so fast that it doesn't get a chance to do its job."

"I went back to the clinicians at St. Jude's and asked, 'Has anybody evaluated just vincristine in kids?' They said nobody had ever done the study, largely because childhood tumors are so rare that you can't ever do the clinical trials that you want to do," he said. "So, to me, the mouse model is really going to be a big part of future treatment advances."

—Robert Longtin