Innovations

Of mice and men
Geron Corporation

Calvin Harley now knows that he has little chance of curing cancer in mice. Whether he can do it in humans remains an open question.

Harley is chief scientific officer of Geron Corporation (Menlo Park, California), a company formed in 1992 to exploit the possible involvement of an enzyme called telomerase in cancer and aging. The simplicity and elegance of the telomerase theory has landed it on the cover of both *Cell* and *Time*, and in the pages of both *Science* and the *New Yorker*.

But it is a theory that was initially based more on correlation than causation, and recent results in mice have left some researchers with doubts. Most are keeping an open mind, given the uncertain translation of mouse results to humans. "The arguments at this stage are purely theological," says Elizabeth Blackburn of the University of California, San Francisco. "There is no scientific evidence, only opinion."

A theory is born

Telomerase solves a problem first recognized by James Watson in 1972. The duplication of DNA is unidirectional, and it starts with an RNA primer. Even if a primer attaches to the very end of a chromosome (the telomere), primer removal makes the new chromosome a little bit shorter. Telomerase corrects this erosion by making more copies of the protective sequence at the end of the chromosome (Figure 1). Telomerase has both an RNA component, which provides the template for catalysis, and a recently discovered protein component with reverse-transcriptase motifs.

In germ cells, telomerase actively maintains the ends of chromosomes. But in most other cells telomerase is turned off. The telomeres shorten, until eventually the cells stop dividing. This fact, correlation number one, suggests that shortened telomeres might signal the cell to become senescent, a stable state in which the cell remains metabolically active but no longer divides. Senescent cells accumulate as organisms age, and their altered gene expression may contribute to various age-related diseases.

The last thing that cancerous cells want to do is to senesce, and sure enough they reactivate telomerase activity (by a mechanism that remains unknown) and maintain the length of their telomeres. The finding that ~85% of malignant cancer biopsies are positive for telomerase, correlation number two, has encouraged Geron to search for specific inhibitors of telomerase as cancer therapeutics.

The mouse that kept on roaring

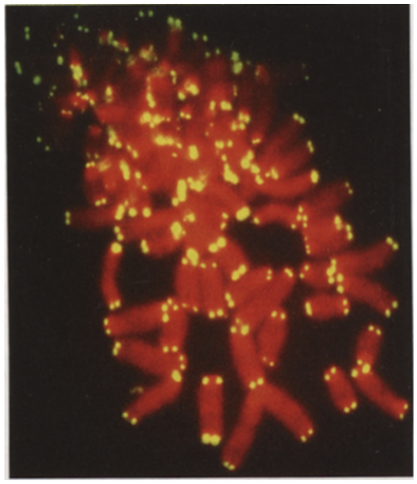
To test the outcome of telomerase inhibition, Maria Blasco of the Universad Autonoma Madrid, and Carol Greider of Johns Hopkins University made knockout mice lacking the gene for telomerase RNA. Perhaps because of the long telomeres that they are born with, the mice live for as long as their parents and can generate cancer-causing cells.

The knockout mice lose telomeric DNA at the rate of ~4.8 kb per generation. By the sixth generation without telomerase, 56% of cells derived from the mice have the wrong numbers of chromosomes, 5% have undetectable amounts of telomeric repeats at one or more chromosome ends, and the average length of telomeric repeats has shrunk from ~44 kb to ~14 kb. Bad things, one suspects, are just around the corner.

But in Blasco and Greider's current description of the sixth generation, they find little evidence of telomerase's importance in cancer and senescence.

Their first test was growth in culture. Cultured human cells must jump over two barriers to avoid senescence and become immortal.

Figure 1



Mouse chromosomes stained for telomeric repeat DNA. Image courtesy of Geron.

The first involves mutation of an oncogene or tumor suppressor to allow unregulated growth. This is enough to keep them going for a while, but then the telomeres shorten and cells enter a state called crisis. The cells that get through crisis have increased levels of telomerase.

Mouse cells have a single period of slowdown which may be equivalent to crisis. The mouse cells lacking telomerase were just as efficient as normal cells at getting through this block, so it seems that re-expression of telomerase is not a critical event in this mouse version of *in vitro* immortalization.

For the *in vivo* test, Blasco and Greider added oncogenes to cells cultured from sixth generation mice, then injected the cells into immunodeficient (nude) mice. Although these cells had divided over 300 times without telomerase, they were still capable of forming tumors.

Greider is concerned that the association of telomerase with some cancers could be just correlation. Last year Titia DeLange of Rockefeller University in New York reported that telomerase levels increased even in those cancerous mouse cells that still had long telomeres. "Just because a tumor expresses telomerase," says Greider, "that doesn't mean it needs it."

Hope for humans (and Geron)

Harley cites results from yeast (telomerase inhibition causes senescence) and cultured human cells (telomerase antisense causes crisis) as a counter to Greider's findings. And he is not deterred by the mouse results. "The initial generations of mice are not a good model for telomerase biology in humans," he says. Humans start off with many fewer telomeric repeats than mice, and some of the cancers that Geron hopes to treat have particularly diminutive telomeres. "I never thought that the telomerase approach was going to be a universal cure for all cancers," says Greider. "I think there will be some specific cancers that will be good targets."

One of the worst-kept secrets in molecular biology is that mice beyond the sixth generation have significant problems, but until this work is published none of the authors are discussing it. These later generations of mice may vindicate parts of the telomere hypothesis, but they also raise the question of how long a telomerase inhibitor will take to start working. Harley has always acknowledged that there will be a lag, a time in which the existing telomere is gradually eaten away even as the cancer grows. He estimates the delay in human cells to be 20 population doublings, equivalent in theory to a million-fold increase in cell number. But Harley has calculated that, with the extensive cell death that is common in tumors, the tumor mass will increase by only several-fold.

This is a disadvantage that Geron claims it can manage. "Every cancer therapy on the market today has a limitation," says Harley. "We didn't expect telomerase inhibitors to be any different — we don't expect them to be a magic bullet. But we do expect them to be more universal and safer than current therapies." Harley plans to use telomerase inhibitors to prevent metastases and relapses that start from just a few cells, not as treatments for large primary tumors.

Another uncertainty involves a poorly characterized pathway named alternative lengthening of telomeres (ALT). Roger Reddel of the Children's Medical Research Institute in Sydney has described this pathway in cultured cells that lack detectable telomerase activity but acquire long telomeres. As in yeast, this lengthening may work by non-reciprocal recombination between telomeric repeats, in which DNA is copied from one telomere to another. What is important for Geron is how often ALT happens *in vivo* in humans. With this and other issues unresolved, David Kipling of the University of Wales at Cardiff says that "treating real people with real tumors is the only true experiment."

Diagnostics

Approximately half of Geron's research is focussed on finding a telomerase inhibitor. A project with less glamour but arguably less risk is the investigation of telomerase activity as a marker for cancerous cells. In this field correlation is enough; even if telomerase activation is a passive bystander in tumor formation it can be a valuable marker.

Telomerase is associated with over 80% of malignant tumors. Only 14% of benign and premalignant growths, and 6% of tissues adjacent to tumors, test positive. Many of these 'false positives' may be true positives, as the telomerase activity assay is especially sensitive, capable of detecting from one to ten telomerase-positive cells. "Telomerase is here to stay," says Jerry Shay of the University of Texas Southwestern. "It's an extraordinarily important new marker."

Telomerase screening of easily available cells could replace invasive biopsies and tissue pathology as an indicator of tumorigenicity. The samples collected would include urine (bladder cancer), sputum (lung, and head and neck cancer), blood enriched for epithelial cells (various metastatic cancers) and nipple-aspirated fluid (breast cancer).

What was old is new again

The idea that telomerase is involved in cell senescence, itself only a small part of the aging process, somehow got translated into the idea that telomerase controls lifespan. "But that was never proposed by anyone," says Greider. Although Geron has images of elderly but spry individuals sprinkled liberally through its promotional materials, Geron is not planning to stop people from aging.

They are, however, looking at how the cells with shortened telomeres affect several age-related diseases. Geron researchers found that telomeres shorten twice as fast in endothelial cells lining arteries compared to those lining veins, probably because the turbulence in arteries causes higher cell turnover. Consistent with this, telomere loss rates are highest for the sections of arteries subject to the most turbulence. Accumulating senescent cells may then lead to atherosclerosis.

Judith Campisi of the University of California, Berkeley, has found that senescent cells accumulate as skin ages. She can see senescent skin cells by staining for the enzyme β-galactosidase at neutral pH (it is active in normal cells only at low pH). Senescent skin cells produce too little collagen and too much collagenase, and the combination may contribute to the fragility of aging skin.

Geron is using the chips made by Synteni, Inc. (see *Chemistry & Biology*, **4**, 157–158) to search for more genes that are expressed differentially in senescent cells, and hopes to modify that expression with small molecules.

These newer research directions may be good insurance if humans turn out to be as indifferent to the loss of telomerase as mice. Meanwhile, supporters like Shay are ready to test the inhibitors. "There's an optimistic and a pessimistic way to spin any story," he says. "But these trials will be done either way."

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