



1 to Shutter

When Cells Grow Old

by **John Davenport**
illustration by Frank Stockton

Just like a whole person, cells change as they age. Clues about what controls cellular aging are revealing puzzles and possibilities for a host of diseases.

Sometimes the evidence of aging hits a little too close to home.

HHMI investigator George Daley, of Harvard Medical School, saw signs of his own mortality as he peered into a Petri dish. Daley has been studying how to coax adult cells into stem cells, using lab members' cells—and his own.

Adding just four genes can turn adult cells back into embryonic-like cells, able to develop into any cell type in the body, according to Daley's studies. In culture dishes, cells from a younger postdoctoral fellow in Daley's group were "youthful and vigorous," he says; many of them morphed into stem cells. But Daley's cells were stubborn, refusing to reverse their clocks. It seems as a person ages, cells get increasingly stuck in their ways.

Daley isn't taking it too personally. "I'm deficient in a lot of things, and reprogramming seems to be one of them," he says. He plans to use the observation to understand how to reprogram cells most efficiently.

His finding points out an important concept: cells might not sprout gray hair, get achy joints, or forget where they put their car keys, but they do age. Several HHMI researchers are just beginning to learn what happens to cells as they grow old, and they're making connections between those changes and cancer, deficiencies in wound healing, and other problems that increase in likelihood as a person ages.

A HELP OR A HINDRANCE?

The first inkling that cells might grow old came in the early 1960s, when gerontologist Leonard Hayflick was playing with cells in culture. The conventional wisdom at the time held that

cells in a culture dish could split an indefinite number of times. But Hayflick found that after 50 or so divisions—now called the Hayflick limit—cells stopped dividing. The cells turned into zombies. They didn't die but remained in a kind of hibernation, a state known as cellular senescence.

Over the following decades, researchers elucidated the environmental conditions that push cells to senesce and defined many of the genes and molecules that control the process. Several signals can put cells to sleep. Accumulating DNA mutations can send cells into senescence. Fraying chromosomes also serve as a trigger. Protective caps of DNA called telomeres keep chromosomes from unraveling, but each time a cell divides, its telomeres get a little shorter. When telomeres get too short, chromosomes can break or fuse with other chromosomes, shuffling the genome and possibly causing cancer. A third signal also links to cancer. In 1997, HHMI investigator Scott Lowe of Cold Spring Harbor Laboratory, New York, and his colleagues found that activating a cancer-causing gene forces cultured cells into senescence.

The cancer connection seems counterintuitive. Typically, cancer-causing genes spur cells to divide uncontrollably, not grind to a halt. But Lowe's result supports the idea that senescence is a cancer-prevention tactic. Cells must accumulate multiple mutations before turning into full-blown cancer. When a cancer gene turns on, senescence might help cells shut down before additional mutations push the cell to become cancerous. However, shutting down cells to fight cancer could hamper health in other ways. For instance, senescence might deplete the pools of cells that replace damaged ones and keep liver, bone, blood, and other tissues working properly. An accumulation of malfunctioning cells and a loss of new ones to replace them could underlie some of the effects of aging. So is senescence good or bad for the body?

In a sense, cancer and senescence are opposite sides of the same coin. To remain robust, tissues rely on dividing cells for replenishment; yet, left unchecked, cell division leads to cancer. Thus, it might seem a Faustian bargain to guard against cancer now at the expense of decrepit tissues later.

Evolution should favor the anti-cancer mechanism, Lowe says. Genes that improve survival early in life should help ensure that an organism reproduces, and thus the genes tend to be passed down to the next generation. That's true even if the same

genes compromise health late in life. “We didn’t evolve during a period when we lived 80 years,” says Lowe. “The system isn’t built to last that long.”

Some studies on the tumor suppressor gene *p53* have supported this idea of a trade-off between cancer and aging. The *p53* gene acts as a master controller of anti-cancer mechanisms, including senescence, particularly when DNA incurs damage or telomeres erode. The gene also governs other processes such as a regulated cell death pathway called apoptosis. Several groups of researchers have shown that producing certain forms of *p53* dramatically reduces cancer in mice, but those animals age more rapidly than normal.

SENDING SIGNALS

For decades, cellular senescence was thought to be an oddity seen only in the lab. Few studies provided any evidence that senescence influenced aging or cancer in a living creature. “[Researchers in the] cancer biology field felt it was a cell culture artifact,” says Lowe. “Senescence hadn’t been observed in tissues.”

But starting in the mid 1990s, researchers discovered ways to track senescent cells in living tissues, not just in cell culture. For instance, senescent cells produce an enzyme called beta galactosidase. When bathed in a particular sugar compound, cells with this enzyme turn blue, providing a way to spot senescent cells. Since then, researchers have used this method to show that tissues from people, as well as from animals such as rodents and monkeys, carried blue cells. And, “the older you got the more blue cells you had,” says Lowe. Other markers exist, and although none of the markers is exclusive to senescent cells—they tag other things too—these studies have provided robust evidence that senescence is important beyond the culture dish.

Recent research supports the notion that senescence helps divert cells when they start down the path toward cancer. Four studies in 2005 revealed that senescent cells accumulate in precancerous growths such as moles, and quieting senescence pathways suffices to trigger melanoma and prostate cancer. “Premalignant tumors are chock full of senescent cells,” says Lowe. The studies’ findings settled the controversy about whether senescence works to prevent cancer in people, he adds.

Senescent cells might seem to be introverted wallflowers, but new work suggests that they are rather social, communicating and influencing their neighbors. HHMI investigator Michael



George Daley (top) aims to efficiently reprogram cells, with help from lessons learned on cellular senescence. Michael Green (bottom) is using senescence-inducing proteins to stop cancer cells from dividing.



Scott Lowe thinks senescence might be a cell's strategy to cope with an immediate threat—even if that tactic proves detrimental later on.

Green, of the University of Massachusetts Medical School in Worcester, made the discovery after looking deeper into how senescence prevents melanoma from taking hold. A cancer-causing mutation in a gene called *BRAF* occurs frequently in melanoma; most benign mole cells also carry the mutation. Like other oncogenes, *BRAF* spurs cells in culture to senesce. Green wondered what spurred cells carrying *BRAF* to senesce rather than grow into tumors.

He and his colleagues identified a set of genes that appear to hold mole cells in senescence; when they shut down any of these genes, the cells grew like crazy. Green was surprised to discover that one of the genes makes a protein called IGFBP7 that gets discharged out of the cell. “We were expecting it to be a purely intracellular event,” he says. The team reported its findings in the journal *Cell* in February 2008. Mole cells didn't need to make this protein themselves to stay quiet. Applying IGFBP7 to the outside of mole cells was enough to keep cells in senescence. The findings suggest that, by spewing IGFBP7, one cell could help its neighbors from turning cancerous.

At about that time, other groups also showed that other types of senescent cells discharge proteins that stall cell division. The results of all the studies are striking, says Lowe. “Senescent cells aren't just sitting there.”

Melanoma cells don't make IGFBP7, says Green, but his studies suggest that they still respond to the molecule. Bathing cancer cells in IGFBP7 prodded them to die. Green is exploring the idea of using IGFBP7 as an anti-cancer drug. He will collaborate with scientists at the National Cancer Institute to conduct preclinical studies that will lay the foundation for clinical trials.

FENDING OFF FIBROSIS

With mounting evidence that senescence is a cancer countermeasure, Lowe and his team wondered if senescence played a part in another disorder: liver disease. Previously, researchers had noticed senescent cells in diseased livers, and Lowe wondered whether they were harmful or beneficial.

Hepatitis infection, alcohol abuse, and fatty liver disease can bring on cirrhosis, a permanent scarring that undermines the liver's normal function. A buildup of connective tissue, known as fibrosis, presages cirrhosis. When a liver is damaged, particular liver cells called stellate cells churn out extracellular matrix, the protein structure that forms fibrous tissue. If the cause of liver damage disappears, the stellate cells help other cells grow, repair, and replenish the liver. But a chronic drinking problem or untreated infection puts a liver under constant stress. Under these conditions, fibrosis spreads, cirrhosis sets in, and, ultimately, the liver fails.

Lowe's recent studies suggest that senescence helps put a damper on fibrosis. His team injected mice with an agent that damaged their livers, spurring fibrosis. In the animals' livers, senescent stellate cells peppered fibrotic regions, the team reported in August 2008 in *Cell*. In animals that can't undergo cellular senescence because they lack *p53*, fibrosis was more pervasive. Lowe posits that senescence hampers fibrosis by preventing stellate cells from growing too heartily and manufacturing a flood of extracellular matrix.

Fibrosis is part of the normal response to repair an injury, and unless a liver suffers continuous insults, fibrosis eventually dissipates. Lowe also found that senescence not only prevents fibrosis, it also helps clear fibrosis once it starts. In normal animals, fibrosis subsided and eventually disappeared once the liver-damaging injections stopped. But in animals without *p53*—and therefore deficient in senescence—fibrosis persisted.

Researchers have known for a while that senescent cells exude sets of molecules that deplete extracellular matrix. His findings suggest one possible reason for this behavior. “It fits nicely with this puzzling gene expression pattern that's been around for some time,” he says.

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SCOTT LOWE

Lowe adds that cirrhosis puts patients at increased risk for liver cancer, and he's trying to understand why, in light of the new findings. Opposing Green's IGF1P7 finding, previous studies have found that senescent cells produce molecules that spur cancer, rather than prevent it. Senescence might be built to cope with an immediate insult, says Lowe, even if, over the

long haul, quieting cells creates havoc. The body "can't stop everything," says Lowe.

Once an obscure artifact of cell culture, cellular aging is emerging as a key force that shapes tissue health. And those who want to help patients are taking note. Harvard's Daley, for instance, wants to understand how to make stem cells and use them to repair malfunctioning tissues. But he's proceeding with caution. "Reprogramming is, in a sense, reversing senescence," he says. Researchers must face the "sobering possibility" that doing so will accelerate cancer. Still, Daley is optimistic that "we can harness the process in a way that's productive." Hopefully, he won't have to age much more before the answers become clear. ■

CONNECTING DOTS

As the role of senescence in health and disease gains prominence, researchers new to the phenomenon are beginning to look for connections to their own work. HHMI investigator Joan Steitz of Yale University has been fascinated by how small RNA molecules control the activity of genes. Now, she's starting to look at how this capability ties to senescence.

One particular type of small RNA molecules, called microRNAs, control how much protein a cell makes from a messenger RNA (see "The Macro World of MicroRNA," page 20). In an actively growing and dividing cell, microRNAs dampen the amount of protein the cell makes. But microRNAs do the opposite and amplify protein production when cells are in a state called quiescence, Steitz's group has found. Like senescent cells, quiescent cells don't divide. But, unlike senescent cells, they can reactivate and start growing and dividing again. "We want to know why microRNAs do one thing when cells are rapidly proliferating and another thing when they withdraw from the cell cycle," says Steitz.

She is now collaborating with Yale colleague Daniel DiMaio to understand how microRNAs work in senescent cells. "Would the same kind of controls be going on in senescent cells?" she asks. "Would it be a different protein that is associated? We don't know." **In addition to the activity** of genes, senescence might tie to the activities of mitochondria. These structures generate power in cells, and David Chan at the California Institute of Technology, who was named an HHMI investigator in spring 2008, scrutinizes how mitochondria merge and divide. He's curious how this dance influences energy production and how missteps might trigger illness. **Mitochondria accumulate** mutations in their DNA over a lifetime. As the glitches pile up, they can cause mitochondria to produce less energy or work less efficiently. It's particularly troublesome for the brain and muscles, heavy users of energy. A blackout in cellular energy production may also contribute to gray hair, weak bones, and other age-related changes. **Chan has found** that the many mitochondria in a

cell split and join, allowing them to mix and redistribute copies of mitochondrial DNA, which means any particular cell is unlikely to remain stuck with a large number of faulty mitochondria. "Fusion is potentially a protective mechanism," says Chan. **Chan suggests** that a connection between mitochondrial dynamics and cellular aging could exist. Old cells may have deficiencies in mitochondrial fusion that help mutations build up, he says. Faults in this process could be particularly important in neurodegenerative diseases. Evidence already links problems in mitochondria to Alzheimer's disease, Parkinson's disease, and Lou Gehrig's disease (amyotrophic lateral sclerosis). Recent studies even suggest that a gene involved in mitochondrial dynamics is crippled in some inherited forms of Parkinson's disease. Chan plans to dig deeper into these disease connections. With this new scrutiny, cellular aging could step farther into the spotlight as a key component of many diseases. —J.D.