HEALTH

QUIET LITTLE TRAITORS

Cells that permanently stop dividing have long been recognized as one of the body's defenses against cancer. Now they are also seen as a sometime culprit in cancer and a cause of aging

By David Stipp

Illustration by Anatomy Blue

CELL SIGHTING: Investigators identify senescent cells—those that have lost the ability to divide—by their color. They turn blue when exposed to a particular chemical.

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IN 1999 JAN M. VAN DEURSEN AND HIS COLLEAGUES AT THE Mayo Clinic in Rochester, Minn., wanted to see whether mangled chromosomes cause cancer. So they engineered mice deficient in a protein that helps to maintain chromosomal integrity. The rodents' coils of DNA were duly deranged. Surprisingly, though, the animals were not particularly tumor-prone. Instead they developed a strange grab bag of ills, including cataracts, dwindling muscles, rapid thinning of fat under the skin and progressive spinal curvature, that made them look like one-humped camels. They also tended to die young.

Van Deursen had no idea why those particular abnormalities showed up. Then, in 2002, he spotted a report on mice afflicted by accelerated aging and was struck by photographs showing that their backs became humped as they aged. Suddenly, it hit him: his camel-backed mice, too, were aging unusually fast. Probing deeper, the Mayo team discovered that cells in a number of the rodents' tissues had prematurely slid into a state called cellular senescence, in which cells permanently lose the ability to divide and become aberrant in other ways. Such failure of cell division would explain the bone, muscle, eye and skin abnormalities observed by van Deursen's group.

The investigators then went beyond explanations and did something about the symptoms: by adding a second genetic alteration to their mice, they eliminated senescent cells as they formed and thereby slowed various aspects of the animals' fast aging. The finding, reported last November, brought the field of cellular senescence to the fore of aging science and breathed new life into a controversial idea proposed more then 50 years ago: that the loss of cells' ability to divide causes the body to deteriorate with time. Other recent research is also drawing new attention to the process for a related reason. Long believed to be a defense against cancer, cellular senescence has been exposed as two-faced—blocking tumor growth in some ways but promoting it in others.

The new findings suggest that slowing our cells' entry into senescence might help postpone late-life cancers and other diseases. Because deletion of senescent cells in the Mayo mice required complex genetic manipulations, the same treatment will not be offered to people anytime soon. Yet all is not lost. A number of simpler interventions could potentially fit the bill.

OLD, TIRED CELLS

THE STUDY OF SENESCENT CELLS has been a story of provocative surprises and extensive revisions. Initially biologists thought of them as cells that simply had exhausted their ability to reproduce. Leonard Hayflick, co-discoverer of the senescent state, established in 1961 that some kind of molecular counter triggers senescence after about 50 replication cycles in human cells. He theorized that this "Hayflick limit" on replication might underlie whole-body aging because stalled proliferation would prevent cells from replacing those lost in damaged tissues. He also posited that cells are programmed to run out of dividing power after some number of replication cycles because having a built-in limit would prevent damaged cells from proliferating uncontrollably and becoming cancerous. Cellular senescence's contribution to aging, in other words, was seen as the price we pay for its help in defending us against cancer.

The theory that senescing cells drive aging gained ground af-

IN BRIEF

Senescent cells—which have permanently lost the ability to divide—were once assumed to contribute to aging by undermining tissue repair. Cells were thought to enter senescence to avoid becoming cancerous when damage put them at risk of proliferating uncontrollably.

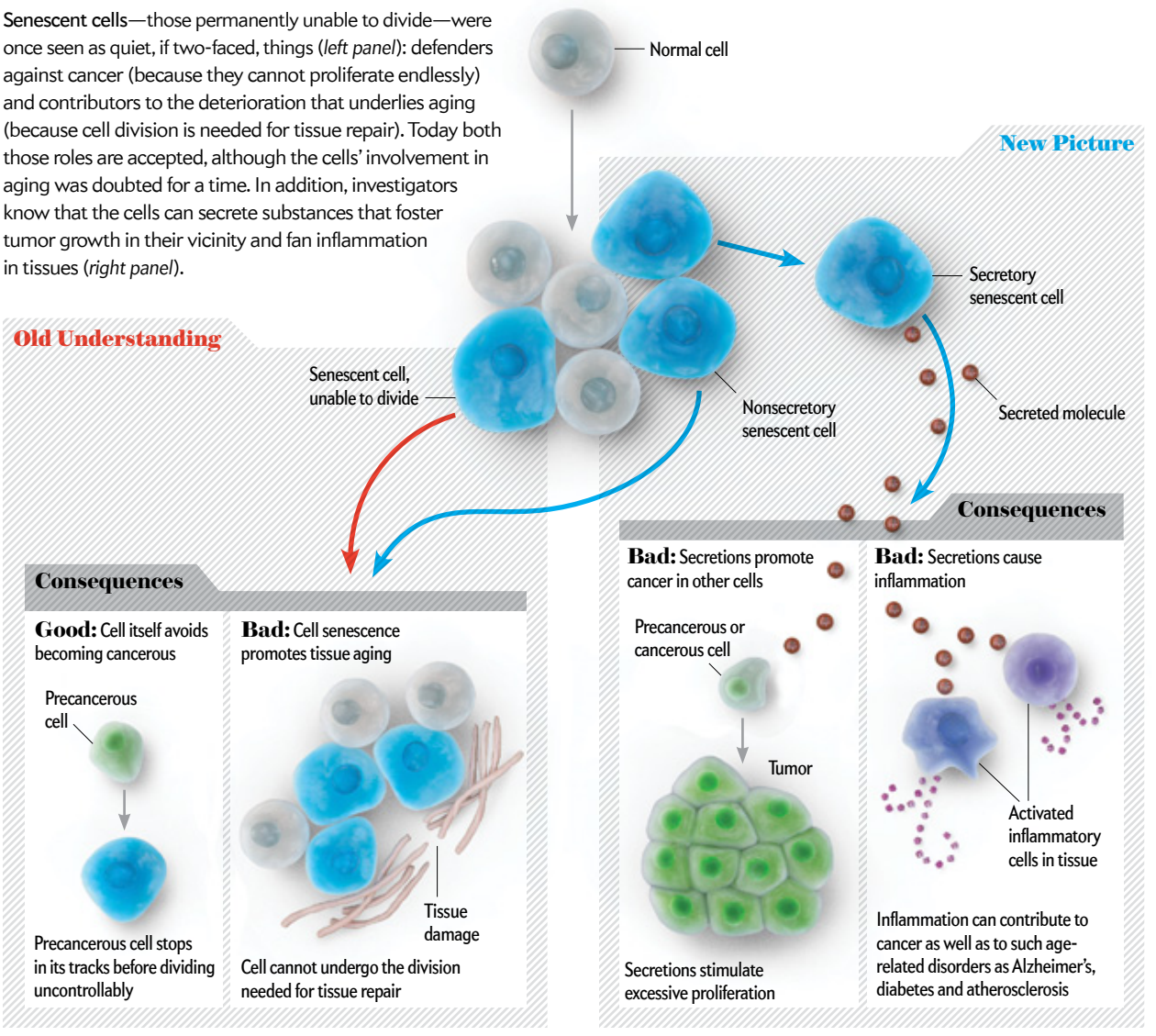
Later, the notion that senescent cells play a part in the aging of tissues and bodies fell out of favor. More recently, though, that idea has gained new support.

Recent research indicates that the cells can contribute to aging in the originally proposed way and also by spurring inflammation. Plus, they can harm nearby cells in ways that promote cancer.

Some evidence in mice suggests that retarding cellular senescence may help slow aging and delay some of the ills associated with it.

Good Cells Gone Bad

Senescent cells—those permanently unable to divide—were once seen as quiet, if two-faced, things (*left panel*): defenders against cancer (because they cannot proliferate endlessly) and contributors to the deterioration that underlies aging (because cell division is needed for tissue repair). Today both those roles are accepted, although the cells' involvement in aging was doubted for a time. In addition, investigators know that the cells can secrete substances that foster tumor growth in their vicinity and fan inflammation in tissues (*right panel*).



ter studies beginning in the 1970s uncovered a molecular clock behind the Hayflick limit. Each time a cell divides, its telomeres—stretches of DNA at the tips of chromosomes—shorten; cells stop dividing when their telomeres shrink beyond some set length. Our cells, it seemed, were programmed to become senescent if we lived long enough.

Later research undercut the theory, though. Multiple laboratories reported in the late 1990s, for instance, that the ability of skin cells to proliferate did not significantly decline with age—a sign that the Hayflick limit was not necessarily reached frequently enough to significantly disrupt tissue repair in a person's lifetime. In line with this view, others established that mice have very long telomeres, apparently preventing their proliferative cells from clocking out before the animals died. In 2001 two gerontologists, Harriet and David Gershon, bluntly declared in a review article that the telomere theory of aging should "be considered irrelevant."

As the tick-tock theory of aging was running down, evidence in favor of cell senescence's other apparent role—as a defense against cancer—was accumulating. By the 1990s it was well known that certain kinds of damage to cells, such as genetic mutations, could trigger uncontrolled proliferation and other changes characteristic of cancers. And, it turned out, various forms of cellular injury could induce senescence—presumably to prevent the damaged cells from becoming malignant. Dousing cells with DNA-damaging oxidizing chemicals, for example, could induce its hallmark proliferative arrest. Tellingly, in 1997 a team led by Manuel Serrano, now at the Spanish National Cancer Research

Illustration by Gert Nielsen

Center in Madrid, found that senescence can be established by a sustained surge of signals within a cell urging it to divide. Oncogenes—mutated genes that help to drive tumors' unchecked growth—are known for pounding out such relentless go-go beats.

These and other discoveries suggested that an anticancer mechanism within cells continually scans for signs of damage that can tip them toward uncontrolled growth. If such signs are sustained and surpass a critical threshold, the mechanism can permanently arrest cell division by triggering senescence, which allows the cell to repair the damage, if possible, and carry on in a kind of semiretired state.

CANCER PROMOTERS

THEN CAME A SHOCK: researchers discovered that senescent cells could sometimes spur on cancer. Among them was Judith Campisi, now at the Buck Institute for Research on Aging in Novato, Calif. She then came up with a hypothesis that has helped quash the idea that senescent cells merely sit quietly in their dotage. The hypothesis holds that the cells can actively both foster tumor growth and cause widespread damage of other kinds.

The first hints that senescent cells might play such an insidious role arose in the late 1990s, as evidence emerged suggesting that senescent cells can disrupt the cells and tissues in their immediate vicinity—in their "microenvironments"—possibly turning the regions into bad neighborhoods that could abet tumor growth. In 2001 Campisi's lab corroborated this idea with a groundbreaking study showing that senescent cells maintained in a culture dish can stimulate precancerous cells in the same culture to form unusually aggressive tumors when injected into mice. The bad-neighborhood effect appeared to stem from the tendency of many senescent cells to secrete a mix of potentially hazardous molecules, including ones that promote cell proliferation and others that break up extracellular proteins surrounding and supporting cells. (Spreading tumor cells are thought to employ the same degradative enzymes to melt through tissues' structural boundaries.) In 2008 Campisi published further support for what she calls the "senescence-associated secretory phenotype," or SASP, using the term to highlight that, in certain contexts, senescent cells secrete hurtful molecules, behaving like catatonic zombies drooling poison.

Why, scientists wondered, would the cells long pictured as cancer preventers actively promote the very malady they seem to have been evolved to block? Campisi drew on studies about wound healing, among other lines of research, to help explain how they came to acquire this role.

One line of work showed that cancer and wound healing, strangely enough, are similar in some ways. Tumors and partly healed wounds, for instance, are both laced with fibrous proteins that form when the precursors of clotting proteins leak from blood vessels and polymerize into a matrix to support rebuilding. Struck by this similarity, in 1986 Harvard Medical School pathologist Harold Dvorak speculated that tumors harness and subvert the body's wound-healing response to aid their abnormal growth. Because of this Machiavellian jujitsu, he concluded, tumors appear to our bodies as an "unending series of wounds that continually initiate healing but never heal completely."

Another line of work demonstrated that senescent cells participate in wound healing. When tissues are damaged, certain cells in the vicinity respond by senescing, after which they fuel an inflammatory phase that initiates healing. The phase involves secretion of chemical messengers called cytokines that attract immune cells and activate them to fight infections and remove dead cells and debris. Later, healthy cells proliferate to replace lost ones, and then the proliferative phase gives way to a remodeling one, during which senescent cells secrete degradative enzymes to tear apart fibrous proteins laid down as an initial scaffold; this destruction limits scar formation.

Fitting these pieces together, Campisi postulated that beyond harnessing cellular senescence to block excessive proliferation by damaged cells, evolution turned to it for wound repair, which entailed adding SASPiness to its repertoire. Unfortunately, the secretory mode makes senescent cells perfect partners in crime for tumors bent on co-opting the wound-healing program for their own growth. Equally regrettable, their ability to fan inflammation may turn the entire body into a bad neighborhood—low-level inflammation is thought to promote the progression not only of cancer but also of atherosclerosis, Alzheimer's disease, type 2 diabetes and many other diseases of aging.

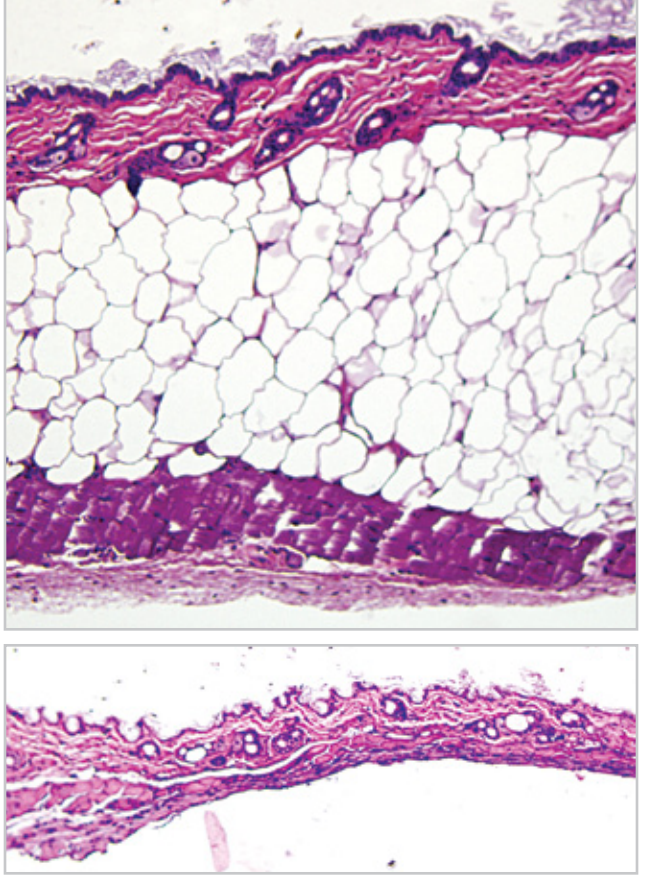
AGING AGENTS, AFTER ALL

INDEED, AS INVESTIGATORS REALIZED that senescent cells could behave in ways that fostered cancer, they also began to accrue fresh evidence for a role in aging. In particular, they found that senescent cells turn up with suspicious frequency in tissues of rodents and humans where things have gone badly awry, as well as in aging bodies as a whole. In 2006, for example, researchers showed that the normal decline of immune function in older mice occurs in tandem with an age-related increase in the senescence of the stem cells that continually generate various kinds of immune cells.

A number of these findings were made possible in part by discovery of features that identify cells as having become senescent. One of the most useful senescence markers is an elevated level of a protein encoded by a gene called $p16^{INK4a}$ (*p16* for short). Discovered in 1993 by David Beach of Queen Mary, University of London, *p16* activity was later found to help force cells to stop dividing when they sense various kinds of damage.

Norman E. Sharpless of the University of North Carolina at Chapel Hill School of Medicine and his colleagues conducted a number of studies correlating p16 protein levels and aging. They demonstrated, for instance, that levels rise with age in rodent and human cells and that this senescence-inducing rise is tied to a diminished ability of the cells to proliferate and repair damaged tissues. In 2004 the team reported that p16 increases markedly in almost all rodent tissues with advancing age and can be slowed by calorie restriction—a form of stringent dieting known since the 1930s to extend life span and promote healthy aging across various species. Five years after the 2004 finding, the Sharpless lab showed that getting older is accompanied by sharply increasing p16 levels in the human immune system's T cells. Intriguingly, p16 levels in the T cells are higher in smokers and people who are physically inactive, suggesting that those behaviors might promote cellular senescence. Anecdotally, Sharpless cheerfully told me that after his lab developed an easy-to-use test for measuring p16, he discovered that his own levels were twice as high as those of his graduate students. He is a young-looking 45-year-old.

Beyond correlating p16 and cellular senescence with features of aging, Sharpless and his colleagues have published a series of experimental findings supporting the idea that cellular senes-



EVIDENCE OF A ROLE IN AGING: In mice able to eliminate senescent cells, the fatty layer in the skin stays lush (*white in top image*), but it dwindles in other mice over time (*bottom*).

COURTESY OF VAN DEURSEN LABORATORY

cence contributes to tissue and organismic aging. In 2006 they reported that aging mice with disabled *p16* genes and thus, presumably, a much reduced tendency to form senescent cells resemble younger mice in their enhanced ability to regenerate pancreatic cells knocked out by exposure to a toxin; that aging mice with suppressed *p16* activity are better able than normal peers to regenerate neurons in certain parts of their brain; and that dialing back p16 levels in blood system stem cells—the ones that give rise to immune and red blood cells—retards the usual aging-related decline in the stem cells' regenerative power.

Other studies conducted over the past five years have suggested that genetic differences affecting the amount of p16 protein people make—and therefore the rate at which their cells become senescent as they age—help to determine their risks of many age-associated diseases, among them atherosclerosis and Alzheimer's. Sharpless says that these "superinteresting" findings have galvanized medical researchers' interest in p16 and that they are the "key to knowing that something real is going on" in research implicating cellular senescence as a culprit in aging-related decline.

Last year's Mayo Clinic study, though, provided the most direct evidence that interfering with cellular senescence might be beneficial, and van Deursen's group did it by taking advantage of p16's role as an ID tag for such cells. The team genetically engineered its mice both to have chromosomal defects that led to premature cellular senescence in various tissues and to carry a gene that made cells susceptible to killing by a particular drug if their *p16* genes were switched on; nonsenescent cells, whose *p16* genes were not activated, were not affected. Drug treatment throughout life erased the senescent cells and delayed the thinning of fat under the skin, loss of muscle, development of cataracts and the onset of other aging-related deterioration that occurred prematurely in untreated mice. Treatment begun later in life slowed age-related losses of fat and muscle.

As exciting as the Mayo findings are, they do not, by themselves, demonstrate that eliminating senescent cells during normal aging will be helpful in people or will extend life. Campisi cautions, for instance, that the study did not definitively prove that senescent cells drive normal aging; the mice in the study suffered from accelerated aging. And not all aspects of their accelerated aging involved rapid cellular senescence. In fact, erasing the senescent cells did not help avert the rodents' main cause of death—early onset of heart and blood vessel dysfunction—and so their life spans were not substantially stretched out.

SIMPLE STEPS

STILL, SUPPOSE THAT at some point scientists find that reducing cellular senescence in people does turn out to retard aging or at least delay wrinkling and some more serious age-related disorders. How might one intervene safely in the senescence process?

Replicating the Mayo study in people would require editing their genomes before birth, so that option that will not be tenable anytime soon, if ever. Simply blocking the activity of *p16* genes with a drug would probably backfire by increasing the risk of unwanted cell proliferation and cancer. Some surprisingly simple options might be open to us, however.

That smokers and sedentary people tend to have higher p16 levels suggests that not smoking and exercising regularly may help prevent the kind of molecular damage that promotes cellular senescence. Losing weight may be another way. Indeed, van Deursen and his Mayo colleague James Kirkland theorize that fat cell precursors called preadipocytes may induce a condition akin to accelerated aging in obese animals and people because the cells tend to become senescent in large numbers and, in keeping with Campisi's theory, promote chronic, low-level inflammation throughout the body.

Some preliminary evidence also hints that a drug called rapamycin can inhibit cellular senescence without fostering cancer. Interestingly, chronically feeding rapamycin to mice has been shown to extend their life spans. And recently Campisi's lab showed that certain anti-inflammatory drugs suppress senescent cells' destructive SASP mode. Yet for the time being, Sharpless says, the most prudent way to oppose deleterious cellular senescence is: "Don't smoke, eat reasonably and take exercise."

No one knows yet whether braking cellular senescence can slow normal aging. The theory that senescent cells are important contributors to age-related deterioration at the tissue and organ levels is, however, now aging with remarkable grace. It seems increasingly likely that this insight will one day lead to potent new ways to promote healthy aging. SA

MORE TO EXPLORE

Four Faces of Cellular Senescence. Francis Rodier and Judith Campisi in *Journal of Cell Biology*, Vol. 192, No. 4, pages 547–556; February 21, 2011. www.ncbi.nlm.nih.gov/pubmed/21321098

Clearance of p16^{Ink4a}-Positive Senescent Cells Delays Ageing-Associated Disorders. Darren J. Baker et al. in *Nature*, Vol. 479, pages 232–236; November 10, 2011.

SCIENTIFIC AMERICAN ONLINE

See researcher Judith Campisi talk about cellular senescence at ScientificAmerican.com/aug2012/senescence