whereas a β_2AR-selective antagonist reduced cerebral plaques.

The implications of this study for understanding Alzheimer disease and treating it may be quite profound. Activation of β_2AR through external influences such as stress may be an important risk factor; if so, reducing stress might delay disease onset. Further, antagonists of β_2AR and other relevant receptors may prevent stimulation of amyloid-β production that would otherwise occur upon endogenous receptor stimulation. Nonselective βAR antagonists include drugs already in use for treating hypertension; perhaps they could be repurposed for prevention of Alzheimer disease.

Before rushing into clinical trials with GPCR antagonists, however, the effects of these agents on other γ-secretase substrates besides APP should be determined. The most notorious substrate from the standpoint of Alzheimer disease therapeutics is the Notch receptor. Does chronic administration of these agents affect regulated proteolysis of the Notch receptor and critical downstream cell signaling events? Such effects should not occur in all cells containing γ-secretase: modulation of activated Notch should be restricted to cells that coexpress the relevant GPCR. Moreover, Notch activation would not be shut down, but only reduced to the degree that GPCR-mediated endocytosis of γ-secretase is a contributor to the amplitude of Notch signaling. But even if Notch signaling is not perturbed, cleavage of other substrates, such as cadherins, by γ-secretase can also have biological consequences[10] and may be unduly affected by GPCR activation.

More generally, it will be important to identify environmental factors that affect the production and aggregation of amyloid-β and toxic events downstream of amyloid-β—such as alterations in the tau protein, which forms neuronal filaments also implicated in Alzheimer disease pathogenesis. Along with reducing stress, other factors under consideration include an enriched environment conducive to keeping mentally and physically active[11] and components of the diet, such as omega-3 fatty acids[12].

Identifying such factors would suggest ways to tailoring one's lifestyle to reduce the risk of Alzheimer disease and may reveal other pharmacological approaches. In the meantime, hitchhiking is still ill-advised, and stressing out about Alzheimer disease will not help.

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Cancer gets the Chk'ered flag

Liu Cao & Toren Finkel

Certain oncogenes seem to be able to trigger cellular senescence and growth arrest, thereby holding cancer at bay. Two new studies suggest that oncogenes trigger senescence through activation of a pathway initially described as sensing DNA damage.

The discovery of human tumor oncogenes in the early 1980s and the subsequent realization of the existence of tumor suppressors seemed to turn a number of cancer biologists into overeducated automobile mechanics. Oncogenes became the cell's stuck accelerator pedal and tumor suppressors were the cell's brakes. The cancer cell, with its accumulation of activated oncogenes and absent tumor suppressors, became the equivalent of a rush-hour cab ride in New York City—accelerator pinned to the floor without the inclination or ability to stop. However, just like any number of things that were so in vogue in the 80s, from Cyndi Lauper to Lionel Ritchie, the car metaphor for cancer may require a bit of a tune-up.

Now, two studies in *Nature* add to the growing body of evidence that oncogenes don't act as simple or pure accelerators for cell growth[1,2]. Furthermore, these two studies provide important insights not only into how we get cancer but also into what may control how quickly we age.

The initial assays for human tumor oncogenes involved transfecting human tumor DNA into the immortalized NIH 3T3 cell line. Oncogene expression transforms this cell type, endowing it with a newly acquired ability to form tumors when injected into mice. The notion that these types of transformation assays require the initially transfected cell to be immortalized was appreciated but—for the sake of convenience—largely ignored. Then, roughly a decade ago, Serrano *et al.* sought to re-examine the oncogene-accelerator paradigm[3].

In those experiments, the *Ras* oncogene, the first human tumor oncogene isolated in the early 80s, was expressed not in an immortalized cell but rather in a normal fibroblast. Surprisingly, these investigators noted that, rather than getting the 'pedal to the metal' phenotype, when normal cells were engineered to overexpress the *Ras* oncogene, they paradoxically stopped growing—and even more unexpectedly—became senescent.

Senescence is a state of permanent growth arrest, in which the cell undergoes several distinct morphological and biochemical changes. It had been known for many years that after a certain discrete number of passages, called the Haflick limit, primary human cells eventually enter a senescent state. This form of senescence is called replicative senescence and is viewed by some as the cellular equivalent of aging. Subsequent experiments suggested that the ability of oncogenes to convert normal growing cells into senescent ones was not unique to *Ras*. How then do oncogenes cause normal cells to stop growing and enter senescence? For other forms of senescence such as replicative senescence, serial passaging gradually shortens telomeres, the specialized ends of chromosomes. When these telomeres reach a critical length, activation of a signaling pathway involving the checkpoint kinases Chk1 and Chk2 ultimately leads to growth arrest[4]. These two kinases, along with ataxia telangiectasia mutated (ATM) and p53, were first shown to be activated in the setting of DNA damage, and this pathway is often termed the DNA damage response.

Perhaps because oncogene-induced senescence appeared to be independent of telomere shortening, the initial focus for exploring how oncogenes caused growth arrest centered on alternative pathways, including activation of the

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tumor suppressor molecule p16INK4A. Now with the publication of these two new studies[1,2], the focus of oncogene-induced senescence has shifted back to the DNA damage response pathway and includes three of its principal players, ATM, Chk2 and p53.

Both groups of researchers collectively examined several different oncogenes, including *RAS*, *MOS*, *CDC6* and *CYCE*. Each of these oncogenes induced rapid senescence when expressed in a normal host cell. In each case, this induction of senescence was accompanied by simultaneous activation of the DNA damage response. Inhibiting the DNA damage response pathway, either genetically or pharmacologically, blocked the senescence program.

Notably, in cells engineered to overexpress an activated *Ras* oncogene, even if senescence was already fully established, subsequent genetic inhibition of the DNA damage response stimulated proliferation. Therefore, the DNA damage response appears to be required for both the induction and the maintenance of senescence.

To understand how oncogene expression triggers the DNA damage response, the authors analyzed their respective models for evidence of DNA replication stress. Using a variety of different techniques, both groups obtained evidence that oncogene expression stimulates inappropriate DNA replication. In some cases, this inappropriate replication resulted in increased DNA double strand breaks—the classic stimulator of the ATM pathway.

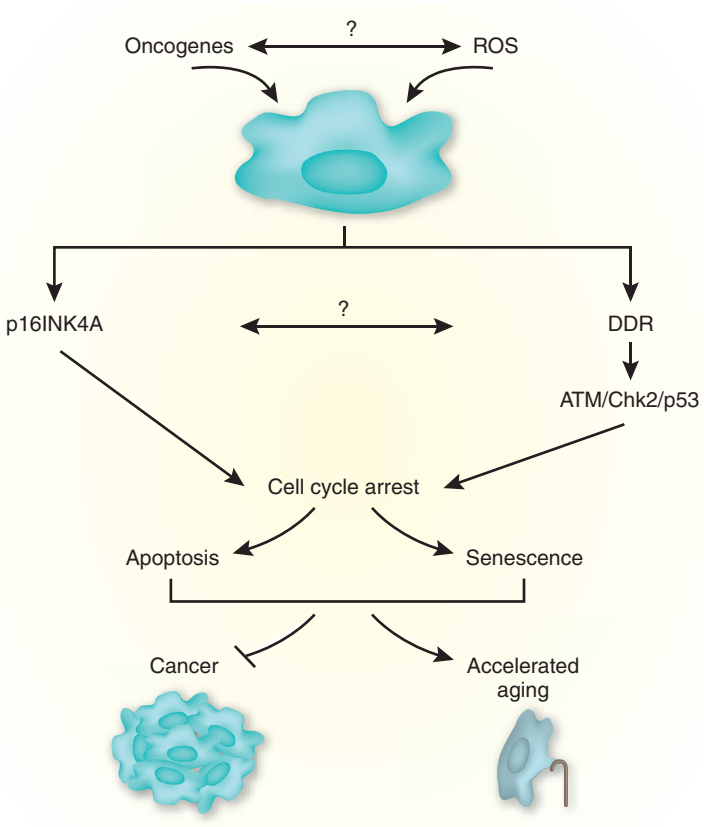
What do these results tell us about tumor formation? Previous results have strongly suggested that oncogene-induced senescence acts as a barrier to cancer[5,6]. These previous studies proposed that by entering either senescence or apoptosis, a normal cell harboring an oncogene is removed from the proliferative pool in its early, preneoplastic stage. If senescence is a cancer barrier, then inhibiting the DNA damage response should remove this obstacle and lead to bigger and faster-growing tumors. This seems to be the case, as both of the two new studies show that pharmacological or genetic disruption of the DNA damage response results in a more aggressive *in vivo* malignancy (see **Box 1**). These results are consistent with an earlier study showing that expression of another oncogene, *c-myc*, stimulates the DNA damage response and that inhibiting this response accelerates tumor growth[7].

Together these studies suggest that the first response of the cell to an oncogene may not be to step on the gas but rather to slam on the brakes. Although growth arrest through the engagement of the DNA damage response may be useful for the immediate prevention of cancer, many believe that the accumulation of senescent cells ultimately drives the aging process. As such, pathways that limit cell growth after perceived damage may help to prevent tumors, but, in the process, their activation may accelerate the rate at which we age (**Fig. 1**). This notion is consistent with other recent results suggesting that deletion of p16INK4A, a common genetic change seen in many tumors, may actually delay the age-related decline of stem and progenitor cell function[8–10]. In addition, animals in which the DNA damage response is continuously engaged, because of DNA damage and genomic instability, appear to suffer from accelerated aging[11,12].

Accurate maintenance of genomic integrity is therefore critical both for suppressing malignancies and for determining the speed at which we age. Both cancer and aging would seem to be linked by the common thread of DNA damage and the inevitable response to this damage.

Although these two studies significantly advance our understanding of oncogene-induced senescence, many questions remain. For instance, how do we reconcile these new findings regarding a pre-eminent role for the DNA damage response pathway with previous observations that the induction of p16INK4A is required for oncogene-stimulated senescence? Do these two growth-arrest pathways intersect in some fashion? Similarly, what do these observations say about other inducers of senescence? In particular, given that oncogene-induced senescence and oncogene-mediated DNA damage seem to be partly mediated by reactive oxygen species[13,14], do exogenous and endogenous oxygen radicals also stimulate senescence by activating the DNA damage response?

Finally, for those who envision new anti-aging therapies, it's unclear whether efforts should be aimed at directly inhibiting DNA damage or at dampening the molecular response to that damage. Indeed, if life is like a car trip, does the magnitude or duration of the DNA damage response determine the speed of our journey? If so, does the response act as the



Kim Caesar

Figure 1 Oncogene expression leads to growth arrest. Depending on the cell or the oncogene, this arrest can occur through a p16INK4A pathway or, as Di Micco *et al.* and Bartkova *et al.* describe[1,2], through a pathway dependent on the DNA damage response (DDR). The engagement of the DNA damage response can lead to either apoptosis or senescence—but in both cases the cell no longer proliferates. The loss of cell division through apoptosis or senescence reduces the risk of cancer but may accelerate the rate of aging. Although the current studies looked at the induction of senescence by oncogenes, perhaps these same pathways may also have a role in senescence induced by other processes, such as exogenously added reactive oxygen species (ROS) or ROS generated secondary to normal aerobic metabolism. In addition, a rise in intracellular ROS levels appears to contribute to both oncogene-induced senescence and oncogene-mediated DNA damage[13,14].

car's engine or the tires? Hmm... sounds like a pretty good metaphor.

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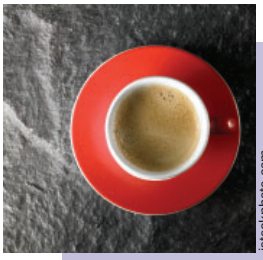

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BOX 1 Caffeine buzz

Caffeine is well known to act as an inhibitor of ATM a component of the DNA damage response—although the beloved substance has not been linked with cancer in epidemiological studies. In one of the newly published reports, Bartkova *et al*. examine the ATM-modifying aspects of caffeine, and present addicts with a potential dilemma[2].

In their experiments, mice were given continuous doses of caffeine that were two or more orders of magnitude higher than you get in your average cup of Joe. Consistent with the notion that the DNA damage response restrains tumor growth, animals given pharmacological doses of caffeine developed larger, more aggressive malignancies than their noncaffeinated littermates.

Therefore, next time you are in line at your local coffee house, remember that if you order enough of those double caramel macchiatos, you could (very theoretically) help remove the last barrier keeping some small preneoplastic cell in check. On the other hand, that same drink, by inhibiting the DNA damage response, might also block cellular senescence and thereby slow your overall aging.

Cancer or aging—pick your poison. Come to think of it, that decaffeinated chai latte looks pretty good.

Osteoclasts, no longer osteoblast slaves

Brendan F Boyce & Lianping Xing

A protein has been identified that promotes the formation of osteoclasts, bone-degrading cells—while also inhibiting osteoblasts, bone-forming cells. The findings could lead to new avenues of drug development to strengthen bone (pages 1403–1409).

Osteoclasts, bone-degrading cells, operate under the control of osteoblasts, bone-forming cells. But recent studies have suggested that osteoclasts don't just passively carry out orders—they in turn seem to influence osteoblasts[1]. In this issue, Lee *et al*.[2] bolster the case for an expanded osteoclast sphere of influence.

In examining how multinucleated osteoclasts develop by fusion of precursor cells, the authors identified a protein key for this activity—a component of a proton pump, Atp6v0d2. Protons in osteoclasts are known to be secreted through this pump to dissolve bone. The authors show that Atp6v0d2 unexpectedly promotes precursor fusion and osteoclast activity, but also somehow inhibits osteoblasts. The findings raise the possibility of therapeutic intervention for osteoporosis by targeting a single gene product in osteoclasts to inhibit bone destruction and stimulate bone formation.

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Teams of osteoclasts continually degrade bone throughout life at over 1 million microscopic sites in the human skeleton; teams of osteoblasts subsequently rebuild bone at the same sites. This process is called bone remodeling[3]. Bone remodeling replaces worn-out sections of bone to maintain skeletal integrity and, importantly, is disrupted in all bone diseases associated with changes in bone mass.

Osteoblast lineage cells regulate multiple aspects of bone remodeling. For example, osteocytes (osteoblasts that get embedded in bone during bone formation) may help recruit osteoclasts to sites of bone destined for removal by signaling to cells on the bone surface through mechanisms that remain poorly understood. Preosteoblastic stromal cells close to these precursors in the marrow (**Figs. 1** and **2**) regulate osteoclast activation and formation from bone marrow macrophages. The stromal cells accomplish this task through expression of membrane-bound receptor activator of NF-κB ligand (RANKL), a member of the tumor necrosis factor (TNF) superfamily, and macrophage colony-stimulating factor (M-CSF)[3].

Multiple factors operate with RANKL to regulate osteoclasts—including NF-κB, c-Fos and NFATc1. NFATc1 is a transcription factor activated by c-Fos in osteoclasts downstream from RANK, the RANKL receptor, and also by intracellular calcium fluxes[4]. NFATc1 mediates RANKL signaling. Osteoprotegerin (OPG) expressed by more mature osteoblasts binds to RANKL, preventing it from interacting with RANK to limit osteoclast formation[3,4]. RANKL/RANK signaling also inhibits osteoclast formation directly by autocrine expression of interferon-β mediated by c-Fos in osteoclast precursors[4]. 'Coupling factors' released from the bone matrix during resorption are thought to attract osteoblasts to the site[5], but this aspect of the process remains poorly understood.

Once formed, osteoclasts attach themselves firmly to the surface of the bone; they form a tight ring-like seal at the ends of cytoplasmic extensions, each containing finger-like processes called the ruffled border (**Fig. 1**). This border serves to increase the surface area contacting the bone. From this ruffled border, the cells secrete protons through the V-type H^+ ATP6i proton pump complex; chloride ions follow passively through the chloride channel ClC-7 to form HCl. The HCl dissolves bone mineral and