Telomeres Limit Cancer Growth by Inducing Senescence: Long-Sought In Vivo Evidence Obtained

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Cellular senescence triggered by telomere dysfunction has long been hypothesized to constitute a tumor suppression mechanism. The evidence has come primarily from in vitro cell culture studies, and more indirectly from analysis of tumor specimens. Two recent studies, published in the current issue of *Cancer Cell* and online at *EMBO Reports*, provide direct mechanistic evidence in cleverly manipulated mouse cancer models. This work shows that telomere-induced senescence is as effective as apoptosis in reducing cancer incidence and is mediated by the tumor suppressor p53.

Cellular senescence is an irreversible cell-cycle arrest that can occur in response to a variety of stresses and signaling imbalances. The first described trigger was simply ongoing cell division resulting in progressive shortening and eventual malfunction of telomeres (Hayflick, 1965). Telomeres are stretches of repetitive DNA, which together with specialized proteins form protective structures at the ends of chromosomes, preventing them from being recognized as DNA double-strand breaks and consequently degraded or fused by DNA repair machineries. Telomeres shorten with each cell division because DNA polymerases cannot fully replicate 3′ termini, a phenomenon referred to as the end-replication problem. Telomeres are maintained by a specialized RNA-templated polymerase, called telomerase, which is composed of a catalytic protein subunit and a small template RNA (Cech, 2004).

Telomerase activity is typically found only in germ cells, some stem cells, and transiently in some proliferative cells of renewable tissues. In the absence of telomerase, telomeres shorten by 50–200 base pairs with each round of replication. When telomeres become critically short and incapable of end protection, most cells undergo senescence (Shay and Wright, 2005). Telomere-induced senescence has been shown to share many components of the DNA damage response triggered by DNA double-strand breaks (von Zglinicki et al., 2005). Soon after the discovery of telomerase, its upregulation was documented in the majority of cancer cells, presumably to confer the capability for extensive proliferation. The role of telomere shortening in senescence was formally demonstrated by the ability of experimental telomerase expression to immortalize normal somatic cells (Bodnar et al., 1998).

In the last decade it became apparent that, in addition to telomere shortening, many stresses and/

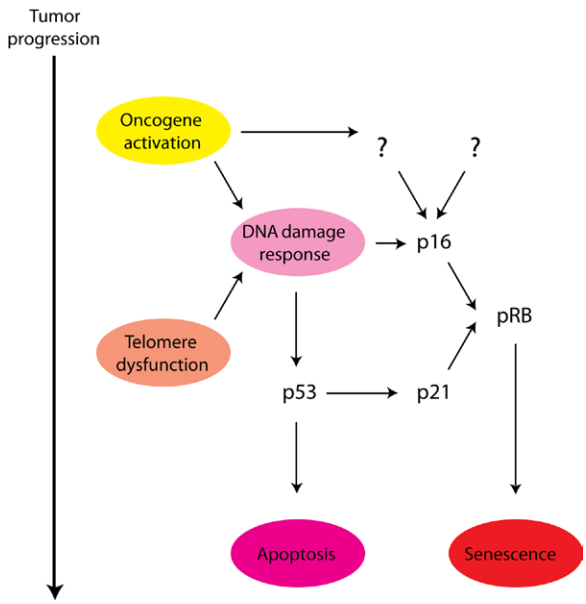

Figure 1. Cancer Suppression Signaling Pathways Leading to Senescence and Apoptosis
Inappropriate signaling elicited by activation of oncogenes, usually an early event during tumor progression, can elicit a DNA damage response resulting in the activation of p53. Oncogene activation often also upregulates p16, either as a consequence of the DNA damage response or through independent mechanisms. The p16 and p21 branches converge on pRB, whose activation strongly promotes senescence. Although the relative contributions of the p21 and p16 branches are not yet well understood, in many cases p16 is believed to play a crucial role. This is underscored by its frequent silencing during tumor progression, and evidence is accumulating that the key selective pressure is to relieve the senescence checkpoint. Telomere dysfunction, expected to occur at later stages of tumor progression, also causes a DNA damage response and strongly activates p53. The manner in which activation of p53 is channeled into either an apoptotic response or senescence is not well understood, but these choices appear to be strongly influenced by cell type and perhaps other contexts.

or signaling imbalances, including activation of some oncogenes, can trigger cellular senescence in normal cells (Ben-Porath and Weinberg, 2004; Collado and Serrano, 2006). In spite of the plethora of stimuli, in the great majority of cases senescence proceeds through one (or both) of two central signaling pathways, leading to the activation of the p53 and retinoblastoma (pRB) tumor suppressor proteins (Figure 1). The two key effectors that distinguish these pathways are the cyclin-dependent kinase inhibitors p21 (CDKN1A) and p16 (CDKN2A) (Campisi, 2005; Herbig and Sedivy, 2006). p21 is transcriptionally activated by p53, and this pathway is the primary mediator of telomere- and genotoxic stress-induced senescence (d'Adda di Fagagna et al., 2004). The p16-pRB pathway mediates many forms of nongenotoxic stress-induced senescence. Oncogene-induced senescence can activate both the p16-pRB and p21-p53 pathways.

The cardinal features of cellular senescence are irreversibility of the arrest, profound changes in gene expression patterns, and the ability of senescent cells to persist for long periods of time. Although both senescence and apoptosis are regulated by the p53 pathway, they are considered to be physiologically distinct phenomena; in fact, many senescent cells display a distinct resistance to apoptotic cell death. It is important to note, however, that the great majority of information on senescence has come from cell culture models, and that until very recently, even the existence of this process in vivo at any appreciable level was on tenuous footing. Solid evidence has now come from mouse models in which expression of activated oncogenes (or loss of a tumor suppressor) resulted in the development of benign lesions comprised of demonstrably senescent cells (Collado and Serrano, 2006). Furthermore, the malignant tumors that grew out of these lesions had inactivated and bypassed the senescence response. Similarly, benign melanocytic nevi in human skin contain cells that express oncogenic BRAF and are senescent.

These findings indicate that oncogene-induced senescence occurs in vivo and suggest that the role of the senescence response is to prevent oncogene-expressing cells from progressing to malignancy. Similarly compelling evidence for the in vivo existence of telomere-induced senescence has been lacking. This is mainly because the laboratory mouse is a less than ideal model system: this species has very long telomeres, and many tissues express significant levels of telomerase. A mouse that completely lacks telomerase activity, due to a germline knockout of the RNA template component, is apparently normal and healthy. After successive generations, allowing for sufficient telomere shortening, the mice display premature aging phenotypes, although their tumor susceptibility is not significantly altered. The latter is due to surveillance performed by the p53 pathway, which was shown to induce apoptosis of cells that have suffered telomere dysfunction.

On the basis of these studies it was widely believed that telomere-induced senescence simply did not exist in the mouse. Carol Greider (Feldser and Greider, 2007) and Sandy Chang (Cosme-Blanco et al., 2007) recently undertook some clever mouse genetics to reinvestigate this issue more closely and investigated consequences of an oncogenic challenge in the presence of shortened telomeres when apoptotic mechanisms were genetically blunted. Both groups used a knockout of the telomerase RNA to generate animals with short telomeres. The Greider group first combined the telomerase knockout with a transgene that drives Myc oncogene expression in B cells. As previously observed, short telomeres strongly inhibited tumorigenesis by inducing apoptosis. To suppress apoptosis, Bcl2 overexpression was engineered in bone marrow cells using in vitro retrovirus vector infection, and the cells were subsequently transplanted into lethally irradiated syngeneic recipient animals. As expected, transplanted bone marrow cells with normal telomeres invariably gave rise to highly aggressive tumors. Surprisingly, when telomeres were shortened by removing telomerase activity, palpable tumors did not develop for over 100 days. The answer was found by histological examination, which revealed small encapsulated tumor masses in lymph nodes that displayed clear signs of senescence, such as senescence-associated β-galactosidase activity and expression of the tumor suppressor p16.

The Chang group used a knockin of a specific allele of p53, Arg172Pro, that abrogates induction of apoptotic responses but maintains competence for cell-cycle arrest, and investigated the occurrence of spontaneous tumors in older mice with normal or short telomeres. In this experimental setting, strong tumor suppression was also observed, with clear evidence of senescence activation in diverse cellular compartments. Interestingly, although the mice displayed profound spontaneous cancer resistance, their life spans were not prolonged. This may be due to the activation of senescence in numerous proliferative compartments, which would be expected to negatively affect survival; indeed, the mice showed some phenotypes of premature aging. Senescence did not, however, effectively suppress chemical carcinogen-induced skin cancer, a situation where both apoptosis and senescence may be needed.

Both groups concluded that in most (although perhaps not all) cases senescence was as effective as apoptosis in reducing cancer incidence, and that in all cases examined the senescence response was mediated by the p53 pathway. The main point of these studies is that telomere-dependent senescence does in fact occur and can effectively suppress cancer in the mouse. The existence of this mechanism has been a key assumption of the senescence tumor suppression hypothesis, so it is very reassuring to have it confirmed in vivo. More importantly, it strongly reinforces the notion that cellular senescence, either oncogene- or telomere-induced, is of equal importance to apoptosis in

mediating tumor suppression. It has been well documented that genotoxic stress, including telomere dysfunction, triggers apoptosis in some cell types, whereas in others senescence is the primary response. While such choices are not well understood, it is comforting that, in B cells, which prefer apoptosis, senescence can act as a robust backup system. Finally, the possibility that oncogene- and telomere-induced senescence may act as reinforcing, two-tiered defense systems, especially in human cancers, needs to be considered.

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Role of *c-Myc* in *Apc* Mutant Intestinal Phenotype: Case Closed or Time for a New Beginning?

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Inactivation of the *adenomatous polyposis coli* (*APC*) tumor suppressor gene occurs in most colorectal cancers. The proto-oncogene *c-MYC* was one of the first genes linked to *APC* inactivation, but the in vivo significance of *c-MYC*'s enhanced expression in intestinal cells with *APC* defects has been uncertain. Sansom et al. recently reported that targeted inactivation of c-Myc in murine intestinal epithelium potently inhibited phenotypical and transcriptional changes seen in *Apc*-deficient intestinal epithelium. While these findings are very interesting, some questions remain about the assignment of *c-Myc* as the pre-eminent β-catenin-regulated gene in intestinal epithelium.

The small intestine's epithelial lining is characterized by invagination of the so-called crypts into surrounding tissue and by the presence of finger-like protrusions (i.e., villi) extending into the lumen (Figure 1A). In the lower region of each crypt, there may be four to six stem cells (Marshman et al., 2002). Following their proliferative expansion in the crypt, progenitors differentiate into enterocytes, goblet cells, and enteroendocrine cells as they migrate upward along the crypt-villus axis to populate the villus surface, or into Paneth cells as they migrate down to the base of the crypt. Enterocytes undergo apoptosis and/or are shed from the mucosal surface only days after their birth.

The β-catenin protein has previously been established to have a central role in regulating proliferation, differentiation, and migration in intestinal epithelium (Batlle et al., 2002; Ireland et al., 2004; van de Wetering et al., 2002). Most of the cell's pool of β-catenin is tethered to E-cadherin as an adherens junction component to mediate intercellular adhesion. A less abundant "free" pool of β-catenin can serve as a transcriptional coactivator upon its translocation to the nucleus and binding to DNA-binding proteins of the T cell factor (TCF) family (Figures 1B and 1C). In most cells, the free pool of β-catenin is tightly regulated by a destruction complex composed of the adenomatous polyposis coli (APC) and Axin tumor suppres-