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HOT TOPICS

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Summary

Cancer is an age-related disease in organisms with renewable tissues. A malignant tumor arises in part from genomic damage, which can also drive age-related degeneration. However, cancer differs from many age-related degenerative diseases in that it entails gain-of-function changes that confer new (albeit aberrant) properties on cells, resulting in vigorous cell proliferation and survival. Nonetheless, interventions that delay age-related degeneration – for example, caloric restriction or dampened insulin/IGF-1 signaling – often also delay cancer. How then is the development of cancer linked to aging? The answer to this question is complex, as suggested by recent findings. This Hot Topic review discusses some of these findings, including how genomic damage might alter cellular properties without conferring mutations, and how some genes that regulate lifespan in organisms that lack renewable tissues might affect the development of cancer in mammals.
Key words: DNA damage response; FOXO transcription factors; inflammation; longevity; p53; PI3 kinases; sirtuins; telomeres; tumor suppression.

Introduction

In most mammalian species that have been examined carefully, cancer incidence rises with approximately exponential kinetics, beginning at about the midpoint of the lifespan. In this regard, cancer is no different in its trajectory and relationship to lifespan from many of the degenerative diseases of aging. Age-related degenerative diseases generally entail a loss of cell function. By contrast, malignant tumorigenesis requires that cells adopt new, albeit aberrant (at least for that cell type) phenotypes, ranging from uncontrolled cell proliferation to the ability to create a nourishing vasculature (Hanahan & Weinberg, 2000). So, is there a common biology that links cancer and other age-related pathologies, as is often suggested (Finkel *et al.*, 2007; Vijg & Campisi, 2008)? Or, is cancer merely a coincidental correlate of aging, controlled indirectly by, or independently of, the major aging processes? Definitive answers to these questions are still lacking. However, recent findings support the idea that cancer and aging are indeed intimately connected, and that this connection depends on the activities of specific 'longevity' genes and also on the specific responses of cells to genotoxic stimuli.

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Telomeres at the crux between cancer and aging

There is little doubt that a major driving force behind the development of cancer is DNA damage and the subsequent acquisition of somatic mutations. Among the important genomic changes acquired by malignant tumors are complicated chromosomal aberrations. These aberrations generally arise from the faulty repair of complex DNA damage, or the failure of protective structures such as telomeres. Mouse models of faulty DNA repair or telomere maintenance support the idea that complex genomic damage can drive cancer, as well as aging phenotypes that are unrelated to cancer (Blasco, 2003; Hasty *et al.*, 2003). Some recent papers have shed light on why this might be the case.

Telomeres are repetitive DNA sequence and associated proteins that cap the ends of linear chromosomes. The telomeric structure protects chromosome ends from being perceived and processed as DNA double strand breaks. This protection prevents telomeric fusion, cell death or senescence, genomic instability and neoplastic transformation, depending on the cell context (Rodier *et al.*, 2005). How might it prevent aging?

Telomeres shorten during cell division and aging because DNA replication machineries incompletely copy 3′ DNA ends, and the G-rich telomeric DNA is susceptible to oxidative damage and hence repair-mediated loss. Critically short telomeres lose the protective structure, triggering a DNA damage response that causes irreversible cell cycle arrest (senescence) in normal cells, or genomic instability in cells with compromised checkpoints (Campisi & d'Adda di Fagagna, 2007). Some cell types – few in humans, more in mice – express telomerase, which can add telomeric DNA to chromosome ends *de novo* (McEachern *et al.*, 2000). Thus, telomerase stabilizes telomere length and structure. In mice, which have longer telomeres and more widespread telomerase expression than humans, germ line inactivation of telomerase causes telomeres to shorten and eventually fail, leading to progeroid-like pathology and cancer (Artandi & DePinho, 2000). These studies indicate that telomere shortening can drive aging and cancer phenotypes, but begs the question of whether

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telomere shortening plays a role in the development of age-related pathology in unmodified mice.

A recent paper suggests it might (Flores *et al.*, 2008). Using quantitative *in situ* measurements, Flores *et al.* showed that the longest telomeres in mice were found in adult stem cell compartments. Further, they showed that, despite somewhat promiscuous telomerase expression in mouse tissues, telomeres do shorten with age in multiple mouse tissues, and that this shortening is evident in multiple stem cell compartments. Most important, telomere shortening coincided with a loss of stem cell function. Thus, apparently, telomeres shorten in mice, as they do in humans, and long telomeres correlate with healthy adult stem cell function.

The importance of telomere maintenance for healthy aging was more directly addressed by a recent paper by Tomás-Loba *et al.* (2008). Telomerase is a double-edged sword. When overexpressed, it can improve processes that require cell proliferation, such as wound healing (Gonzalez-Suarez *et al.*, 2001); on the other hand, it allows cancer cells to stabilize their telomeres and proliferate indefinitely. Thus, telomerase overexpressing mice are generally cancer prone, but superior in physiological processes such as wound healing (Gonzalez-Suarez *et al.*, 2001). To counter the cancer-prone tendencies of telomerase overexpressing mice, Tomás-Loba *et al.* crossed them to transgenic mice that carry extra copies of the tumor suppressor genes encoding the p53, Arf and Ink4a (p16) proteins. These super tumor suppressor-mice were previously shown to be remarkably cancer free, although their lifespan was similar to, or only slightly longer than, that of wild-type mice. Telomerase overexpression in these cancer-resistant mice substantially extended the mean lifespan, although it had no effect on the maximum lifespan. Of particular interest, the combined overexpression of tumor suppressors and telomerase markedly improved the integrity and function of proliferative tissues, probably by preserving stem cell function. Further, this combination of genetic manipulations rectangularized the lifespan curve, causing most of the animals to die over a relatively short interval. Together, the data suggest that elevated telomerase activity in the context of heightened tumor suppression can improve the health span, if not the maximum lifespan, of mice. Improved health span is a major goal of aging research, and so the important question now is do these principles pertain to other mammals, in particular humans?

How might long telomeres contribute to stem cell function? A definitive answer to this question is lacking, but a recent paper by Beliveau *et al.* (2007) provides an intriguing possibility. Working with human mammary epithelial cells in culture, Beliveau *et al.* showed that as telomeres shorten during repeated cell division, they elicit a chronic low level DNA damage signal before they become so short as to lead to growth cessation and cell senescence. This signal leads to slight, chronic elevation of p53 activity, which in turn markedly sensitizes the cells to the growth arrest caused by epidermal growth factor or insulin insufficiency through mechanisms still to be determined. These findings raise the possibility that short, but not obviously dysfunctional, telomeres can compromise the function of cells – including stem cells – by sensitizing them to stresses such as growth factor insufficiency. The findings might also explain why chronically active, but not transiently elevated, p53 can accelerate certain aging phenotypes in mice (reviewed in Hot Topics: Aging and cancer cell biology, 2007) (Campisi, 2007).

Cellular senescence, inflammatory cytokines, and the tissue microenvironment

Aging is characterized by sometimes striking changes in tissue structure and function, which can also fuel the development of cancer. Aging and cancer also share inflammation as an important risk factor. Three recent papers link the tumor suppressive senescence response to the secretion of inflammatory cytokines and other modulators of the tissue microenvironment both in cell culture and *in vivo*.

Cellular senescence is the irreversible arrest of cell proliferation that occurs in many mammalian cells in response to potentially oncogenic stimuli (Campisi & d'Adda di Fagagna, 2007). This tumor suppressive mechanism has been proposed to contribute to aging by reducing the regenerative capacity of tissues, as nondividing senescent cells accumulate with age, and by degrading local tissue structures, as senescent cells chronically secrete inflammatory cytokines and tissue remodeling enzymes. Coppe *et al.* (2008) provided the first extensive characterization of the senescence-associated secretory phenotype (SASP). This phenotype, a robust feature of senescent human fibroblast and epithelial cells, entails the secretion of more than 40 distinct molecules in response to DNA damage, whether elicited by dysfunctional telomeres, ionizing radiation or the expression of certain oncogenes. Ironically, SASP components – notably the pro-inflammatory cytokines interleukin (IL)-6 and IL-8 – stimulated malignant phenotypes in nearby premalignant or malignant cells, supporting the idea that the senescence response can be antagonistically pleiotropic (Campisi, 2007). Further, the SASP was suppressed by p53, suggesting that p53 suppresses cancer by both cell autonomous and cell nonautonomous mechanisms. Importantly, human prostate cancer cells expressed a robust SASP after DNA damaging chemotherapy, indicating that this phenotype occurs in humans *in vivo*.

Two related papers identified surprising cell autonomous functions for the IL-6 and IL-8 secreted by senescent human cells (Acosta *et al.*, 2008; Kuilman *et al.*, 2008). Both these cytokines reinforce the senescence growth arrest via an intricate network comprising the IL-6 and IL-8 transmembrane receptors and the CEBP-β and NF-κB transcription factors. Unexpectedly, the secreted cytokines were less important for the growth arrest than activation of the receptors, which presumably are also activated by intracellular binding of the ligands. The activated receptors, in turn, stimulated the nuclear activities of the transcription factors, and both

the receptors and the transcription factors were required for an efficient senescence response to telomere dysfunction or certain oncogenes. These findings suggest a model whereby senescence-causing stimuli upregulate expression of IL-6 and IL-8, which then act intracellularly via their receptors to signal nuclear events that reinforce the senescence growth arrest. Senescent cells are frequently found in preneoplastic lesions in human tissues (Prieur & Peeper, 2008), consistent with the senescence response being a barrier to the development of cancer. Notably, these lesions also showed high levels of IL-6, IL-8 and/or their receptors, suggesting that the senescence-associated cytokine network also operates *in vivo*.

The findings by Acosta *et al.* (2008) and Kuilman *et al.* (2008) suggest the SASP can have beneficial effects – in this case, reinforcement of the tumor suppressive senescence-associated growth arrest by IL-6 and IL-8. Even more striking evidence for potentially beneficial effects of SASP components was recently reported by Krizhanovsky *et al.* (2008). Liver fibrosis is a precursor to liver cirrhosis, which in turn is a major risk factor for liver cancer. This pathology is initiated by hepatic stellate cells, which proliferate and produce the fibrotic extracellular matrix in response to liver damage. Krizhanovsky *et al.* found that senescent cells accumulate in the fibrotic scars of mice treated with liver-damaging CCl_4, and do so prior to resolution of the fibrotic scar following CCl_4 withdrawal. Loss of the senescence response by genetically abrogating the p53 and pRB/p16 tumor suppressor pathways, which are required for cellular senescence, significantly exacerbated the fibrosis. These findings suggest that cellular senescence limits the extent of fibrosis, thereby reducing the risk of cirrhosis, and, ultimately, liver cancer. The mechanisms by which senescent cells act in this context are incompletely understood. Although the cells likely stimulate inflammation, at least initially, the cytokines they secrete also appear to activate the innate immune system to eliminate senescent cells. Further, senescent cells also secrete high levels of matrix metalloproteinases, which may help resolve the fibrotic scar.

Longevity pathways intersect with cancer pathways

The last decade or so of aging research has seen remarkable progress in identifying the components of pathways that, when dampened, extend lifespan. These longevity pathways are most robust in simple model organisms such as nematodes, but also act, albeit less robustly, in more complex organisms such as mice (Vijg & Campisi, 2008).

One of the best studied longevity pathways is the insulin/IGF-1 signaling (IIS) pathway. This pathway entails insulin or IGF-1 receptor-mediated activation of downstream kinases, ultimately causing the phosphorylation and inactivation of FOXO transcription factors. In simple organisms, reduced IIS and heightened FOXO activity increase stress resistance and promote longevity. In mice, globally reduced IGF-1 signaling, or reduced insulin signaling in fat cells, results in a small increase in lifespan. Moreover, heightened activity of IIS kinases and reduced FOXO activity are thought to promote cancer. A recent study, for example, showed that cancer stem cells have high levels of the IIS kinases PI3K p110 α and β, and that knock down of the FOXO3A transcription factor increased the tumorigenic potential of the cells (Dubrovska *et al.*, 2009). Thus, genes encoding IIS components, including FOXO proteins, are candidates for co-ordinating longevity and tumor suppression in mammals (Greer & Brunet, 2005). Does reduced IIS and/or increased FOXO activity promote longevity and cancer-free survival in humans? Two recent studies suggest this might be the case.

Studying genetic variation among components of the IIS pathway in a well-characterized human population of centenarians, Suh *et al.* (2008) found that nonsynonymous mutations in the IGF-1 receptor (*IGF1R*) gene were over-represented in female centenarians. Female centenarians heterozygous for these mutations had reduced IGF1R protein levels, and reduced IGF-1 signaling activity, as determined by the levels of phosphorylated AKT, a downstream target of IGF1R activation. The cancer incidence in this population was not reported. However, using a different human population, Willcox *et al.* (2008) found that genetic variation in the human *FOXO3A* gene associated strongly with longevity in males, and that these long-lived males had a lower prevalence of cancer (and cardiovascular disease). Neither the functional status of the FOXO3A variants, nor the activities of upstream components of the IIS pathway were reported for this population. Nonetheless, together, these studies are consistent with the idea that reduced IIS and heightened FOXO3A activity might contribute to cancer resistance and longevity in humans.

SIRT1 enigmas

Mammalian SIRT1 is a protein deacetylase and close relative of the yeast protein SIR2 (silent information regulator 2). Overexpression of SIR2, its *C. elegans* homolog sir2-1, or its *Drosophila* homolog dSir2, extends lifespan in yeast, nematodes and fruit flies, respectively. In mice, SIRT1 overexpression or activation confers a metabolic state that resembles caloric restriction (Bordone *et al.*, 2007), but, so far, there have been no reports of lifespan extension.

In human cancers, it is presently unclear whether increased SIRT1 expression exacerbates or impedes the development and/or maintenance of malignancy. As succinctly summarized recently (Liu *et al.*, 2009), SIRT1 expression and deacetylase activity are repressed in nonmalignant cells by tumor suppressor proteins including p53, Chk2, HIC1, and DBC1. During aging, the HIC1 promoter can undergo hypermethylation and epigenetic silencing (Chen *et al.*, 2005). As a consequence, SIRT1 expression is expected to rise in aging tissues, where, possibly, it might increase the survival of damaged cells and cancer risk.

Experimental expression of oncogenes, or inhibition of tumor suppressor genes, causes SIRT1 overexpression and

activation, resulting in aberrant histone modification, promoter CpG island methylation and transcriptional repression, in addition to deacetylation of nonhistone proteins (e.g. p53, which is activated by acetylation). SIRT1-mediated transrepression can inactivate tumor suppressor proteins such as p53, Rb, FOXO, and Ku-70. Because these proteins, in turn, regulate senescence, differentiation and stress-induced apoptosis, SIRT1 might be expected to promote tumor initiation, progression and drug resistance. However, despite the facts that SIRT1 deacetylates p53 and can silence other tumor suppressor genes, transgenic SIRT1 overexpressing mice did not show an increased incidence of tumor formation (Banks *et al.*, 2008; Pfluger *et al.*, 2008; Zhang *et al.*, 2008).

On the contrary, recent studies suggest that SIRT1 itself can serve as a tumor suppressor, at least in certain contexts. Among its activities, for example, SIRT1 plays a key role in recruiting RAD51 and NBS1 to DNA double strand breaks to initiate the DNA damage repair process (Oberdoerffer *et al.*, 2008). As recently summarized (Deng, 2009), SIRT1 overexpression suppresses age-related transcriptional changes (Oberdoerffer *et al.*, 2008). Moreover, it reduces the incidences of colon cancer in APC+/min mice (Firestein *et al.*, 2008), BRCA1-associated mammary cancer (Wang *et al.*, 2008b), spontaneous cancers in multiple tissues of Sirt1+/−; p53+/− mice (Wang *et al.*, 2008a), and ionizing radiation-induced lymphoma in p53+/− mice (Oberdoerffer *et al.*, 2008). Thus it is possible that the elevated levels of endogenous SIRT1 observed in certain cancers are consequences (perhaps adaptive responses), rather than causes, of tumorigenesis.

The relationship between cancer and aging continues to prove complex and multi-faceted. As our understanding of both the aging process and the initiation and progression of malignant tumorigenesis progress, this relationship is likely to deepen.

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