Telomerase is critical for the integrity of stem cell compartments. Mutations in telomerase components lead to telomere shortening and hematopoietic stem cell failure in autosomal dominant dyskeratosis congenita and aplastic anemia. Telomerase activity is readily detected in most cancers but not in adult somatic cells. The telomerase hypothesis for cancer states that telomerase is reactivated in late stages of carcinogenesis. However, recent evidence has suggested that a stem cell origin for certain cancers, implying that the genetic aberrations that lead to cancer accumulate in tissue-specific stem cells and not in adult somatic cells. In these cancers, stem cells would already have telomerase and it would not need to be reactivated. Hence, we reconsider the telomere hypothesis in view of this evidence and propose that, rather than telomerase reactivation, enzyme activity may increase in later stages of carcinogenesis due to increased expression or efficient assembly of telomerase components. Understanding these mechanisms will refine approaches to telomerase inhibition in cancer.

Telomeres protect chromosome ends and distinguish them from DNA breaks. In humans, the telomeric complex consists of 5–10 kb of TAAGGG repeats bound to specialized telomere-binding proteins. Telomerase synthesizes telomeric repeats onto chromosomes to maintain telomere length (Greider and Blackburn 1985). Telomerase contains a catalytic protein component hTERT and an intrinsic RNA component hTR (Greider and Blackburn 1987; Feng et al. 1995; Nakamura et al. 1997). The RNA contains a small region that serves as a template for telomere repeat addition (Greider and Blackburn 1989). In the absence of telomerase, telomeres shorten because of the end replication problem (reviewed in Greider 1996). Critically short telomeres activate a DNA-damage response that can lead to cell cycle arrest or apoptosis (Lee et al. 1998; Hemann et al. 2001a,b; d’Adda di Fagagna et al. 2003; Hao et al. 2004). Cancers appear to have an unlimited replicative capacity and must develop mechanisms of maintaining telomere function. The telomere hypothesis for cancer proposes that normal somatic cells lack telomerase; however, in cancer cells, telomerase is reactivated and can compensate for telomere loss as cancer cells proliferate (Harley et al. 1990). Here, we reexamine the telomere hypothesis in view of accumulating evidence that the genetic events which lead to some cancers take place, not in somatic cells, but in primitive cells that have the capacity to self-renew and differentiate (Reya et al. 2003; Taipale and Beachy 2001). The fact that telomerase is already present in stem cells and need not be reactivated has implications for the ongoing pursuit of anti-telomerase therapies in cancer.

THE TELOMERE HYPOTHESIS

The telomere hypothesis was first articulated in the 1990s and was based on the fact that telomerase is active in germline cells, not readily detectable in somatic cells, and must be reactivated in cancer. This idea was based on multiple observations in human cells. First, in primary human fibroblasts, which appear to lack telomerase activity, telomeres shorten in vitro and in vivo with aging (Harley et al. 1990). Telomere shortening eventually leads to senescence, which can be bypassed by expressing hTERT, the protein component of telomerase (Bodnar et al. 1998). Second, transformation of primary cells with SV40 bypasses senescence and leads to further telomere shortening (Counter et al. 1992). The immortal cells that then arise after crisis have activated telomerase and can maintain telomere length. Unlike in primary fibroblasts, telomerase activity is readily detectable in germ cells and hematopoietic stem cells (Kim et al. 1994). The majority of cancers also have telomerase activity (Kim et al. 1994). On the basis of this evidence, it was hypothesized that cancers, presumably arising as clonal populations from somatic cells, activate telomerase, and those clones where it is active have a selective advantage in that they can divide indefinitely. In this model, telomere shortening occurs initially in the absence of telomerase and provides an environment of genomic instability, which then leads to the activation of oncogenes, silencing of tumor suppressors, and reactivation of telomerase (Hackett and Greider 2002; Feldser et al. 2003).

STEM CELLS AND CANCER

In recent years, interest in cancer as a disorder of stem cells has emerged. The stem cell theory of cancer holds that the initial events which lead to cancer occur in primitive or stem cells. By definition, these cells divide over long periods of time and are more likely to accumulate genetic defects that can lead to neoplasia. In addition, similar to cancer, stem cells possess the capacity to both self-renew and differentiate (Reya et al. 2001; Taipale and Beachy 2001). The idea gained support when it became apparent that in acute myelogenous leukemia, only rare cells occurring at a frequency of 1–100 in 10^4, and not blasts, are capable of inducing leukemia in immunodeficient mice (Lapidot et al. 1994; Guenechea et al. 1996).
In solid tumors, similar findings have established the likely presence of a cancer stem cell in breast cancer and glioblastoma multiforme (Al-Hajj et al. 2003; Singh et al. 2004). These cancers consist of heterogeneous populations of cells with only a minority capable of sustaining tumor growth. These subpopulations of cells may represent expansions of tissue-specific progenitors (Pardal et al. 2003). Additionally, many cancers show constitutive activation of pathways that maintain stem cell identity and number. For example, up-regulated hedgehog pathway activity has been documented in upper gastrointestinal cancers, small cell lung cancer, and prostate cancer (Herman et al. 2003; Watkins et al. 2003; Karlaudkar et al. 2004). In colorectal cancers, mutations in either APC or β-catenin lead to aberrant Wnt pathway activity (Morin et al. 1997). Current research is aimed at isolating and identifying the genetic determinants of cancer stem cells with the hope of designing targeted therapies that can lead to durable clinical responses.

The cancer stem cell theory is intrinsically at odds with a model of telomerase activation in cancer. Indeed, stem cells normally express telomerase, and tumors that arise from stem cells would already have telomerase and it need not be reactivated. If this is so, then how do we reconcile the fact that telomere shortening occurs in most tumors? Short telomeres appear to be present in early neoplastic lesions (Meeker et al. 2004). For example, in prostatic intraepithelial neoplastic lesions, telomeres are short compared with neighboring benign prostatic epithelium in the same patient (van Heek et al. 2002). This is surprising, since these lesions are relatively static and do not have high mitotic rates. Advanced prostate cancers also have short telomeres, implying that there may be little change in telomere length between early and late lesions despite the higher mitotic rates in advanced neoplastic lesions (Meeker et al. 2004). If neoplastic lesions represent genetic alterations in stem cells that are telomere-positive, why are the telomeres short? New insights come from the rare inherited syndrome, autosomal dominant dyskeratosis congenita, where telomere shortening occurs despite the presence of telomerase and leads to stem cell failure.

STEM CELL FAILURE IN SYNDROMES OF TELOMERE SHORTENING

Dyskeratosis congenita is a rare hereditary syndrome initially described on the basis of a triad of mucocutaneous features: skin hyperpigmentation, oral leukoplakia, and nail dystrophy (Dokal and Vulliamy 2003). The main cause of morbidity is aplastic anemia, a failure of hematopoiesis due to the loss of bone marrow stem cells. In recent years, germ-line mutations in both hTR and hTERT have been identified in subsets of patients with apparently sporadic aplastic anemia (Vulliamy et al. 2001; Armanios et al. 2005). Affected individuals develop idiopathic pulmonary fibrosis, liver fibrosis, hypopigmentation, and premature graying, which characterize this disease complex. Families with autosomal dominant dyskeratosis congenita display genetic anticipation, an earlier and more severe onset of phenotypes with successive generations. This anticipation correlates with telomere shortening, as was initially noted in the telomerase knockout mouse (Blasco et al. 1997; Vulliamy et al. 2004). The presence of heterogeneous mutations in autosomal dominant dyskeratosis congenita and aplastic anemia patients suggests that haplinsufficiency of telomerase underlies the loss of stem cells.

DYSKERATOSIS CONGENITA IS A CANCER-PREDISPOSING SYNDROME

Both aplastic anemia and dyskeratosis congenita patients have an increased predisposition to malignancies, which occur in 10% of cases (Dokal 2000). The cancers that arise are generally limited to tissues of high turnover where stem cell failure is also present. For example, aplastic anemia patients are predisposed to developing acute myelogenous leukemia and myelodysplasia. In dyskeratosis congenita, there is an increased incidence of squamous cell carcinomas of the skin and upper aerodigestive tract, which arise where hyperpigmentation and oral leukoplakia also appear. Taken together, these observations imply that in humans, telomere shortening preferentially leads to stem cell loss, which is most prominent in tissues of high turnover: the skin and bone marrow. Despite the limited number of remaining stem cells, genomic instability may occur and can lead to tumor initiation.

We recently described a mouse model of autosomal dominant dyskeratosis congenita on a wild-derived C57/B6J background that has telomere lengths similar to those of humans (Hao et al. 2005). The cancers that occur in 10% of cases (Dokal 2000). The cancers that arise are generally limited to tissues of high turnover where stem cell failure is also present. For example, aplastic anemia patients are predisposed to developing acute myelogenous leukemia and myelodysplasia. In dyskeratosis congenita, there is an increased incidence of squamous cell carcinomas of the skin and upper aerodigestive tract, which arise where hyperpigmentation and oral leukoplakia also appear. Taken together, these observations imply that in humans, telomere shortening preferentially leads to stem cell loss, which is most prominent in tissues of high turnover: the skin and bone marrow. Despite the limited number of remaining stem cells, genomic instability may occur and can lead to tumor initiation.

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TELOMERE SHORTENING IN THE PRESENCE OF TELOMERASE

Since autosomal dominant dyskeratosis congenita patients are heterozygous for telomerase, we examined successive generations of CAST/EiJ mTR−/− mice. These mice developed progressive telo-mere shortening in later generations, which correlated with worsening cytopenias and germ cell loss (Hao et al. 2005). The evidence for haplinsufficiency of telomerase in the CAST/EiJ mTR−/− mouse, along with evidence of hTERT haplinsufficiency in patients with autosomal dominant dyskeratosis congenita.
dykeratosis congenita and aplastic anemia, implies that telomere maintenance in stem cells is exquisitely sensitive to the level of telomerase. Armanios et al. 2005; Hao et al. 2005; Ly et al. 2005; Yamaguchi et al. 2005. Despite the presence of half the dose of telomerase, telomeres shorten, and defects in tissue renewal arise because of stem cell depletion. The fact that telomere shortening can be seen in cells that express telomerase supports the concept that cancers which arise from stem cells may show extensive telomere shortening even though telomerase is present.

TELOMERE ACTIVATION OR TELOMERE DETECTION?

The telomere hypothesis for cancer suggests that most tumors have short telomeres because they arise from telomerase-negative cells, and that telomerase is activated during tumorigenesis. Here, we suggest that tumors may arise from telomerase-positive stem cells. If telomerase is present in stem cells that give rise to a tumor, rather than being activated later, how then do we account for the absence of telomerase activity in the majority of precursor lesions (Blasco et al. 1996)?

There are at least two explanations for this. First, telomerase activity may be present in a few cells within the bulk of a premalignant lesion but may escape detection by conventional methods. As the number of cells that are telomerase-positive within a tumor expands, the level of telomerase activity detected will increase but may only reflect differences in sampling within a tumor, rather than an "off-to-on" phenomenon. Second, the level of telomerase per cell may increase as tumors progress from premalignant to malignant states. Telomerase activity is usually measured by the telomere repeat amplification protocol assay, which is not ideal for precise measurement. The lack of reliable in situ assays for telomerase activity does not allow quantitation of activity at the cellular level. One study using an in situ assay for TERT expression showed low mRNA levels in premalignant lesions, and there was an increase in both the amount of TERT per cell and the total number of cells expressing the transcript during tumor progression (Kolquist et al. 1998). This supports the idea that both an increase in level and an increase in the number of cells expressing telomerase may occur during tumorigenesis. As methods to identify cancer stem cells improve, along with better cell-based assays of enzyme activity, these hypotheses can be further tested.

TELOMERE LEVELS IN CANCER STEM CELLS

As we reconsider the telomere hypothesis in light of the presence of cancer stem cells, we propose that altered telomerase levels may be integral to events of tumorigenesis. The stem cells from which tumors are derived have telomerase, but telomere shortening still occurs, giving rise to premalignant lesions that have short telomeres. As cells accumulate mutations that activate oncogenes or inactivate tumor suppressors, expression of hTERT, which is generally tightly controlled, can be deregulated and can lead to an increase in telomerase activity (Lin and Elledge 2003). Increased telomerase will then allow maintenance or even lengthening of telomeres in tumors. There is also evidence that localization of telomerase components within the nucleus is altered in cancer cells and may lead to more efficient assembly of telomerase components and, thus, increased enzyme activity (Zhu et al. 2004). Further support for the idea that increased levels of telomerase can prevent telomere shortening comes from transgenic models where TERT overexpression stabilizes telomere length in hematopoietic stem cells (Allsopp et al. 2003). Thus, an up-regulation of telomerase activity to levels at which telomeres can be maintained in cancer stem cells may be an essential step in tumorigenesis.

SUMMARY

In summary, telomerase is critical for the integrity of stem cell compartments. In autosomal dominant dyskeratosis congenita, where half the dose of telomerase is limiting, stem cell failure predominates. However, despite the presence of telomerase, telomeres shorten and likely contribute to genomic instability that leads to tumor initiation. The evidence that many cancers originate in tissue-specific stem cells contradicts the original telomere hypothesis which assumes a somatic cell origin for cancer. Here, we propose that in cancer stem cells, telomerase rather than being reactivated, is more efficient at telomere maintenance. A better understanding of these molecular events will help refine approaches to targeting telomerase in cancer stem cells.

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M. ARMANIOS and C.W. GREIDER

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