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Review

Telomerase activity is not enough for tumor initiation in human cells

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Studies have reported that the telomerase could be detected in a majority of human tumor tissues, but not in most normal tissues. In tumorigenesis, the activation of telomerase seems to be an important step for somatic cells to gain the ability of indefinite proliferation and become immortal by a way of maintaining telomere length. In clinical, telomerase activity is correlated with outcomes of patients with different types of tumor. Research data have also indicated that the inhibition or absence of telomerase activity may result in cell crisis of cancer and tumor regression. However, several reports have demonstrated a telomerase-negative status in some immortalized human cells and the absence of telomerase activity in certain kind of human tumors. Additionally, previous studies have found that the expression of the human telomerase catalytic component, hTERT, in normal human somatic cells can reconstitute telomerase activity, but does not appear to induce phenotypic changes related to malignant transformation, to explain these inconsistencies, we hypothesize that telomerase activity is not enough for tumorigenesis. Multiple mutations are required for cells to acquire malignant characteristics and telomerase should be viewed as a part of multistep tumor development process. For better outcomes of malignant tumor treatment, ongoing studies should also consider about telomerase-independent mechanism in tumorigenesis.

Key words: Telomere, telomerase, somatic cells, tumorigenesis.

INTRODUCTION

Telomeres, which are specialized structures consisting of TTAGGG nucleotide repeats, are essential for genomic stability (Golubovskaya et al., 1999). With each cell division, loss of telomere repeats from chromosome ends averages 50 - 200 bp (Allsopp et al., 1992; Counter et al., 1992). After progressive shortening, the telomere length reaches a critical threshold so that cells stop dividing and enter growth arrest (Counter et al., 1992; Harley et al., 1990). In most organisms, telomere shortening can be prevented by activation of telomerase, a ribonucleoprotein that could add TTAGGG sequences to the ends of the chromosomes by using an RNA molecule as a template (Chan and Blackburn, 2004).

Except for embryonic and hemopoietic cells, most human somatic cells were telomerase-negative, therefore, they would lose telomeric DNA during each round of DNA replication and finally doom to death. However, compared to normal somatic cells, telomerase is activated in most human tumor cells (Kim et al., 1994), which often exhibit an apparently unlimited proliferation potential and thus are termed immortalized. For example, it has been demonstrated that telomerase activity exists in 80% of lung cancers (Hiyama et al., 1995), 84% of prostate cancers (Sommerfeld et al., 1996), 85% of liver cancers (Tahara et al., 1995), 93% of breast cancers (Hiyama et al., 1996), 72% of astrocytic gliomas (Le et al., 1998), 94% of neuroblastomas (Hiyama et al., 1995), 95% of colorectal cancers (Tahara et al., 1995), and 98% of bladder cancers (Kyo et al., 1997). The measurement of

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telomere length and telomerase activity shows significant clinical values when used as potential diagnosis markers for most malignant tumors (Boultwood et al., 1999; Brummendorf et al., 2000; Bechter et al., 1998). Also, antitumor therapy is practiced by inhibiting the expression of hTERT (Zhang et al., 1999) or binding of TRF2 (Karlseder et al., 1999) to telomere. These observations have led to the notion that activation of telomerase may be required as a critical step in the process of tumorigenesis.

Previous studies have also reported that the expression of telomerase catalytic component (hTERT) in normal human somatic cells could reconstitute telomerase activity, but does not appear to induce phenotypic changes related to malignant transformation (Xu-Rong Jiang et al., 1999; Burger et al., 1998), that telomere shortening is a common but not must-be phenomenon in tumors originated from soft-tissue (Schneider-Stock et al., 1998), that telomerase inhibitors used in tumor therapy could not work well on hematological malignancies (Sen et al., 1999) and that eliminating telomerase in mice has no effect on telomere length or tumor formation for many generations (Chiang et al., 2006). In addition, cancer development could also occur in $mTR^{-/-}$ mouse cells that lack telomerase activity (Blasco et al., 1997). These results imply that other telomere maintenance mechanism can substitute for telomerase.

To well understand the role of telomerase in tumorigenesis, we hypothesize that telomerase activity is not required for tumorigenesis in human somatic cells and the per se effector is the length of telomere that can also be elongated by other mechanism, suggesting that anti-tumor therapy should be mainly focused on the development of telomere-based approaches.

LINKS BETWEEN TELOMERE LENGTH, TELOMERASE AND TUMOR

When the length of a small part of telomeres are adequately reduced to a critical threshold, cells stop to further divide and replicative senescence (M1 stage) occurs (Bodnar et al., 1998; Greider, 1998; Wright and Shay, 2001). Then, cell division is blocked by checkpoint signaling factors associated with both acute and chronic DNA damage, such as p53 and $p16^{INK4a}$/pRB. If these cell-cycle checkpoint factors are damaged by mutations or viral oncogene expression, cells would bypass M1 senescence and telomeres would continue to shorten, eventually resulting in cell crisis. Crisis (M2 stage) is characterized by many 'uncapped' chromosome ends, where end-to-end fusions occur. Furthermore, chromosome breakage-fusion cycles result in mitotic catastrophe and cell apoptosis.

Multiple mutations are required for cells to acquire malignant characteristics. As most mutations are recessive, the original mutant cell must divide enough times for an additional clonal expansion to the remaining wild-type alleles (usually through loss of heterozygosity) and then many more times to reach a population size large enough to have a reasonable probability of incurring the next oncogenic mutation. If the times of available cell division are limited by cellular senescence, premalignant cells will eventually be prevented from continuing to divide after accumulating only a few mutations and thus tumor progression is inhibited (Shay and Roninson, 2004; Campisi, 2003). Obviously, the most efficient strategy to prevent tumor initiation would be to have few or no available cell divisions. Within this theory, escaping from normal cellular senescence because of alteration of telomerase activity and then becoming immortal constitutes an additional step in oncogenesis, which are required for ongoing proliferation in most tumor cells (Wright and Shay, 2001).

HYPOTHESIS

Is telomerase critical for tumorigenesis? Although human somatic cells can spontaneously become immortalized and telomerase activity can be detected in many tumor cells, we suggest telomerase alone is not enough for tumorigenesis or tumor initiation. To further understand the mechanism of tumor formation, the future works should be focused on other initiating factors rather than telomerase.

THE RATIONALE FOR TUMORIGENESIS INDEPENDENT OF TELOMERASE ACTIVITY

Telomerase activity does not seem to be an absolute requirement for *in vitro* or *in vivo* tumor growth, as shown in tumor cells derived from telomerase-deficient mice (Rudolph et al., 1999) and some reports of immortalized telomerase-negative human cells (Bryan et al., 1995; Whitaker et al., 1995). In addition, there is considerable evidence that cellular senescence, which is considered as anti-tumor mechanism, can occur through other mechanisms rather than telomere dysfunction (Itahana et al., 2004; Belair et al., 1997). Previous studies also showed that enhanced telomeric recombination in human cells with mismatch-repair defects might contribute to telomerase-independent immortalization of cells and consequent tumorigenesis (Rizki and Lundblad, 2001). These observations provide direct evidence for the hypothesis that telomere length, not telomerase, determines the proliferative capacity of cells.

Ectopic expression of telomerase alone has not resulted in increased growth rate, cytogenetic abnormalities and p53 and pRb-mediated cell cycle changes (Morales et al., 1999). Cells with force-expressed telomerase maintain normal chromosome complements for a considerable period and continue to normally grow

(Morales et al., 1999). However, mutations in p53 gene and the acquirement of multiple LOH are specific for the malignant phenotype (McCluskey and Dubeau, 1997). Recent data suggest that the severe chromosomal instability of telomere crisis promotes secondary genetic changes that facilitate tumorigenesis and that stabilization of telomere ends allows the continuous tumor growth (Cosme-Blanco and Chang, 2008).

Most malignant tumor tissues have shorter telomeres than the original tissue, and telomerase activation is detected in late-stage of tumor (Blasco et al., 1996; Mutirangura et al., 1996). These phenomenons indicate that telomerase is activated by some mechanisms after tumor formation already happened.

DISCUSSION

Our hypothesis has been partly confirmed by works on telomerase activity in tumorigenesis. Previous studies have showed that there is another regulator of growth arrest in cultured fibroblasts, ARF/p53 pathway, which is frequently inactivated in human cancers (Carnero et al., 2000). In mammal cells, p53 plays same role like telomerase in maintenance of genomic integrity. Wu X et al. (1999) found that p53 protein overexpression might be common in individuals genetically susceptible to carcinogen exposure and p53 status might be related to telomerase expression. Established data indicates that $p16^{Ink4\alpha}$, along with $p19^{Arf}$, functions as a tumour suppressor in mice (Sharpless et al., 2001). In addition, using both normal and tumorous human uroepithelial tissues, Belair et al. (1997) demonstrated that telomerase activity is a marker of cell proliferation, not malignant transformation.

The expression of hTERT immortalized normal human urothelial cells (NHUC); however, the expression of a modified hTERT without the ability to act in telomere maintenance did not immortalize NHUC, confirming that effects on telomeres are required for urothelial immortalization (Chapman et al., 2008). More interestingly, although hTERT expression has been detected in different human malignancies with a poor prognosis, a low expression of hTERT at mRNA levels was found to be associated with a worse prognosis in pancreatic ductal adenocarcinoma, whereas undetectable expression of this molecule showed an intermediate risk of tumor-related death (Grochola et al., 2008).

In another study, Deng et al. (2007) demonstrated the potential of enhancer-binding protein-2beta (AP-2beta) as a novel tumor marker or a cancer therapeutic target. AP-2beta that specifically binds and activates the hTERT promoter was activated in human lung cancer cells rather than in normal cells. The hTERT promoter has been shown to promote hTERT gene expression selectively in tumor cells but not in normal cells.

All data indicate that complete understanding on the role of telomerase in tumorigenesis through well-designed clinical studies will have a significant clinical impact on the treatment and diagnosis of malignant tumors. Ongoing studies that were aimed at elucidating the roles of telomerase in tumor therapy should also consider about telomerase-independent mechanism in maintenance of telomere length, thus allow for the establishment of strategies to improve treatment of clinical malignancies.

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