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TELOMERASE ACTIVITY AND TELOMERIC STATES IN CELL PROLIFERATIVE AND DIFFERENTIATIVE MECHANISMS.

L'ATTIVITÀ TELOMERASICA E GLI STATI DEI TELOMERI NEI MECCANISMI DI PROLIFERAZIONE E DIFFERENZIAMENTO CELLULARE.

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REVIEW

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Abstract.
Telomeres are DNA-protein complexes playing an important role in the maintenance of genome integrity. Telomerase is the enzyme acting as a template for addition of new telomeric repeats; this addition is essential for those cellular populations that have proliferative and differentiative potential.
Telomerase and associated proteins are essential in response to DNA damage. Moreover, telomere-associated proteins as TRF2 are involved in all signalling transduction pathway which drive cellular proliferation and differentiation. In somatic cells, shortening of telomeres contributes to the onset of senescence or apoptosis; tissues which require cellular renewal express telomerase activity in order to compensate telomeric shortening. Telomerase activity remains detectable in adult stem cells populations and in cells with high proliferative potential. Reactivation of telomerase activity may be a pathogenetic characteristic of neoplastic cellular population favouring tumoral proliferation and progression.

KEYWORDS: telomerase, telomere, stem cells, apoptosis, se-

Riassunto.
I telomeri sono complessi DNA-proteine che giocano un ruolo importante nel mantenimento dell'integrità genomica. La telomerasi è l'enzima che agisce da templato per l'addizione di unità ripetitive telomeriche; quest'addizione è essenziale per il mantenimento del potenziale proliferativo di diverse popolazioni cellulari. La telomerasi e le proteine associate risultano fondamentali nella risposta al danno genomico. Inoltre proteine associate ai telomeri come TRF2 sono coinvolte in tutti i pathway di trasduzione del segnale che dirigono la proliferazione e il differenziamento cellulare. Nelle cellule somatiche l'accorciamento dei telomeri contribuisce all'insorgenza di patologie legate alla senescenza e all'apoptosi; i tessuti che richiedono un rinnovamento cellulare esprimono l'attività telomerasica che compensa il progressivo accorciamento telomerico. L'attività telomerasica rimane rilevabile nelle popolazioni staminali adulte e nelle cellule che hanno un alto potenziale proliferativo. La riattivazione dell'attività telomerasica può anche essere una caratteristica patogenetica di popolazioni cellulari neoplastiche, favorendo la proliferazione e progressione tumorale.

PAROLE CHIAVE: telomerasi, telomere, cellule staminali, apoptosi, senescenza, cellule tumorali.

Introduction to the telomere biology

Telomeres are DNA-protein complexes located at the ends of eukaryotic chromosomes that play an important role in maintaining the integrity of the genome. They are characterized by guanine-rich repetitive DNA and associated proteins. During cell division, telomeric DNA (30-100 base pairs) is lost because of the end-replication problem. Therefore, when normal cells reach a critical telomere length, they way out the cell cycle, enter M2 (mortality stage 2) crisis and undergo senescence (1). This phenomenon renders telomere length as a mitotic clock for cellular life span.

Highly dynamic telomeres play a critical role in the maintenance of chromosomal integrity, their length as well as their dynamics are heterogeneous in human cellular populations, but every cell division is accompanied by a loss of 30-100 base pairs of telomere repeats.

Telomerase is a ribonucleoprotein reverse transcriptase. It has a RNA component (human telomerase RNA component) called hTERC (gene t*TERT*, 5p15.33) which acts as a template for addition of new telomeric repeats, and a catalytic subunit (human telomerase reverse transcriptase) called hTERT (gene h*TERC*, 3q26). Many cellular populations (as germ and stem cells) continue dividing throughout their life, therefore requiring the addition of new telomeres to their chromosomes to replace sequences lost during cell divisions (2).

Each chromosome needs to be capped at its ends by a minimum number of telomere repeats to prevent activation of a DNA damage response, leading to genome instability or cell death. This capped structure consists of a T-loop, in which the single-stranded 3'-end of the chromosome folds back into double-stranded telomere repeats (3).

In normal somatic cells, mean telomere length is 10 kilobases (kb), stem cells of tissues undergoing cyclic renewal have an average telomere length of 12 kb, while germ cells and cells from fetal tissues have an average length of 15-20 kb and maintain their telomeres; cancer cells have a mean telomere length of about 5 kb (range 2-9kb) (4).

Mechanisms of telomeric ends maintenance in cellular physiology and pathology

Cancer cells, which have acquired a high proliferative potential, go beyond replicative senescence by activating the enzyme telomerase, which reactivates synthesis of TTAGGG hexanucleotide repeats onto shortened telomeres, hence stabilizing their length.

All human tumor cell lines and 85% of human cancer tissues have been shown to possess own telomerase activity; by contrast normal tissues nearby tumor and human somatic cells, other than stem cells, do not possess detectable levels of telomerase. Accordingly, tumor growth depends on the acquisition of the immortal phenotype by the malignant cells (2).

Telomere protection depends on several factors, including the precise composition of telomere-associated proteins, the level of telomerase activity and telomere length itself. When the cells have long telomeres, telomerase activity is not requested, but when the cells reach a critically short telomere length, restoration of the telomerase activity becomes essential to avoid chromosomal end-to-end fusions, replicative senescence and apoptosis (5).

The synthesis of telomeric DNA requires the activity of specialized telomere-associated proteins (TRF1 and TRF2, telomeric binding factors 1 and 2) together with the telomerase activity compensates telomere shortening, stabilizing chromosomal length. In contrast to somatic cells, germ, stem and tumor cells, as well as immortalized cell lines, maintain high levels of telomerase activity. As a result, these cells have longer telomeres and preserve a relative proliferative potential. Telomerase-dependent transcriptional regulation of genes involved in cell growth has been recently suggested as an additional mechanism by which telomerase promotes cell proliferation independently of telomere length maintenance (6).

Regardless of transcriptional regulation of hTERT expression, TERT and TRF2 have a key role in response to genomic damage in differentiation and maturation processes. So telomere associated proteins, like TRF2, are involved in transduction pathways that direct cellular proliferation and differentiation. As a general rule, we observe greater level of telomerase activity during embryonic development and low or undetectable levels soon after birth (7).

Human telomerase activity (hTERT) remains detectable in adult stem cell populations, and in cells with high proliferative potential, such as activated lymphocytes. TRF2 binds directly to double-stranded telomeric DNA and facilitates T-loop formation, thus avoiding cellular senescence and apoptosis by protecting telomeres and/or inhibiting telomere-associated DNA damage response pathways.

Telomere damage can trigger apoptosis by activating a DNA damage response pathway involving ATM and p53, with subsequent mitochondrial changes resulting in the release of cytochrome C and caspase activation; apoptosis related to telomere damage may or may not require release of cytochrome C from mitochondria and caspase activation. An interesting study showed that newly generated neurons

(NGNs) are highly sensitive to telomere damage compared with neural progenitor cells (NPCs) and mature neurons (MNs). While the overexpression of TRF2 in NGNs suppressed DNA damage response and protected NGNs against apoptosis, knock-out of TERT sensitized NPCs to DNA damage. This study suggested that telomere damage-induced cell death of NGNs require PARP1 activation but is independent of caspase activation (8). The upregulation of TRF2 during brain development indicates that there is an evolution of telomere structures from a dynamic state (NPC stage) to a stable state and TRF-dependent T-loop structure in postmitotic neurons, indicating TRF2 as an important protein involved in cell differentiation and survival.

In telomere checkpoint response, p53, a tumor suppressor, has a key place. For example it has been shown that p53 inactivation enhances survival of cells with short and dysfunctional telomeres leading to neoplastic process (9). Moreover, p53 deficiency accelerates tumorigenesis in vivo (10). In human colon and pancreatic cancer it has been observed that genetic instability during carcinogenesis should be linked to telomere dysfunction (as shortening) as early event in tumorigenesis (11). Therefore, telomere length can be used as a predictor of benign-to-malignant progression, and its use as a diagnostic marker for cancer diagnosis or prognosis has been proposed (12). In support of this evidence there is the finding that shortened telomeric DNA results in nonhomologous end joining of telomeric DNA, leading to loss of telomere function and genomic instability (13).

Telomere shortening in somatic cells is thought to contribute to aging and associated disorders, therefore when normal tissue function maintenance requires cellular renewal, telomerase allows cells overcoming one of the fundamental limitations to mammalian cell immortality, the progressive loss of telomeric DNA (4).

Many studies have shown that telomere length of peripheral blood lymphocytes (PBL) is linked with the risk of developing atherosclerosis, premature myocardial infarctions and coronary disease. Further evidences link telomere states with Alzheimer's disease progression and with overall mortality. In particular, for neoplastic diseases, this progressive shortening appear statistically significantly associated with an increased risk to develop human carcinomas of the head, neck, kidney, bladder, lung and renal cells (14).

There are other possibilities by which telomere length influence tumorigenesis, since further shortening processes, genetic differences in telomere degradation kinetics or in cell turnover should be connected with greater risk for cancer. Environmental stresses (as smoking) may accelerate likely the shortening process and finally immune-cells senescence derived from shortened telomeres should lead to decreased immune surveillance of emerging cancer (15).

DNA damage in the form of telomere shortening can be linked to either apoptosis or senescence, depending on a functional p53-dependent DNA arrest pathway; a relation between telomerase and apoptosis is clear, in the sense of nuclear fragmentation events observed in apoptosis influenced by telomerase, however telomerase activation itself is important in preventing, for example, neoplastic transformation of large vessel human endothelial cells (16).

Telomeres and stem cells

Organism homeostasis requires for several organs a continuous process of cellular loss and renewal. When cellular loss exceeds proliferation, there is a decline and failure in organ function. This process leads to progressive functional compromision that clinically means frailty, accelerated aging and death. The maintenance of a proliferative cell reservoir, namely adult stem cells, allows tissue renewal and organ function.

Since stem cells have extended proliferative capacity, they should bear a mechanism that preserves telomere length for many cell divisions. Multiple evidences showed low but detectable levels of telomerase activity in human adult stem cells, haematopoietic (HSC) and non-haematopoietic stem cells (NHSC), such as neuronal, skin, intestinal crypt, mammary epithelial, pancreas, adrenal cortex, kidney and mesenchymal stem cells (17) and consequently the telomeres of these cell types slowly shorten.

Human mesenchymal stem cells (MSCs), as example of NHSC, can replicate around 40-50 population doublings and they don't exhibit high level of telomerase activity, but they have significant proliferative and differentiative ability even at early passages. Moreover overexpression of telomerase, in hMSCs, result in the elongation of telomeres, so the maintenance of a minimum level of telomerase activity preserves regenerative capacity and differentiation potential. MSCs show a telomere profile similar to lymphocytes, they can be grown until senescence (18), but most importantly they have a low degree of random fluctuation in the telomere dynamics, and in addition telomerase activity, while elongating telomeres, maintains a telomere profile similar to that of lymphocytes (19).There are many characteristics of stem cells that could diminish telomere dynamics during proliferation, as asymmetric cell division

and immortal strand segregation, telomere elongation by regulation of telomerase activity and furthermore alternative lengthening pathway (ALT) based on replicative mechanisms or both the last two.

Embryonic stem cells are likely to be potentially immortal and capable of indefinite self-renewal, with the ability to differentiate and contribute to the germ line. Embryonic stem cells and undifferentiated embryonal carcinoma (EC) cell display high levels of telomerase activity and hTERT expression that are downregulated during differentiation (20); the maintenance of telomerase activity during differentiation of ESCs give several advantages like proliferation, resistance to apoptosis and oxidative stress, and enhanced differentiation by expansion of the progenitor cellular population.

On the other hand, the high proliferative rate of ESC should cause the formation of tumors in vivo, and tumorigenesis is one of the major non-ethical obstacles to ESC use in regenerative medicine.

Another point that should be focused is the possibility that somatic stem cells, during the adult life, should accumulate mutations, due for example to the prolonged exposition of the parental DNA strand to mutagenic injuries. Some of these mutations may target cancer relevant genes, and several literature reports suggested that cancers may arise from the malignant transformation of normal stem cells, generating the so-called "cancer stem cells" (21). When telomeric maintenance systems are involved in this transformation process, several evidences demonstrated that tissue stem cells with telomere dysfunction can react physiologically in two ways: first of all, cells can undergo apoptosis or enter in a senescence pathway. Otherwise, cells can maintain their proliferative activity with subsequent generation of genomic instability.

In most cases, the response is the progressive loss of stem cells, and accordingly loss of tissue regenerative ability and aging. Apoptotic loss of progenitor cells has been showed in some models, for example as an increase of apoptosis of germ cells of testes and crypt cells of the intestine in response to shortened and dysfunctional telomeres (22).

Conclusions

Telomeres and telomerase represent sophisticated cellular homeostatic systems, by which telomere length and integrity are tightly supervised. Telomere dysfunction may be one of the molecular causes of genetic instability because on the other side telomere function could be pictured as regulating and channelling the active and sensitive surveillance of DNA damage response, detecting a single break and modulating an appropriate cellular response. Shortening of telomeres is associated with telomerase activity for the reason that high telomerase activity results in increased proliferation of cells with DNA damage. Telomeric integrity is essential for the stability of chromosomes and so telomerase is a target for therapeutic approaches, with telomerase inhibitors for cancer treatment being developed (23). Research on telomere-telomerase dynamics in stem cells can show the way to the knowledge on therapies in ageing-related diseases and cancers, giving important cues for clinical transplantation approaches.

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