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Review

Cutaneous Melanoma and Telomerase

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Abstract

There are cells that suffer uncontrolled growth with loss of contact inhibition and alterations in the nucleus, affecting the maintenance of telomeres in the chromosomes, forming a tumor. Telomeres have a tendency to merge, making repairs of DNA impracticable, taking the loss of genetic information and producing chromosomal aberrations. In relation to telomeres, there is a protein known as telomerase, hyperactive in several neoplasms, such as melanoma. The integrity and stability of chromosomes is one of the key factors that must be maintained for good functioning and propagation of an organism. In this revision, only aspects related to melanoma, telomeres and telomerase are addressed, pointing to the contribution of genetic and mutagenic environmental factors for carcinogenic development.

Keywords: Melanoma; Telomerase; Genes; Skin Neoplasms.

Introduction

When it was first reported by Hippocrates in the fifth century B.C. as one of the neoplasms of poor prognosis, melanoma occupies third place in incidence among the malignant skin neoplasms worldwide, with embryologic origin in the neuroectodermal tissue, resulting from uncontrolled proliferation of melanocytes of unknown etiology.[1-4]

Associated with several types of neoplasms, there are cells that suffer uncontrolled growth with loss of contact inhibition and alterations in the nucleus, normally occurring in a single somatic cell, which is then divided and continues developing until forming a tumor, affecting the maintenance of the size of the telomere in the chromosome.[5-6] Described for the first time in 1930, telomeres are specialized structures located in the ends of linear eukaryotic chromosomes, consisting of nucleotide sequences (5´ -TTAGGG- 3´) repeated in tandem, whose principal function is to maintain the integrity and stability of the chromosomes, preventing senescence.[7-11]

In normal somatic cells, although compensatory restoration mechanisms occur, the telomeres are shortened in successive divisions, leading to senescence and suffering apoptosis. Possibly, they emit replicative senescence signals, being able to interfere in mechanisms involved with p53, also detected in melanomas.[2,11-15]

Cellular replication cannot be fully reproduced, with a gradual loss in the order of 100 to 150 nucleotide of the telomere occurring.[16]

This malfunctioning contributes to the reduction of cellular viability, altering differentiation and damaging

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response and regeneration. In the absence of a mechanism that maintains the telomeres, a progressive reduction to each replication leads to a compromising of cellular proliferation, aberrations and carcinogenesis.[17]

To circumvent this deficiency, telomerase is an enzyme responsible for the synthesis of the telomere. Telomerase, a holoenzyme that consists of several subunits, including human telomerase RNA (hTR), supplies a RNA template so that the fragment corresponding to a primer in the end 5' line is not degraded, avoiding the incident of fusions of the ends and guaranteeing the correct duplication in the terminal regions of the chromosomes, as they are necessary for the indefinite proliferation of human cells.[18-19]

Telomerase is a specialized reverse transcriptase which can be associated with cellular immortalization and cancer. It was described for the first time in 1985 by Greider and Blackburn; its activity is absent in most normal human somatic cells and present in almost all stem, immortal and germinative cell lines. It is also present in approximately 90% of human tumors, including the neoplasms of the skin such as melanoma.[2,8,18,20-26]

The present revision aims to summarize the current knowledge on the importance of cutaneous melanoma, telomeres, telomerase and its association with neoplastic processes.

Material and Methods

A bibliographic search of the electronic archives PubMed, Regional Library of Medicine (BIREME), Scientific Electronic Library Online (SCIELO) and National Institute of Cancer (INCA), in the period from 1969 to 2008, in the Portuguese and English language, was carried out using the keywords 'cutaneous melanoma', 'telomeres', 'telomerase' and 'genes'.

Melanoma

Melanoma is characterized as a type of cancer derived from melanocytes, from a less common neuroectodermal origin, with mortality reaching elevated levels.[1,12,27-28]

The tumor presents great metastatic capacity, even in initial phases, since invading and disseminating can be considered the innate prerogative characteristic of this cellular type. Most times it originates in the skin, although it can appear from mucous membranes or in other locations, such as leptomeninges and ocular globe.[29]

In Australia, the estimated risk for developing melanoma before 75 years of age is 1 in 27 for men and 1 in 36 for women.[5,27-28,30-31] In Brazil, a study based on anatomopathologic reports revealed that melanoma corresponds to 0.15% of all malignant neoplasms, and among malignant skin tumors, cutaneous melanoma responds to nearly 5% of the cases.[3-4,28,30,32-33]

As much in melanomas as in other neoplasms, evidence demonstrates that the process of malignant transformation involves several alterations and genetic predispositions, which modify the important cellular processes including proliferation, differentiation and programmed cell death, besides physical trauma in nevi previously existent and immunodeficient.[4,6,12,34-36]

In particular, the cycle of cellular division seems altered in malignant cells, especially in skin tumors that are frequently a consequence of DNA damage resulting among other factors, ultraviolet rays. Solar radiation is recognized as a complete human carcinogen. Among the skin cancers related to the exhibition to this radiation, the most serious is cutaneous melanoma, in virtue to its lethality.[6,12,34,36]

Studies in vitro using melanoma were described for the first time in 1914. However, the success of the culture of this neoplasm occurred only in 1966 by Brown and collaborators, demonstrating the difficulty in culture of this type of cell and the necessity to search for new techniques that facilitate its proliferation, and in this way, collaborate in studies that facilitated its understanding and possible cure.[37-38]

Telomeres and Telomerase

The interest for understanding the properties of telomeres began in the end of the 1930s with pioneer work of the North American geneticists Hermann Müller and Barbara McClintock16. This study had prior origins to a study carried out by the group of Oswald Avery, who later in 1944 identified DNA as hereditary material. Muller and McClintock defined the telomeres as functional structures that protect the chromosome terminals.[16,39]

The integrity and stability of chromosomes is one of the key factors that must be maintained for good functioning and propagation of an organism, as the telomeres have a tendency to merge, making repairs of the DNA impracticable, carrying the loss of genetic information and producing chromosomal aberrations.[39]

Cellular proliferation in a superior animal is not

governed simply by the cellular environment, but also depends on the history of the cell in the long term: each differentiated type of cell, to each stage of animal development, obey rules with small differences and reflects differences in the internal control of their machinery. Perhaps the simplest thing, but also the most mysterious example of effects in the long term on cellular division, is observed in the phenomenon of cellular senescence. A possible interpretation for cellular senescence is in the behavior of telomeres.[24]

Telomeres are complex nucleoproteic structures that form the physical ends of chromosomes, comprised of preserved and repetitive double-stranded DNA sequences followed by a guanine-rich single-strand end. They are treated as a substrate for telomerase and for telomeric proteins involved in the maintenance of telomeres, impeding phenomena as degradations of telomeric regions, fusions and loss of genetic information.[9,13,40-43]

Such specialized structures are comprised of tandem repetitions of nucleotide sequence TTAGGG. Their functions include the stabilization and protection of chromosomes against nuclease degradation and the prevention of aberrant recombination.[31,42,44-45]

After a determined number of cellular divisions, significant reductions occur in the size of telomeres, which results in chromosomal instability and cellular death.[42,44,46]

The ends of chromosomes are not replicated by normal action of polymerase DNA, but by a special mechanism that involves activity of a reverse transcriptase, which loads with its own RNA template, to complement the telomere repetitive sequences of DNA.[47-48]

Its adequate function is vital for cellular growth and segregation of the cell lines48. The progressive loss of telomeres causes senescence, so when there is great shortening of the telomere, the cell stops to be divided. Thus, the telomere is recognized as a "mitotic clock", being responsible for the cellular replicative capacity. However, germinative cells and stem cells escape from telomere shortening by telomerase expression.[42] The dysfunction of the telomeres contributes to the reduction of cellular viability, altering its differentiation and damaging its response and regeneration.[17] The problem of DNA replication in eukaryotes revolves around the fact that DNA is linear and not circular as prokaryotes and the enzymes responsible for the process are not able to finish the replication of chromosome terminals, leading to the gradual sequence loss of nucleotides that steadily compromise its chromosomal structure , replicating this, realized by several enzymes, of which the most important is DNA polymerase III, which synthesizes complementary strands of DNA from single-stranded DNA and of an initiator (primer), composed of RNA. This primer is formed by the enzyme RNA primase and contains normally from 8 to 12 nucleotides.[13]

The telomeric region is also responsible for the anchoring of the chromosomes to the nuclear membrane, guaranteeing the three-dimensional structure of the nucleus and the correct space distribution of the chromosomes, suggesting function in the chromosomal positioning for cellular replication.[14]

DNA polymerase is not able to replicate the terminal segment of the discontinued repetition strand of linear chromosomes. In the end of the strands that will be replicated in a discontinued mode (in all telomeres there will be one) there will not be a DNA template strand to allow the binding with a new primer after the last Okazaki fragment has the RNA primer removed.[18-19] The replication of the telomeres is facilitated by the sequence type of nucleotides present in this chromosomal region. The telomeres of the human chromosomes are composed of repetitions of a set of 6 nucleotides repeated in tandem, and telomerase recognizes the end of this sequence, binding with the DNA and extending the strand template in the direction of 5' for 3, adding a new TTAGGG repetition each time.

Telomerase is a ribonucleoprotein enzyme which synthesizes the repetitions in telomeres. Its activation allows the cells to acquire unrestricted proliferative capacity with cellular immortalization as consequence. This enzyme is activated in most tumor cells and inactivated in a great part of normal somatic cells.[46] It is treated as one enzyme with the sole characteristic of possessing a RNA strand in its interior that serves as a template for telomeric extension, containing a RNA component that synthesizes repetitions of telomeric DNA, compensating for the loss of telomeres during cellular division.[42]

In a way, this enzyme does a "reverse transcription" since, from the RNA template, it constructs a new DNA segment at the end of the chromosome.[18-19]

Most human somatic cells do not express telomerase activity, signaling a mechanism of limited replication, which protects against the development of a clonal tumor.[49]

The absence of telomerase in normal cells results in progressive erosion of the telomeres, leading to an incomplete replication, which causes chromosomal instability and shortening of the telomeres, and consequently, senescence.[42]

There is still the possibility of an increase in mutation, taking into account the factors that stimulate

tumor growth as one of the possible consequences.[42]

Without telomerase activity, the chromosomes become shorter with each replication cycle because of partial loss of the telomeric region. With time, the telomeres are entirely lost and the deletions start to take place on encoded regions. This progressive shortening of the chromosomes is considered one of the factors that limits or makes the continuous cellular division impossible and is probably associated to the normal aging process of cells, tissues, and consequently, the organism as a whole.[18-19]

Proto-oncogenes and Gene Suppressors

Associated with telomerase is the activation of genes and proteins, as the p53 gene, a known tumor suppressor gene, a transcription factor that controls the integrity of the cellular genome acting in the DNA repair. Cells that lose p53 activity become vulnerable to malignant transformation, since accumulated chromosomal damage results in the exhibition of harmful agents, as radiation, drugs or other stress. The mutation of p53 is considered a late event in tumor progression.[50]

The genes are composed by molecules of DNA in the cellular nucleus. They specify sequences of amino acids that must bind one to another to form a protein, which will carry out the biological effect of the gene. When a gene is activated, the cell responds, synthesizing an encoded protein. Mutations in a gene can disturb the cell, altering the quantity of protein or the activity of the synthesized protein.[51-52]

Two classes of genes have key roles in the development of cancer, and in their normal configurations, they direct the cellular cycle in an intricate sequence of events, for which the cells grow and are divided. The proto-oncogenes stimulate, while gene suppressors inhibit the processes of cellular division. Collectively, these two classes of genes are related with uncontrolled cellular proliferation found in human cancers.[53-54]

When mutations occur, proto-oncogenes become oncogenes, which are carcinogenic and cause excessive cellular multiplication. These mutations carry the proto-oncogene to express in excess of its growth stimulator protein. The tumor gene suppressors, in contrast, contribute to cancer development when they are inactivated by mutations. The result is the loss of the gene suppressor functional action, which deprives the cell of crucial controls for the inhibition of inappropriate growth.[55-56]

Relation Between Telomerase and Melanoma

Telomerase is abundant in embryonic cells but is absent in most adult somatic tissues. However, in tumor cells there is a frank activity of its expression.[57]

The knowledge of the regulation mechanisms of this enzyme in humans is extremely important for a better understanding of the processes that lead to tumorigenesis.[10,26]

In pioneer studies carried out by Morin,[58] telomerase was observed for the first time in human tumor cells, which has contributed to the increase of studies with focus on this enzyme in several neoplastic tissues such as colon, breast and skin, among others, besides research directed to pre-established cell lines.[38,59-61]

Strong evidence indicates association between telomeres and the development of the majority of diverse types of cancer in humans, including skin neoplasms such as melanoma.[6,62]

Zamolo et al.[63] proposed that the lengthening of the telomere is one of the first events that lead to the process of transformation and immortalization of melanoma cutaneous cells.

Gagos et al.[64] reported that the maintenance of length of telomeres is a key factor for the expression of the telomerase enzyme in melanoma and that its activity can be related to the aggressiveness of the tumor.

Studies carried out by Villa et al.[65] in several samples of melanomas detected the activity of this enzyme; however, they did not indicate its association between cellular differentiation processes.

The development of cutaneous melanoma is associated with a gradual increase of telomerase activity during the transformation of isolated nevi in metastasis, with an increase of practically all the cell lines.[66]

The activity levels of this enzyme and RNA establish a simultaneous variation with the degree of malignancy of cutaneous melanoma, making the determination of the aggressive nature of individual tumors possible, thus enabling therapies and more appropriate prognoses since it has been demonstrated that tumor cell resistance to chemotherapy can also be attributed to elevated telomerase expression.[45]

Correlations show an association between the high activity of this reverse transcriptase and an early metastatic dissemination in melanomas linked to telomerase and

the tumor biology of this neoplasm64. Additionally, studies suggest that the genetic transcription of hTERT is a mechanism that regulates the telomerase activity in human melanomas.[65]

An eventual progression of melanocytic nevi in combination with analysis of quantitative measurement of this enzyme might help in the early identification of the malignant transformation, imperceptible to conventional morphology in initial stages.[66]

Some studies indicate that the development of malignant diseases depends on the reactivation of telomerase and that an inhibiting agent of this enzyme might be an effective antitumor drug if induced to senescence cells in response to the shortening of the telomeres, a situation that can be applied to cutaneous melanoma.[5,67-69]

Additionally, telomerase might be used as a complementary tool to distinguish malignant and benign tumors.[66] In the specific case of melanoma, diagnosis can be clinically made in 80% to 90% of the cases, while the other 10% need histologic examinations because of a certain degree of ambiguities, and due to this, the use of detection techniques of the presence of this enzyme would potentially help in the diagnosis.[45]

Investigations of telomerase enzyme activity in human melanoma tissues began with Taylor et al.[70] and each year has seen the number of studies in reference to this subject increase, as much in cell lines as in samples of primary or metastatic melanoma, in the attempt of determining the variability of the activity of this enzyme and its role as an initiating agent and in malignant tumor progression.[21,71-73]

Parris et al.[55] demonstrated the activation of telomerase in premalignant skin lesions and also in all forms of skin cancer. In normal skin, this event is rare, and in only 2 out of 16 (12.5%) samples, 1 with a history of chronic solar exhibition, presented telomerase activation. Other samples with positive telomerase activity: 1 out of 16 (6.25%) proliferative benign injuries; 11 out of 26(42%) preneoplastic injuries; 10 out of 13 (77%) basal cell carcinomas; 22 out of 31 (69%) cutaneous metastatic melanomas; and 1 primary melanoma. Only 4 out of 12 (27%) squamous cell carcinomas presented positive telomerase activity.

Ueda et al.[74] verified the telomerase activity in malignant tumors (91%), many skin tumors benign (60%) and premalignant (89%), proposing the involvement of telomerase activation as a crucial biological phase for the carcinogenesis of the melanoma in humans.

Other considerations refer to its usefulness as a possible prognostic indicator,[68]as diagnostic marker [66] or as treatment.[75]

Due to that, countless strategies are being developed with the aim of more efficient therapies in the combat of cancer, hence, the search of new genes associated with melanoma tumorigenesis are of extreme importance, as well as better understanding the molecular alterations, for which more precise methods of diagnosis help in the establishment of therapeutic conduct differentiated by distinct types of neoplasms.[76-77]

Conclusion

Because of being treated as a heterogeneous tumor, with characteristics still not completely elucidated and whose incidence has been increasing in an alarming way, the attempts of a better understanding of the biology and evolution of melanoma must help in the introduction of new therapeutic strategies. Each time, more and more studies are seen demonstrating that telomerase can fulfill an important role in the process of immortalization of cell lines, having its activity identified in many human cancers.

Necessary studies are being done with drugs that present inhibition action in the enzymes related to the process of cell immortalization, which will be able to act as co-adjuvant in treatments against this neoplasm, contributing to the improvement and increase of patient survival.

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