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# Alterations of Telomerase reverse transcriptase and its promoter in the melanocytic lesions of the skin

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## Abstract:

Malignant melanoma is a deadly neoplasm, which has recently shown a dramatic increase in global incidence. It evolves and progresses through a multistep process during which there is a sequential transition from the precursor lesions to early indolent stages and finally to the late aggressive stages. Tumorigenesis is related to telomerase alterations. Although these changes were recently examined in the advanced stages of melanoma, similar investigations in the precursor lesions (some dysplastic nevi) as well as in the earliest stages of these lesions are lacking. An understanding of these changes will provide valuable insights into the pathogenesis of these tumors. It may also have some therapeutic ramifications.

***Keywords:*** Melanoma, dysplastic nevus, TERT, Telomerase

## 1 Introduction:

Melanoma is a fatal neoplasm with a dramatically increased recent incidence worldwide [1]. The precursor lesions (some dysplastic nevi) and the earliest stage of melanoma (radial growth phase) are indolent and can be effectively cured. Alternatively, its advanced stages (vertical growth phase) are metastasizing, highly aggressive and cannot be effectively cured [2-4]. The authors consider that this difference in the biologic characteristics may be due to the alterations of telomerase reverse transcriptase protein expression as well as to alterations of Telomerase reverse transcriptase (TRET) promoter.

The incidence of cutaneous malignant melanomas is growing faster worldwide. At the histologic level, melanomas seem to develop from transformed melanocytes by a series of transitions that represent a morphologic continuum of lesional steps (dysplastic nevi→ to in situ melanoma → radial growth phase melanoma → vertical growth phase melanoma → metastatic melanoma. At the molecular level, these transitions seem to involve a series of poorly understood genetic alterations. These include loss of tumor suppressor genes, hypermutability, microsatellite instability and alterations of housekeeping genes [2,3, 5-8].

Melanocytic dysplastic nevi were first described in both patients and their relatives

who had one or several cutaneous malignant melanomas [2]. Most of these dysplastic lesions are biologically stable, but some of them have severe histological atypia bordering on melanomas in situ. Some dysplastic nevi harbour molecular changes (allelic loss, alterations of the mismatch repair proteins, microsatellite instability) that can be found in malignant melanomas [6,7,9,10].

Hypothetically, transformed melanocytes can give rise to any lesion in the hierarchy of melanocytic tumours. Based on these hypothetical perspectives and on the molecular profile of the dysplastic nevi, some of these lesions may represent precursors of malignant melanomas [2-4]. Malignant mmelanomas include two sequential phases: radial growth phase and vertical growth phase. As addressed by Clark, the transition of radial growth phase to vertical growth melanoma is the most critical step in melanoma tumorigenesis [5]. During this transition, radial growth phase melanoma cells acquire partial growth autonomy and progress to vertical growth melanoma. Therefore, radial growth phase melanoma represents the first step of cancer, and their presence signals further progression as the rule rather than the exception. Biologically, these lesions are incompetent for metastasis and early excision correlates with good prognosis. Although a wealth of information is still unfolding about the molecular changes in the vertical growth melanoma, little is known about these changes in radial growth phase melanoma [2, 3, 5-8]. Our previous studies raised several notions. Some molecular changes are shared between dysplastic nevi and melanomas, suggesting that some dysplastic nevi represent intermediate steps or sequential phases in melanoma tumorigenesis. These changes are more common in radial growth phase melanoma and their cells have the ability to grow both indefinitely and independently. The subsequent progression of radial growth phase melanoma to vertical growth melanoma is the most important step in melanoma tumorigenesis [2, 3, 5-8].

Telomerase, a highly conserved enzyme, is a eukaryotic ribonucleoprotein complex that contains both an essential RNA and a protein reverse transcriptase subunit. It is a specialized cellular reverse transcriptase catalyzing the synthesis and extension of telomeric DNA with its own RNA template. By reverse transcription, the telomerase ribonucleoprotein maintains the stability of the telomere length in almost all cancer cells [11,12]. There is a strong correlation between telomerase activity and the development of cancer with about 90% of cancers being characterized by increased telomerase activity [13].

Several studies have examined telomerase reverse transcriptase (TERT) protein expression and TERT promoter mutations in the uveal [14-16], conjunctival [15, 16] and invasive cutaneous melanomas [15-19]. In cutaneous melanomas, recent studies identified novel recurrent mutations in the promoter region of TERT, coding for the catalytic subunit of the telomerase holoenzyme, in up to 71% of these tumors [18,19]. The mutations showed a characteristic UV signature (C4T and CC4TT) [20]. Functional studies found that these mutations lead to increased gene expression, most likely by creating ETS transcription factor binding sites [18, 19]. A follow-up study screening a panel of different neoplasms identified TERT promoter mutations in a number of other common cancers, that is, in high frequencies in hepatocellular cancer, bladder cancer, and gliomas [21]. To date studies and to the best of our knowledge, studies investigating the alterations of telomerase protein expression and TERT promoter mutations in dysplastic nevi and in radial growth phase of cutaneous melanomas are lacking.

**2 Hypothesis:**

This author hypothesizes that “The progression among the sequential phases of melanoma tumorigenesis (dysplastic nevi with severe dysplasia/melanoma in situ → radial growth phase melanoma →vertical growth

phase melanoma →metastatic melanoma) is associated with alterations of the telomerase reverse transcriptase protein expression as well as mutational changes of the telomerase reverse transcriptase promoter." This author would predict the presence of such alterations, but with different magnitude, in the entire spectrum of the lesional steps of melanomagenesis. To test this hypothesis and prediction, this author propose the following specific aims and experimentations: i) analysis of the telomerase reverse transcriptase protein expression in the entire spectrum of the melanocytic lesions including: dysplastic nevi (with mild, moderate and severe dysplasia bordering on melanoma in situ), non-tumorigenic radial growth phase melanoma, vertical growth phase and metastatic melanomas using immunohistochemical staining methods [6, 9, 10, 22-24] and ii) analysis of the mutational changes of TERT promoter in the in the entire spectrum of the melanocytic lesions including: dysplastic nevi (with mild, moderate and severe dysplasia bordering on melanoma in situ), non-tumorigenic radial growth phase melanoma, vertical growth phase and metastatic melanomas by Direct (Sanger) Sequencing PCR amplification of a 474 bp region of the TERT promoter region [15, 16, 18, 19, 21] [3, 6, 9].

## 3 Evaluation of The Hypothesis:

Telomerase activity (as reflected by hTERT protein expression) is up regulated in several tumors. During squamous carcinogenesis, there is an increased telomerase expression with progression from squamous dysplasia to invasive squamous carcinoma [23, 25-28].

Some studies examined telomerase activity in melanomas. In uveal melanomas, there is upregulation of telomerase activity. In situ hybridisation visualized a moderate to high upregulation of telomerase RNA component in the melanoma cells but not in the admixed reactive cells [14]. Horn et al examined the TERT promoter in sporadic melanoma and observed recurrent ultraviolet signature somatic mutations in 125 of 168 (74%) of human cell lines derived from metastatic melanomas, 45 of 53 corresponding metastatic tumor tissues (85%), and 25 of 77 (33%) primary melanomas. The majority of those mutations occurred at two positions in the TERT promoter and also generated binding motifs for Ets/TCF transcription factors [19]. Huang et al reported two independent mutations within the core promoter of telomerase reverse transcriptase, which collectively occur in 50 of 70 (71%) melanomas examined. These mutations generate de novo consensus binding motifs for E-twenty-six (ETS) transcription factors, and in reporter assays, the mutations increased transcriptional activity from the TERT promoter by two- to fourfold [18]. Griewank and his colleagues examined mutations in the TERT promoter by Sanger sequencing in uveal and conjunctival melanomas. This group reported mutations of the TERT promoter in 32% of the conjunctival melanomas. These mutations had UV signatures identical to those found in cutaneous melanoma [15, 17-19].

## 4 Discussion and Consequences of The Hypothesis:

Human telomerase consists of two major components: human telomerase RNA (hTR), which consists of a 451-base integral RNA providing the template for the synthesis of the human telomeric repeat (TTAGGG)n [29], and human telomerase reverse transcriptase, hTERT, which is a 127-kDa protein providing catalytic function to replicate the ends of linear DNA [30, 31]. Telomerase reverse transcriptase (TERT, or hTERT in humans) is a catalytic subunit of the enzyme telomerase, which, together with the telomerase RNA component, comprises the most critical unit of the telomerase complex [32, 33].Telomerases are part of a distinct subgroup of RNA-dependent polymerases. Telomerase lengthens telomeres in DNA strands. Therefore, telomerase allow senescent cells that would otherwise become postmitotic

and undergo apoptosis (programmed cell death) to exceed the Hayflick phenomenon **(**the number of times a normal human cell population will divide until cell division stops) [34] and become potentially immortal, as is often seen in cancerous cells. TERT is responsible for catalyzing the addition of nucleotides in a TTAGGG sequence to the ends of a chromosome's telomeres [35]. This addition of repetitive DNA sequences prevents degradation of the chromosomal ends following multiple rounds of replication [36].

Alterations of hTERT could have some therapeutic ramifications. In this regard, if increased telomerase activity is associated with tumorigenesis, then possible cancer therapy could involve inhibiting its catalytic component, hTERT, to reduce the enzyme's activity and induce apoptosis. As non-neoplastic somatic cells do not express TERT, telomerase inhibition in neoplastic cells can cause senescence and apoptosis without affecting normal human cells [37]. Additionally, dominant-negative mutants of hTERT could reduce telomerase activity within the cell [13]. This forces the cells with short telomere lengths to undergo apoptosis, a promising avenue for cancer therapy [13]. In melanoma, Hunger et al examined the safety, tolerability, and immunological responses to vaccination with a combination of telomerase-derived peptides GV1001 (hTERT: 611-626) and p540 (hTERT: 540-548) using granulocyte-macrophage colony-stimulating factor or tuberculin as adjuvant in patients with cutaneous melanoma. They found that immunity to hTERT can be generated safely and effectively in patients with advanced melanoma and therefore encourage further trials [38].

**CONFLICT OF INTEREST STATEMENT: None**

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