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UV light-induced dual promoter mutations dismantle the telomeric guardrails in melanoma

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Abstract

Understanding how benign nevi can progress to invasive and metastatic melanoma is critical for developing interventions and therapeutics for this most deadly form of skin cancer. UV-induced mutations in the telomerase TERT gene promoter occur in the majority of melanomas but fail to prevent telomere shortening despite telomerase upregulation. This suggests additional "hits" are required to enable telomere maintenance. A new study in *Science* identified somatic variants in the promoter of the gene that encodes telomere shelterin protein TPP1 in human melanomas. These variants show mutational signatures of UV-induced DNA damage and upregulate TPP1 expression, which synergizes with telomerase to lengthen telomeres. This study provides evidence that TPP1 promoter variants are a critical second hit to prevent telomere shortening and promote immortalization of melanoma cells.

Genomic instability and mutagenesis are hallmarks of cancer. Every cell division provides an opportunity for the cell to acquire genetic mutations arising from DNA replication errors, especially in the face of mutagenic DNA damage. Indeed, recent whole-genome sequencing analyses reveal that mutations increase with age across lifespans [1, 2]. Telomeres at chromosome ends act as guard rails to restrain the number of cell divisions, and thereby reduce the chances that cells acquire mutations that set them on a path to carcinogenesis.

In humans, telomeres consist of approximately 10 kb of GGTTAG repeats coated with a six-member protein complex termed shelterin [3]. Telomeres shorten during each cell division due to the end replication problem. When telomeres become critically short, they are falsely recognized as chromosomal breaks, activating a DNA damage response

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Conflict of interest

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(DDR) and p53, which trigger replicative senescence [4]. Senescent cells enter a state of growth arrest, generally thought to be irreversible, which prevents potentially pre-malignant cells from proliferating and progressing to cancer. Therefore, telomere length determines replicative capacity, and cells with short telomeres will enter replicative senescence after fewer divisions compared to cells with long telomeres. The reverse transcriptase telomerase adds telomeric repeats to extend and restore telomeres to prevent shortening [5]. While most somatic and adult stem cells lack sufficient telomerase to prevent shortening with aging, nearly 90% of cancers upregulate telomerase to achieve unlimited cell proliferation [6].

Melanomagenesis is generally understood as a series of progressions from precursor lesions to metastatic melanoma. Somatic mutations in melanocytes, including BRAF and NRAS oncogenes, occur early and are proposed to drive oncogene-induced senescence in these cells, which gives rise to benign nevi [7, 8]. Onogene-induced senescence occurs independently of telomere attrition, and a lack of significant telomere shortening in benign nevi suggests these senescent melanocytes retain replicative potential [9]. Oncogene-induced senescence is very effective since the majority of benign nevi never progress to melanoma [10]. However, evidence suggests epigenetic changes may allow pre-malignant melanocytes to re-enter the cell cycle since inactivating mutations in tumor suppressors that enable senescence bypass (*TP53, PTEN*, and *CDKN2A*) either occur later in melanomagenesis or are rare [7, 11]. Once the pre-malignant cells proliferate again, the telomeres shorten, and telomeric guard rails go up. Critically short telomeres promote chromosome fusions and rampant chromosomal instability that eventually kill most of the cells [12]. This may explain the clinical observation of nevi regression [10].

Progression from intermediate lesions to melanoma *in situ* requires escape from telomere crisis, and this is the stage when *TERT* promoter mutations often arise [7]. *TERT* encodes the telomerase catalytic component, and mutations in the *TERT* promoter are the most common noncoding mutation in cancer [13] and occur in nearly 75% of melanomas [14, 15]. While these mutations upregulate telomerase, they are insufficient to prevent telomere shortening and confer immortalization. When the Hockemeyer lab introduced *TERT* promoter mutations into cells, telomeres continued to shorten, eventually driving genomic instability, which led to further telomerase upregulation to stabilize the ends [16]. They proposed a two-hit model. A *TERT* promoter mutation as the 1st hit is insufficient to extend bulk telomeres but delays replicative senescence or telomere crisis by lengthening the shortest telomeres. This delay increases the chances of acquiring a 2nd hit that enables sustained telomere maintenance and immortalization. Aviv and Shay invoked a similar 2-hit model to explain why individuals with long telomere lengths have a greater risk of developing cancer, especially melanoma [17]. Cells with constitutively longer telomeres are more likely to sustain a 2nd hit during an extended replicative capacity.

Dysregulation of telomere biology as a “first hit” is a common theme in melanogenesis. Although germline *TERT* promoter mutations are rare, they primarily predispose to melanoma [14]. Collectively, germline variants in the coding region of shelterin genes *TPP1*, *RAP1*, and *POT1* account for 9% of melanomas [18]. Germline POT1 variants in melanoma impair telomere binding, resulting in longer telomeres through enhanced telomerase access [19–21]. Interestingly, the TPP1 variants result in truncated proteins or missense mutations

in the POT1 binding domain, essentially mimicking the loss-of-function POT1 variants. While germline variants in shelterin proteins that confer telomere lengthening, or *TERT* promoter mutations, are often the 1st hit to extended replicative capacity, the identity of 2nd hits to impart sustained telomere maintenance and proliferation are largely unknown.

A recent study led by Jonathan Alder and Carol Greider uncovered evidence that *TPP1* promoter mutations represent a critical "2nd hit" toward sustained telomere maintenance in cutaneous melanoma cases [22]. This team mined the International Cancer Genome Consortium database for somatic variants in genes related to telomere maintenance in melanoma. They identified a cluster of variants in the *TPP1* gene (termed *ACD*) in the predicted promoter region of the TPP1-short isoform. Although a TPP1-long isoform has been described [23], Chun-On et al [22] showed that only TPP1-short is expressed in melanocytes based on RNA-Seq analysis of 12 melanoma cell lines and 61 nevi and melanoma samples from patients. The *TPP1* variant promoters also arose in tumors with *TERT* promoter mutations, hinting at cooperation. The result that these were non-coding variants in the *TPP1* gene was a key finding.

Similar to the *TERT* promoter mutations, the *TPP1* promoter variants arise from C to T mutations indicative of previous UV exposures [22]. But the similarities do not stop there. The authors went on to show that, similar to *TERT* promoter mutations, the *TPP1* promoter variants generate binding sites for the E-twenty-six (ETS) family of transcription factors. Interestingly, the introduction of the *TPP1* promoter variants in a luciferase reporter assay yielded disappointing results in HEK293 cells but increased expression in melanoma cell lines. Through mining RNA-Seq data, the authors discovered ETS members ETV5, ETS1, and ETV4 are the most abundant in melanoma and could increase expression in the luciferase reporter gene when driven by the mutated *TPP1* promoter when introduced into HEK293 cells. When the authors introduced *TPP1* promoter variants directly in the genomes of melanoma cell lines, this increased TPP1 expression. These results further underscore the unique relationship between telomere maintenance genes and melanoma.

For *TPP1* promoter variants to be a 2nd hit, enabling sustained telomere maintenance, the enhanced TPP1 expression should stimulate telomere lengthening. Previous studies showed that TPP1 enhances telomere extension by recruiting telomerase to telomeres and by forming a complex with POT1 to increase telomerase repeat addition processivity (the number of GGTTAG repeats added per telomerase binding cycle) [24, 25]. When Chun-on and colleagues over-expressed TERT alone or with TPP1 in human fibroblasts, they found both conditions bypassed RS, but the combined TERT and TPP1 expression extended telomeres to longer lengths, compared to TERT alone [22]. Furthermore, their genome-edited melanoma cell lines with the *TPP1* promoter variants showed increased synthesis of new telomeric repeats. Collectively, their results indicate that *TPP1* promoter variants and upregulation cooperate with telomerase to increase telomeric DNA synthesis and lengthen telomeres in melanoma cell lines.

This study by Chun-on and colleagues uncovered a critical missing piece in melanomagenesis and opens up new lines of inquiry. The authors identified *TPP1* promoter variants in about 5% of the cutaneous melanomas, suggesting there are more "2nd hits" to

be discovered. These hits are potential new targets for therapeutics that treat melanoma. For example, recent cryo-EM structures reveal detailed interactions between telomerase and TPP1 that could be targeted to disrupt the ability of TPP1 to recruit telomerase and enhance its activity [26]. Future studies are needed to address when TPP1 promoter mutations arise during melanomagenesis, and whether they occur after TERT promoter mutations consistent with the 2-hit model. The model predicts that pre-malignant melanocytes with TERT promoter mutations could bypass telomere crisis and maintain telomere lengths for sustained proliferation upon acquiring TPP1 promoter variants. New model systems and engineered cell lines will be required to rigorously test this model further.

In summary, this recent study further underscores the special relationship between melanomagenesis and telomere-driven replicative capacity. We have yet to fully understand why longer telomeres increase the risk of developing cutaneous melanoma. One possibility that warrants further investigation relates to the high mutation burden in melanoma and the role of UV-induced mutagenesis in driving melanoma. Previous work shows that mutational signatures for UV radiation clearly increase with progression from benign nevi to metastatic melanoma, and strongly implicate UV radiation in both the initiation and progression of melanoma [7]. Moreover, the *TERT* and *TPP1* promoter variants are consistent with mutations caused by DNA replication past UV photoproducts. An extended replicative lifespan conferred by longer telomeres in a setting of mutagenic lesions (i.e. photoproducts) increases the chances for polymerase errors and translesion synthesis-induced mutations in critical genes during genome replication. Future studies identifying the acquired mutations that dismantle the telomere guard rails should aid in the development of interventions that reinforce these guards to prevent melanoma progression and drive regression of pre-malignant lesions.

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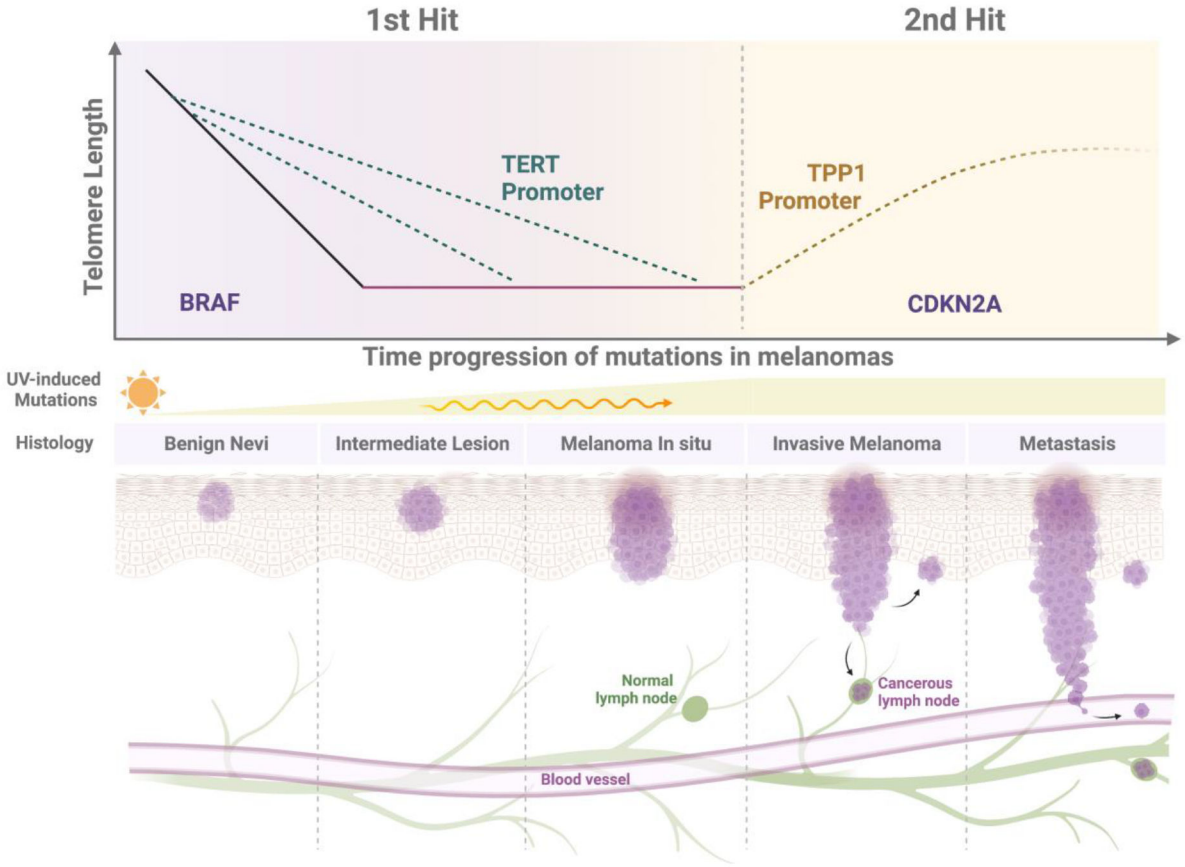

Fig. 1.

Model for melanoma development and progression in the context of UV-induced mutagenesis. *TERT* promoter mutations as a 1st hit can extend replicative capacity by lengthening the shortest telomeres, but do not prevent bulk telomere shortening and crisis (red line). More cell divisions prior to crisis increases the chances of acquiring a 2nd hit due to UV DNA damage being converted to mutations during replication. *TPP1* promoter variants as a 2nd hit act synergistically with *TERT* promoter mutations to lengthen bulk telomeres, and are proposed to enable sustained telomere maintenance and immortalization. *CDKN2A* mutations often arise later in melanomagenesis and promote invasion and metastasis. Image created with BioRender.com.