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AUTHOR'S VIEW

Telomere maintenance: regulating *hTERC* fate through RNA modifications

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ABSTRACT

Disturbances in telomere maintenance are common in cancer. We recently showed that Single-strand-selective monofunctional uracil-DNA glycosylase 1 (SMUG1) promotes telomere homeostasis by regulating the stability of the telomeric RNA component (*hTERC*). SMUG1-mediated recognition of base modifications may function in a regulated process serving to fine-tune the levels of *hTERC*.

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Chemically modified bases are constantly generated in nucleic acids.[1] While only a handful of the DNA modifications are known to have regulatory functions, more than 140 types of functional modifications are described in RNA.[2] Some modifications are stable components of long-lived RNA, e.g., pseudouridine modifications in ribosomal RNA (rRNA) and the telomeric RNA component (*hTERC*). Others are regulatory modifications transiently introduced in a small number of transcripts in defined contexts. In addition, modifications exist for which it is unclear whether they have functional roles or, simply, are damaged bases. One such modification, which is present in both DNA and RNA, is 5-hydroxymethyluridine (hmU). In DNA, it is believed that hmU forms by Reactive Oxygen Species (ROS)-mediated oxidation of thymine or is introduced enzymatically by the Ten-eleven translocation (TET) proteins.[3] How hmU is introduced in RNA is not known, but our group found appreciable amounts of hmU in rRNA in human cells. hmU levels increased when we depleted Single-strand-selective monofunctional uracil-DNA glycosylase 1 (SMUG1),[4] an enzyme that can recognize and process hmU in both DNA and RNA (Figure 1). In DNA, SMUG1 initiates Base Excision Repair (BER) of hmU,[5] but SMUG1 also has an RNA-processing function (Figure 1).[4,6] The recognition of RNA modifications by SMUG1 seemed to be coupled to RNA degradation: the accumulation of hmU in rRNA was accompanied by an increase in misprocessed rRNA species targeted for degradation and a reduction in the corresponding mature species. We, therefore, concluded that SMUG1 functions in rRNA quality control by regulating the presence of hmU.[4]

In our recent study,[6] we showed that SMUG1 is required for maturation of *hTERC* in human cells by regulating the levels of modified bases located between the CR4-CR5 domain and the H-box, a region important for binding of Dyskerin pseudouridine synthase 1 (DKC1). In the absence of SMUG1, the balance between mature *hTERC* and its processing intermediates was disturbed. Mature *hTERC* levels were reduced down to a level insufficient to support adequate telomerase activity, leading to dramatic telomere attrition in SMUG1-knockout human cells. Due to the dual activity of SMUG1 on DNA and RNA, we used complementation assays in order to test which SMUG1 function was required for telomere maintenance. SMUG1 substrates were detected at telomeres in SMUG1-knockout cells, showing that SMUG1 does initiate BER at telomeres in human cells. However, the reduced *hTERC* levels were the limiting factor for telomerase activity and responsible for telomere shortening. Thus, the RNA-processing activity appears to be the main function of SMUG1 in telomere homeostasis. To further characterize the mechanism of SMUG1 in *hTERC* biogenesis, we checked *hTERC* transcription in SMUG1-knockout cells. No differences in transcription initiation or kinetics could be observed suggesting that SMUG1 may act in downstream steps, such as RNA degradation. *hTERC* levels could not be rescued after silencing of the main components of the RNA decay machinery, indicating that SMUG1 may play a role in targeting modified *hTERC* molecules to the exosome and/or to another yet-to-be-identified degradation pathway.[6]

Some questions must be answered before we completely understand the role of SMUG1 in *hTERC* maturation. Firstly, the precise nature of the RNA modification recognized by SMUG1 in *hTERC* has not been identified. However, we know it is a lesion specifically recognized and processed by SMUG1.[6] The most likely candidate is hmU because this modification accumulates in SMUG1-depleted cells, and there is a possible biochemical route for its introduction. Secondly, we could not conclude as to whether the modification recognized by SMUG1 is a functional modification or damage. The SMUG1 substrates in *hTERC* are not randomly distributed along the RNA molecule but enriched between CR4-CR5 domain and the H-box. This suggests that the modification might be a part of a regulated process aimed to fine-tune the levels of mature *hTERC* in human cells.[6] Targeting of SMUG1 to

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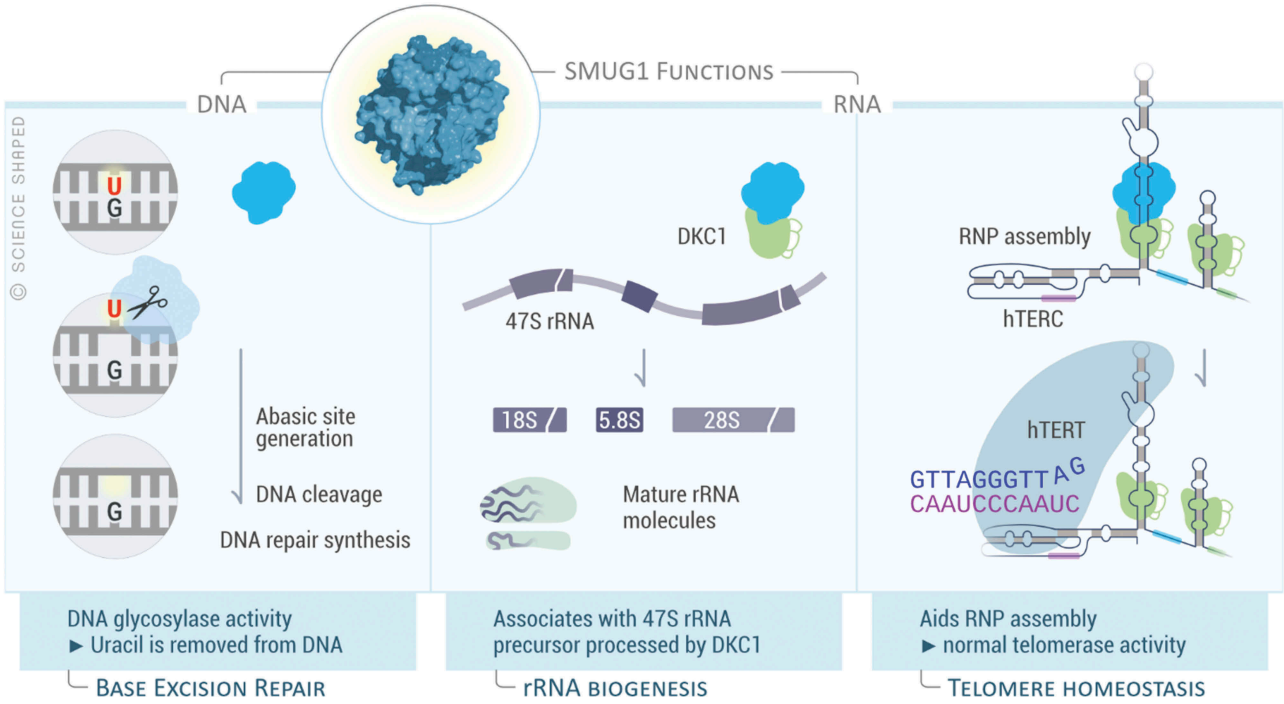

Figure 1. Effects of SMUG1 on DNA and RNA. Dual role of Single-strand-selective monofunctional uracil-DNA glycosylase 1 (SMUG1) in DNA repair as DNA glycosylase in the Base Excision Repair (BER) pathway and in RNA processing with functional relevance for ribosomal RNA biogenesis and telomere maintenance. Dyskerin 1 (DKC1), ribosomal RNA (rRNA), ribonucleoprotein (RNP), telomerase RNA component (*hTERC*), telomerase reverse transcriptase (hTERT). Figure by Ellen Tenstad/Science Shaped.

specific RNA species is likely mediated through direct interaction with DKC1[4] which, as part of an H/ACA ribonucleoprotein complex, contributes to the biogenesis and post-transcriptional processing of many types of RNA molecules, including *hTERC*.[7]

Telomerase insufficiency in SMUG1-knockout cell led to dramatic telomere shortening. May inhibition of SMUG1 limit growth of cancer cells? This is probably highly context dependent. The cell line we used seems to rely on the RNA-processing function of SMUG1. In other cell types, the DNA repair function of SMUG1 might be more important. In mice, BER activity of Smug1 seemed to be the predominant function in telomere maintenance. *Smug1*$^{-/-}$ MEFs and mice showed increased telomere defects, as observed previously in other DNA-glycosylase deficient mice,[8–10] but we found no statistically significant differences in *Terc* levels. Thus, the data currently available supports a dual role for SMUG1 in telomere maintenance both as DNA repair enzyme controlling base damages at telomeres and as an RNA-processing protein that regulates mature *hTERC* levels. The balance between these two functions might differ between species and cell types.[6]

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Disclosure of Potential Conflicts of Interest

The authors declare no competing interests.

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