News Release

Title

TUG1-mediated R-loop resolution at microsatellite loci as a prerequisite for cancer cell proliferation

Key Points

• Oncogene-induced DNA replication stress and consequent pathogenic R-loop formation is known to impede S phase progression.
• Nonetheless, cancer cells continuously proliferate under such high-stressed conditions through incompletely understood mechanisms.
• We found taurine upregulated gene 1 (TUG1) long noncoding RNA (lncRNA), which is highly expressed in many types of cancers, as an important regulator of intrinsic R-loop in cancer cells.
• Under RS conditions, TUG1 was rapidly upregulated via activation of the ATR-CHK1 signaling pathway, interacted with RPA and DHX9, and engaged in resolving R-loops at certain loci, particularly at the CA repeat microsatellite loci.
• Depletion of TUG1 led to an overabundant R-loops and enhanced replication stress, leading to substantial inhibition of tumor growth.
• Our data reveal a crucial role of TUG1 as an indispensable molecule for resolving the problem of R-loop accumulation in cancer cells and a strong rationale for targeting TUG1 as a potent therapeutic approach for cancer treatment.

Summary

Cancer cells exhibit elevated levels of ”replication stress,” wherein DNA replication is frequently interrupted. A primary cause of replication stress is the formation of R-loops, caused by the conflicts between RNA transcription and DNA replication on the DNA strand, leading to DNA damage. However, the mechanisms by which cancer cells resolve R-loops and continue DNA replication under such high replication stress have remained unclear. In this study, we focused on lncRNAs, which are highly expressed in cancer cells and known to be involved in cancer development and malignancy. We discovered that TUG1 (Taurine Upregulated Gene 1), a lncRNA of which expression is rapidly induced under the replication stress, binds to the proteins RPA and DHX9 and resolves R-loops containing microsatellite sequences. Suppression of TUG1 led to the accumulation of R-loops and severe DNA damage, inducing apoptotic cell death. We made a brain tumor mouse model using human glioblastoma cells expressing

a high level of TUG1 and treated with an oligonucleotide therapeutics called TUG1-DDS, which specifically suppresses TUG1, in combination with temozolomide, a standard glioblastoma treatment. The results demonstrated significant inhibition of tumor growth and a remarkable improvement in survival time. Overall, this study reveals a role of TUG1 as molecule important for resolving R-loop accumulation in cancer cells and suggests targeting TUG1 as a potent therapeutic approach for the treatment of glioblastoma.

Research Background

In cancer cells, the high level of replication stress leads to DNA damage and mutation, contributing to cancer development and rapid cancer growth. However, the mechanism by which cancer cells reduce replication stress and continue growing despite such high replication stress is not fully understood. Recently, lncRNAs, which are not translated into proteins, have been linked to cancer development, malignancy, and drug resistance, but their functions remain unclear. This study aimed to identify lncRNA that reduces replication stress in cancer cells and enhances cell proliferation.

Research Results

When we exposed cancer cells to chemicals that induce replication stress (hydroxyurea and camptothecin), the expression of lncRNA TUG1 significantly increased within 2 hours (Figure 1). This upregulation of TUG1 was revealed to be a downstream event of the ATR/Chk1 pathway, which is activated in response to replication stress. Then, we investigated where the newly expressed TUG1 molecules go in the nucleus when replication stress occurs. We found that TUG1 localizes at a DNA structure called R-loop (Figure 2). R-loops consist of RNA/DNA hybrid, leaving the other strand to form a loop-like structure (Figure 2). The single-stranded DNA in the R-loop is prone to breaking and causing DNA damage. TUG1 appears to play a role in resolving the R-loop to prevent this

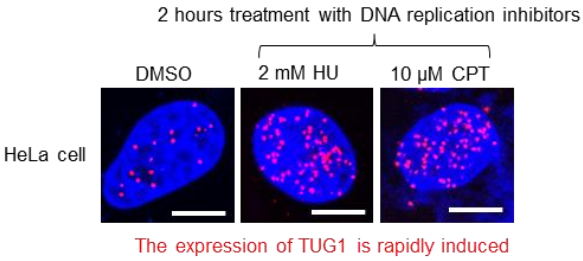

Figure. 1 Discovery of lncRNA induced by replication stress

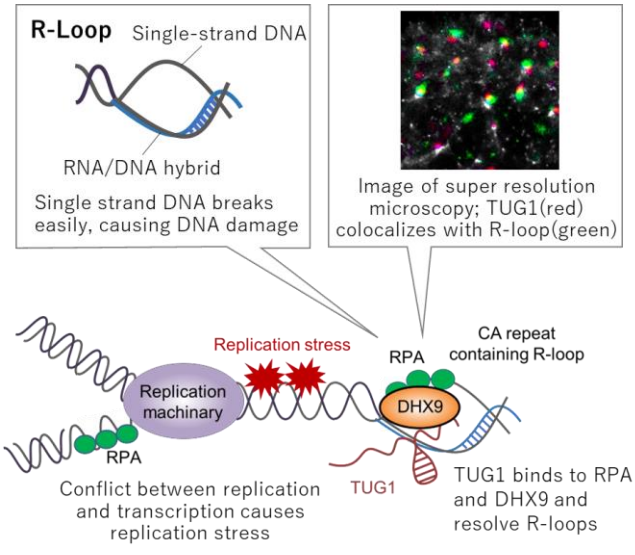

Figure 2. TUG1 is induced by replication stress and resolve R-loops

damage. To understand how TUG1 resolves the R-loop, we identified proteins interacting with TUG1. These proteins include RPA, which binds to R-loops and stalled replication forks, and DHX9, an RNA helicase that is known to resolve R-loop structures (Figure 2). We found that the TUG1/RPA/DHX9 complex is particularly effective at resolving R-loops in microsatellite regions, especially those containing CA repeats. These microsatellite regions are known to be susceptible to DNA damage and mutations.

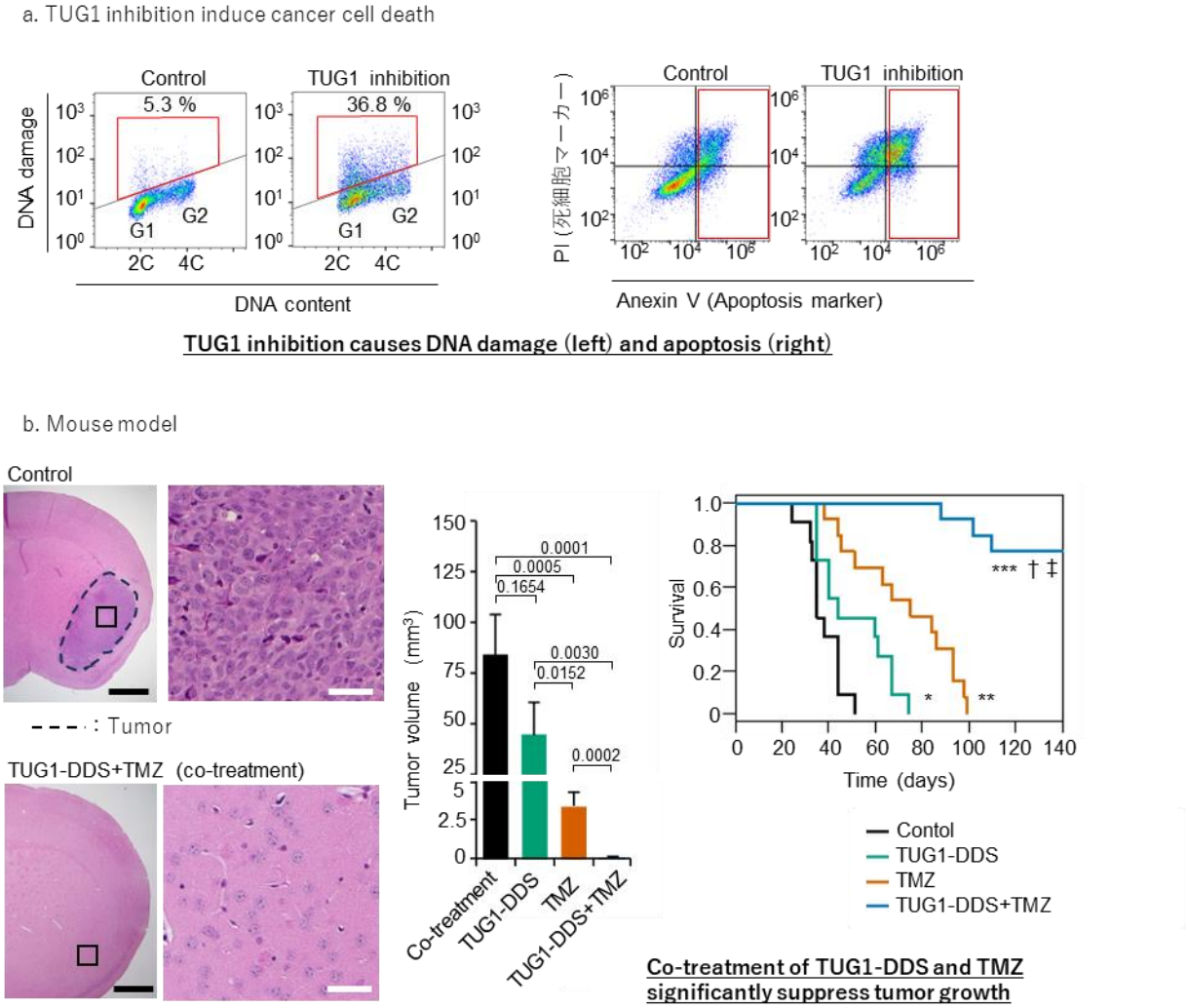

Figure 3. TUG1-DDS is a potentially effective therapeutic agent for treating glioblastoma

In cells where we reduced the expression of TUG1 (TUG1 knockdown), we observed severe DNA damage and the induction of cell death through apoptosis (Figure 3a). Since TUG1 is highly expressed in glioblastoma, an aggressive brain tumor, we tested the effects of TUG1 knockdown in mouse models of brain tumors. Using an antisense oligonucleotide therapeutics called TUG1-DDS, which specifically targets cancer cells by drug delivery system, we found that it significantly suppressed tumor growth and improved survival,

especially when administered in combination with the standard treatment, temozolomide (TMZ) (Figure 3b).

In conclusion, our study revealed that TUG1 is essential for protecting the genome DNA of cancer cells from damage and assists in cell proliferation by resolving R-loops. The TUG1-DDS is a potentially effective therapeutic agent for treating glioblastoma, refractory brain cancer.

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