

News Release

Title

Targeting the notch-regulated non-coding RNA TUG1 for Glioma treatment

Key Points

- Notch-regulated long non-coding RNA (lncRNA), TUG1 is highly expressed in glioma stem cells (GSCs).
- TUG1 promotes glioma stem cell (GSC) self-renewal by sponging miR-145 in the cytoplasm and recruiting polycomb to repress differentiation genes in the nucleus.
- Inhibition of TUG1 reduced tumor growth in intracranial xenograft mouse model along with decreased GSC population.

Summary

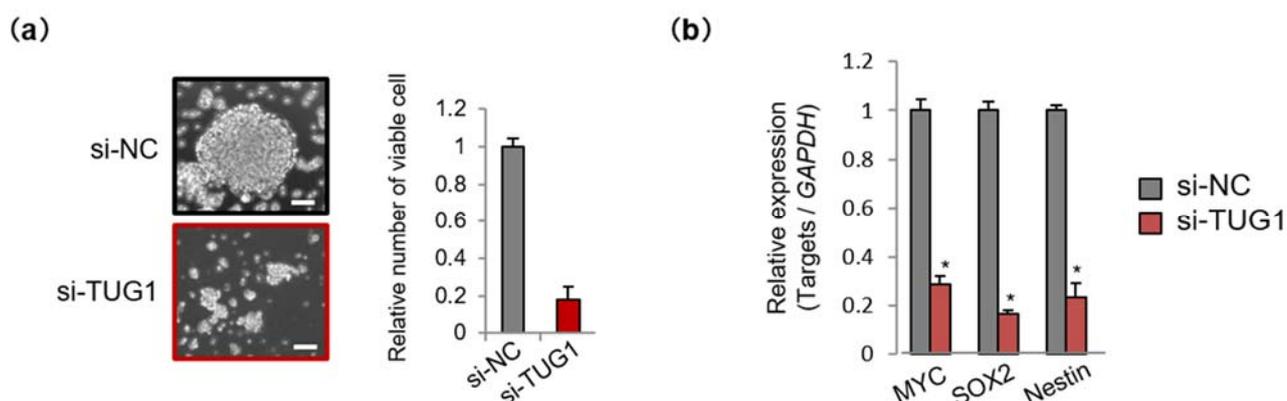
Targeting self-renewal is an important goal in cancer therapy and, to that end, recent studies have focused on Notch signaling in maintenance of the stemness of GSCs. Understanding cancer-specific Notch regulation would improve specificity of targeting this pathway. We found that Notch1 activation in GSCs specifically induces expression of the lncRNA, Taurine Upregulated Gene 1 (TUG1). TUG1 coordinately promotes self-renewal by sponging miR-145 in the cytoplasm and recruiting polycomb to repress differentiation genes by locus-specific methylation of histone H3K27 in the nucleus. Our data indicate that the Notch-regulated lncRNA, TUG1, plays pivotal roles in regulating self-renewal of GSC.

Research Background

Notch signaling has been shown to promote GSC self-renewal and to suppress GSC differentiation. However, the mechanism by which Notch signaling and its downstream effectors maintains the stemness properties of GSCs through the function of a certain set of genes, such as SOX2, MYC and Nestin, remains unresolved. Studies have shown that long non-coding RNAs (lncRNAs) affect the chromatin structure, mRNA stability, and miRNA-mediated gene regulation by acting as miRNA sponges. In the current study, we show that TUG1, the expression of which is regulated by the Notch signaling pathway, was highly expressed in GSCs and maintained the stemness features of glioma cells.

Research Results

We investigated the lncRNA in GSC, whose expression is induced by Notch signaling. Global lncRNA expression analysis revealed that TUG1 was regulated by the coordinated actions of Jagged1 and Notch1 in GSCs. Inhibition of TUG1 by siRNA efficiently reduced GSC proliferation together with downregulation of the stemness-associated genes (SOX2, MYC and Nestin) (Figure 1). RNA-FISH analysis defined that TUG1 localized to both the nucleus and the cytoplasm in GSCs (Figure 2). In the cytoplasm, TUG1 increased the expression of the stemness-associated genes (MYC and SOX2) through miR-145 sponge mechanism (Figure 3). Modified RNA pull-down assay revealed that TUG1 physically interacted with PRC2 and tethered PRC2 to a number of neuronal differentiation-associated genes in GSC. Inhibition of TUG1 reduced the histone H3K27 trimethylation level and increased expression of its target genes (BDNF, NGF, and NTF3) (Figure 4). Finally, we investigated the molecular effect of TUG1 inhibition on an intracranial xenograft mouse model. GSCs were inoculated into the brain of NOD-SCID mice. After 30 days of inoculation, TUG1-DDS was administered intravenously twice a week for 4 weeks. Treatment with TUG1-DDS markedly reduced tumor growth compared to control-DDS (polymeric micelle



containing ASO targeting luciferase gene) (Figure. 5).

Figure 1. TUG1 maintains stemness features of GSCs.

(a) Phase-contrast images of GSCs treated with either si-NC or si-TUG1. Bars, 100 μ m. (left) Viable cells were assessed by trypan blue staining. Values are indicated relative to abundance in si-NC-treated cells (right). Error bars indicate s.d. (n=3). * $P < 0.01$. (b) Effect of TUG1 depletion on expression of the stemness-associated genes (SOX2, MYC, and Nestin). Y-axis indicates relative expression level compared to that seen in si-NC-treated cells. Error bars indicate s.d. (n=3). * $P < 0.01$.

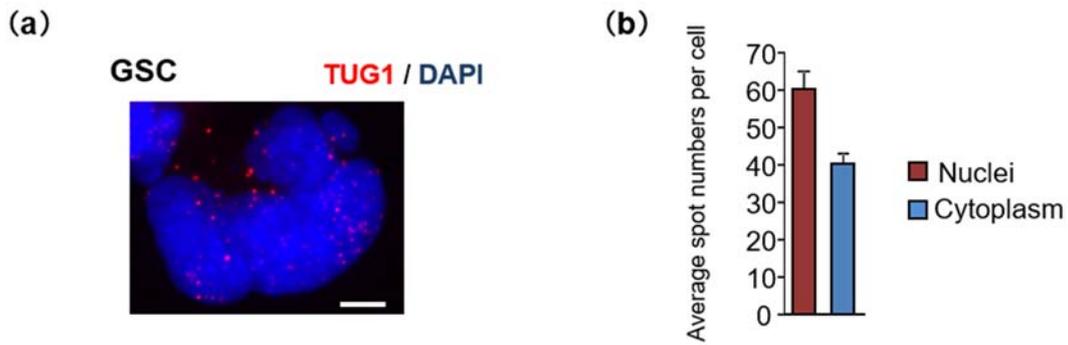


Figure 2. Cellular localization of TUG1 in GSCs

(a) RNA-FISH analysis of TUG1 (red) in GSCs. Nuclei are stained with DAPI. Bar, 10 μ m. (b) The spot numbers relating to TUG1 were quantified per cell. Error bars indicate s.d.

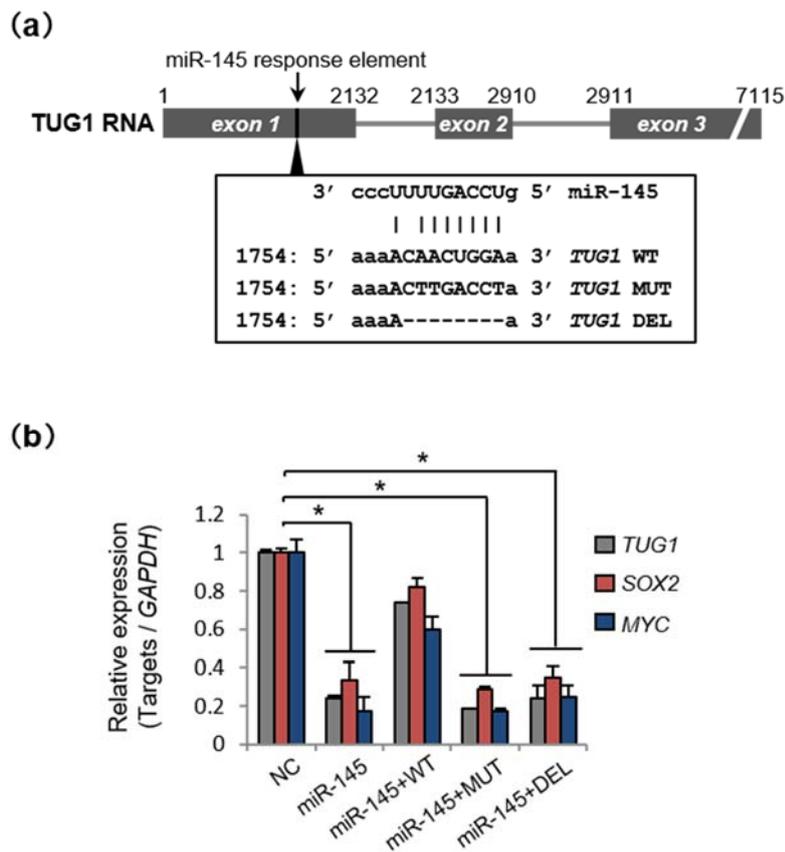


Figure 3. TUG1 antagonizes miR-145 and maintains expression of stemness-associated genes. (a) Schematic of mutated (TUG1 MUT) or deleted (TUG1 DEL) TUG1 transcripts with miR-145 response element. (b) Effect of exogenous miR-145 on TUG1, SOX2 and MYC expression in GSCs. Partial TUG1 transcripts (TUG1 WT, TUG1 MUT and TUG1 DEL as shown in a) were added to GSCs treated with the precursor molecule of miR-145 (miR-145). Expression levels of endogenous TUG1, SOX2, and MYC were measured. Values are indicated relative to abundance of negative control miRNA precursor (NC) treated cells. Error bars indicate s.d. (n=3). * P<0.01.

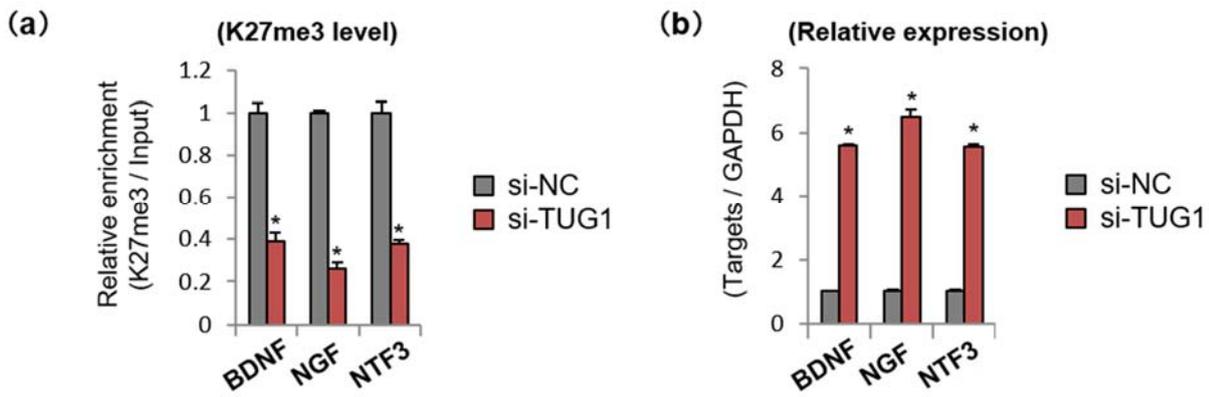


Figure 4. Suppression of neuronal differentiation-associated genes by the TUG1-PRC2 complex (a) Enrichment of K27me3 in the BDNF, NGF and NTF3 promoter regions and their mRNA expression levels (b) in GSCs treated with either si-NC or si-TUG1. K27me3 enrichment is expressed as a percentage of input DNA (left). Relative expression level to si-NC is shown (right). Error bars indicate s.d. (n=3). * P<0.01.

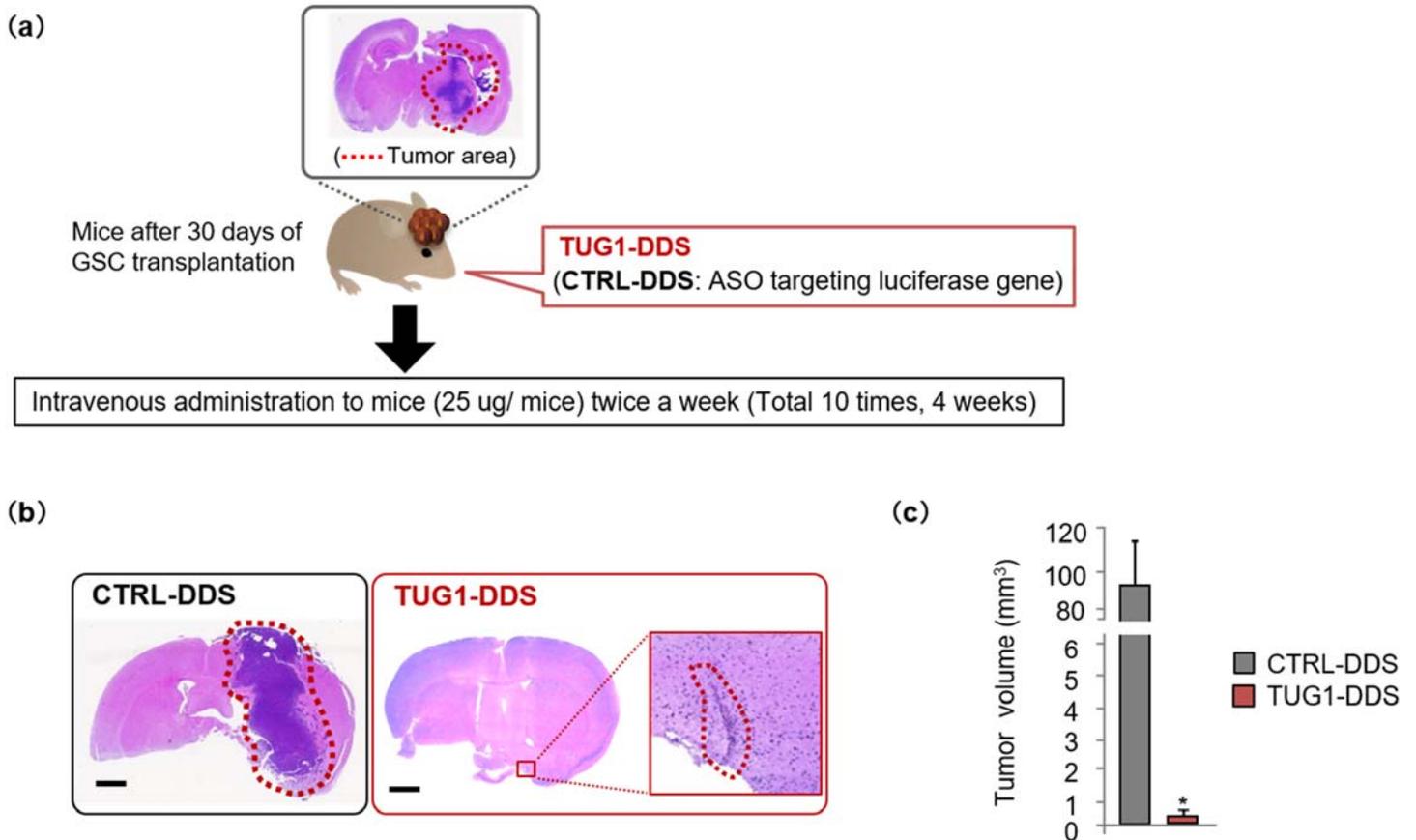


Figure 5. TUG1 is a critical mediator of tumorigenicity in GSC in vivo.

(a) GSCs were transplanted intracranially in NOD/SCID mice. After 30 days of transplantation, CTRL-DDS or TUG1-DDS were intravenous injected twice a week for 4 weeks. (b)

Representative HE stained whole brain sections at 4 weeks post-treatment. Tumor areas are surrounded by red dotted line. Bars, 1 mm. (c) Quantification of tumor volume at 4 weeks post-treatment. Error bars indicate s.e.m. (n=4). *, P<0.001.

Research Summary and Future Perspective

Emerging insights into gliomagenesis have revealed that GSCs have the potential to initiate and maintain the growth of gliomas, and may be crucial for their resistance to conventional therapies. Our results demonstrated that both nuclear and cytoplasmic TUG1 cooperatively promote the stemness and tumorigenicity of GSCs in vivo. Although further investigations including side effects of TUG1-DDS are required, we provide a strong evidence for targeting TUG1 as a specific and potent therapeutic approach for GBMs.

Article

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