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Spotlight

PUS7: a targetable epitranscriptomic regulator of glioblastoma growth

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Pseudouridine is the most abundant yet unexplored RNA modification in glioblastoma. Cui and coworkers find that PUS7, a pseudouridine depositing enzyme, promotes tumor growth and can be targeted by small molecule inhibitors. Mechanistically, PUS7 modifies tRNAs, reduces TYK2 translation, and downregulates a proliferation-restricting interferon-STAT1 pathway in glioblastoma.

Glioblastoma (GBM) is an aggressive and rapidly fatal primary brain cancer, and the poor outcomes that have remained largely unchanged over the past few decades demonstrate a clear need for improved treatments. Despite landmark advances in genetically targeted therapies, biologic agents, and immunotherapies for various cancers, these approaches have not yielded broad success in GBM. Better insight into the underlying biological processes is required to reveal more clinically meaningful opportunities for intervention. One of the major challenges presented by this disease is its treatment resistance and rapid growth, both of which are believed to be mediated by glioma stem cells (GSCs) through their intrinsic cellular plasticity and capacity to give rise to a variety of different tumor cell states [1]. However, the intrinsic biology of GSCs remains poorly understood and these critical gaps in knowledge have spurred the exploration of novel disease mechanisms that are newly accessible with emerging biochemical and genomic techniques and with the development of more physiologically faithful laboratory models, including patient-derived GSC cultures.

More than 100 different RNA modifications have been identified and found to play important functions in normal physiology and in disease states, including cancer [2,3]. Pseudouridine (Ψ) is the most abundant cellular RNA modification and occurs in most classes of RNA [4]. Its role is best understood in tRNA, where it maintains the structure and stability necessary for regulating ribosome assembly and translation. Pseudouridine is also found in rRNA, snRNA, and mRNA, where it is believed to participate in ribosome biogenesis and function, RNA splicing, and post-transcriptional regulation, respectively. Despite its ubiquity, the biological functions of pseudouridine remain largely unexplored in GBM.

Pseudouridine is generated upon isomerization of RNA-incorporated uridine by box H/ACA ribonucleoproteins and pseudouridine synthase (PUS) enzymes. Cui and coworkers observed that PUS7 expression was higher in GBM tissue compared to non-tumor tissue and its expression was linked to worse clinical prognosis across several large GBM genomic databases [5]. Functional studies, including *in vitro* proliferation and glioma sphere formation assays, as well as *in vivo* mouse orthotopic xenograft experiments, demonstrated decreased growth in GSCs with reduced PUS7 expression and with loss of enzymatic activity. A small molecule screen identified several compounds, (C17 and its analog), that could inhibit PUS7 activity and impair GSC growth *in vitro* and in mouse xenografts.

Cui and coworkers further performed mechanistic studies to identify the function of PUS7 in GSCs. Mass spectrometry analyses revealed that PUS7 largely acts on <200 nt small RNAs, though some PUS7-dependent modifications were also found in mRNAs within the context of a previously identified UGΨAR motif. Analysis of small RNAs by DM-Ψ-seq [6] localized PUS7-dependent pseudouridine modifications to 13 sites across eight tRNA types. Interestingly, upon comparison of pseudouridine modification profiles in GSCs and in neural stem cells, a few cell type-specific and PUS7-dependent pseudouridine modifications could be identified, such as one at position 50 of tRNA-Arg-CCG-2-1 (tRNA for arginine, anticodon CCG, transcript ID 2, gene locus 1). Functionally, knockout of PUS7 did not impact overall tRNA levels or global protein translation; however, at the individual tRNA level, PUS7 activity was found to inhibit codon-specific translation involving tRNA-Arg-CCG.

At the cellular level, PUS7 reduction led to upregulated gene expression of interferon (IFN) response pathways as well as upregulated protein expression of TYK2, a nonreceptor tyrosine kinase of the JAK family that associates with IFN receptors and activates the downstream effector molecule STAT1. Codon usage in TYK2 was enriched in PUS7-dependent tRNAs, including tRNA-Arg-CCG, which was consistent with the observed and experimentally corroborated post-transcriptional regulation of TYK2. Finally, TYK2 expression was linked back to the early observation of PUS7-dependent GSC proliferation as TYK2 and STAT1 inhibition increased GSC proliferation, whereas IFN stimulation of this pathway decreased GSC proliferation.

Overall, this study describes a novel role of pseudouridine in GBM, where PUS7-dependent pseudouridine modification of tRNAs regulates GSC proliferation via attenuation of TYK2 translation and inhibition of IFN-STAT1 pathway activity (Figure 1). This connection between pharmacologically targetable pseudouridine modifications and IFN signaling leads to several

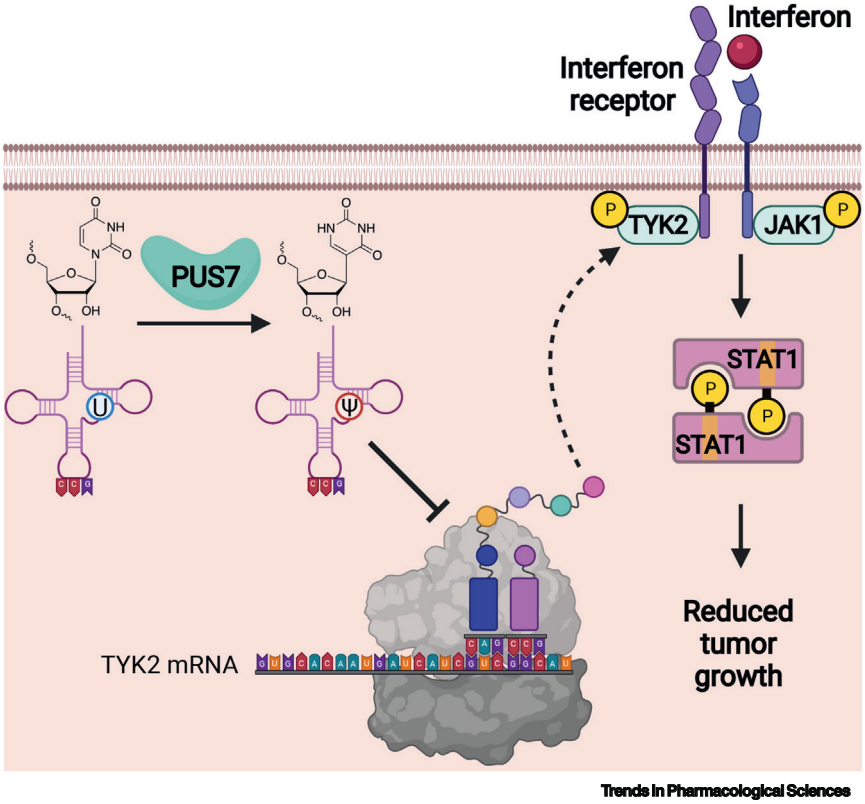

Figure 1. A cartoon depicting PUS7-dependent pseudouridine modification of tRNA-Arg-CCG at position 50, leading to inhibition of TYK2 translation in glioblastoma. The broken arrow connects the nascent TYK2 polypeptide with the mature protein, which associates with the interferon receptor. Interferon signaling through the interferon receptor leads to TYK2-mediated activation of STAT1 and overall reduced tumor growth. Figure was created with BioRender.com.

open questions and potential implications for translational efforts. Interestingly, small molecule inhibition or depletion of PUS7 alone can upregulate gene expression of IFN-stimulated genes, indicating that activity through this STAT1 pathway in GSCs may be partially independent of exogenous IFNs. In this case, PUS7 inhibition may act additively or synergistically together with exogenous IFNs to inhibit GSC proliferation. Similarly, PUS7 inhibition may be useful as an adjunct to immunotherapies to sensitize GSCs to IFNs produced locally and reduce cellular proliferation. The broad applicability of these approaches would be further supported by exploring the biology of PUS7, and of pseudouridine modifications in general, in GBMs with different somatic alterations and clinical contexts (e.g., *de novo* versus recurrent), as well as in tumor cells of different cell states and degrees of 'stemness'. Recently established patient-derived xenografts and organoid cultures that maintain this inter- and intratumoral heterogeneity would be useful systems for these lines of investigation [7].

Beyond its mechanistic insights, this study also establishes the first catalog of pseudouridine modifications in GSCs and identifies GSC-specific pseudouridine modification sites that may be linked to its tumorigenic activity. The development of small molecule inhibitors of PUS7 could be of clinical significance as it advances the emerging pursuit of epitranscriptomic-targeted therapies and offers candidates for ongoing translational efforts, though concerns exist about potential toxicities of systemic therapy given the ubiquity of pseudouridine in core cellular machinery and known roles of PUS7 in brain development and normal physiology [8]. Additional RNA modifications, such as *N*6-methyladenosine and its corresponding methyltransferase complex METTL3-METTL14, are also of active interest as promising targets for the treatment of GBM and other cancers, including acute myeloid leukemia [9,10]. This growing body of work examining epitranscriptomic mechanisms in GBM may offer opportunities to improve the treatment of patients with this deadly disease.

Declaration of interests

The authors declare no conflicts of interest.

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