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HMGB3: A Potential Immunotherapeutic Target in Glioblastoma Multiforme—Current Strengths, Existing Limitations, and Future Perspectives

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Abstract

Glioblastoma multiforme (GBM), the most aggressive primary brain malignancy, continues to pose an insurmountable therapeutic challenge due to its profound intratumoral heterogeneity, inherent drug resistance, and highly immunosuppressive tumor microenvironment [1]. The urgent need to identify actionable molecular targets to overcome these barriers has driven intensive research in GBM immunology and precision oncology. As core regulators of chromatin dynamics, inflammation, and immune modulation, the high mobility group (HMG) family is well-studied in cancer. Within it, HMGB1 and HMGB2 have been extensively investigated in GBM: HMGB1 drives GBM's immunosuppressive TME via TLR4/Akt signaling, while HMGB2 correlates with GBM stem cell maintenance and radioresistance [2–4]. In contrast, the undercharacterized HMGB3 has only recently emerged as a potential onco-immunological target. Studies in non-small cell lung cancer (NSCLC) and breast cancer show HMGB3 overexpression links to tumor immune evasion and poor prognosis [5]. Against this backdrop, Wang et al.'s study, titled "Comprehensive bioinformatics analysis identified HMGB3 as a promising immunotherapy target for glioblastoma multiforme," published in Discover Oncology, represents a significant step forward [6]. By leveraging multi-omics data and robust bioinformatics tools, the study systematically characterizes the role of HMGB3 in GBM pathogenesis, immunosuppression, and therapeutic responsiveness. This commentary evaluates the study's core strengths, critically examines its limitations, and proposes targeted future directions to fully realize the translational potential of HMGB3 in GBM treatment. All analyses are strictly based on the content of the referenced manuscript throughout the study.

Keywords: Bulk RNA-seq, Glioblastoma multiforme, HMGB3, scRNA-seq, Tumor microenvironment

Core Strengths of the Study

A key strength of Wang *et al.*'s study lies in its rigorous integration of single-cell RNA sequencing (scRNA-seq) and bulk RNA-seq, which overcomes the inherent limitations of each technology alone [6]. ScRNA-seq dissects GBM cellular heterogeneity to identify 21 cell clusters and 1,150 cell-type-specific markers, while bulk RNA-seq captures transcriptomic patterns across large cohorts. Cross-validation using authoritative databases (TCGA, GEO, HPA, TIP) confirms HMGB3 overexpression in GBM at both transcriptomic and protein levels, enhancing the reliability of conclusions. The study further excels in systematically elucidating HMGB3's functional role, linking it to GBM immunosuppression, TME

remodeling, and therapeutic resistance via WGCNA and GO/KEGG analyses—establishing its association with a non-inflamed TME, a major barrier to immunotherapy [6]. This study identifies 140 chemotherapeutic agents correlated with HMGB3 expression and demonstrates a negative correlation between HMGB3 and immune checkpoint molecules, thereby establishing HMGB3 as a predictive biomarker for therapeutic stratification. Unlike purely descriptive bioinformatics studies, the present work prioritizes clinical translation by proposing a feasible therapeutic strategy: targeting HMGB3 to convert a non-inflamed tumor microenvironment to an inflamed phenotype, in combination with immune checkpoint blockade (ICB). This approach addresses the unmet clinical needs in GBM, expands the translational implications, and accommodates the heterogeneous therapeutic requirements of diverse patient populations.

Key Limitations of the Study

Overreliance on bioinformatics without experimental validation

A central limitation of Wang *et al.*'s study is its exclusive dependence on *in silico* analyses—specifically, mining of TCGA and GEO datasets—without experimental validation to confirm HMGB3's functional role in GBM. While TCGA/GEO are invaluable for hypothesis generation in neuro-oncology, their inherent biases pose unique challenges for GBM research that directly undermine the study's conclusions and their relevance to clinical practice. Notably, the gap between TCGA/GEO-derived correlations and functional causality is well-documented in GBM immunotherapy: for example, the authors hypothesize that HMGB3 suppresses immune cell infiltration by inhibiting cytokine-cytokine receptor interactions and IL-17 signaling, but this mechanism was not tested using *in vitro* assays (e.g., HMGB3 silencing/overexpression in GBM cell lines) or in vivo models (e.g., GBM xenografts). Similarly, the proposed combination therapy lacks preclinical data to support its efficacy. Bioinformatics predictions, while powerful, are prone to false positives due to dataset biases or confounding variables; experimental validation is therefore essential to confirm that HMGB3 is not merely a correlative marker but a causal mediator of GBM immunosuppression.

Limitations in sample size and heterogeneity capture

This study utilizes a limited scRNA-seq dataset (only two GBM samples: GSM7011674 and GSM7011676), which compromises its ability to comprehensively reflect the full range of intratumoral heterogeneity in GBM [6]. GBM is renowned for its interpatient and intratumoral diversity, with distinct molecular subtypes that exhibit unique immune profiles and therapeutic responses. Analyzing only two samples risks overgeneralizing findings to a narrow subset of GBM cases, limiting the study's external validity. Additionally, the bulk RNA-seq analysis uses median HMGB3 expression as a binary cutoff (high/low) to stratify patients. This approach oversimplifies the continuous nature of HMGB3 expression and may obscure dose-dependent relationships between HMGB3 levels and clinical outcomes. Adopting a more refined analytical approach, such as quartile-based stratification or continuous expression metrics, could yield a more precise evaluation of HMGB3's prognostic and predictive value.

Incomplete mechanistic characterization of HMGB3

Although the expression of HMGB3 is higher in GBM tissues than in normal brain tissues, the mechanism underlying HMGB3 dysregulation is still not well understood. Additionally, the use of publicly available datasets might introduce biases owing to variations in sample collection and processing methods. There is also a need for multi-center validation to support the findings across diverse populations. The authors show that HMGB3 correlates with pathways such as cell cycle regulation and complement activation, but they do not elucidate the specific downstream targets or signaling cascades through which HMGB3 exerts these effects [6]. For example: How does HMGB3 suppress MHC molecule expression, a critical step in antigen presentation? What upstream regulators drive HMGB3 overexpression in GBM? While the associations between the expression of HMGB3 and the infiltration level of tumor-infiltrating immune cells (TIICs) are noted, GBM has a unique immune microenvironment characterized by a high frequency of myeloid cells, a high macrophage predominance, a low frequency of T cells, and low levels of cell surface inhibitory markers. These characteristics enable immune escape reducing the effectiveness of immune checkpoint inhibitors in GBM. However, the underlying biological mechanisms driving these correlations remain largely unexplored, limiting the interpretability of the results. Without this mechanistic depth, the development of targeted therapies against HMGB3 remains challenging, as it is unclear which downstream pathways to co-target or how to avoid off-target effects.

Lack of prognostic significance for overall survival

A surprising finding of the study is that HMGB3 expression does not correlate with overall survival (OS) or disease-specific survival (DSS) in GBM patients, despite being associated with progression-free survival (PFS) [6]. This contrasts with previous studies in other cancers (e.g., non-small cell lung cancer, breast cancer) where HMGB3 overexpression is linked to poor OS [7,8]. The lack of OS/DSS correlation undermines HMGB3's utility as a prognostic biomarker—a key clinical need in GBM, where predicting patient outcomes remains difficult. Possible explanations include the small sample size, the use of median expression cutoffs, or unaccounted confounding variables (e.g., patient age, treatment history, tumor location). However, the study does not investigate these factors, leaving the clinical relevance of HMGB3 as a prognostic marker uncertain.

Future Directions to Advance HMGB3 Research in GBM

To address the study's limitations and translate its findings into clinical practice, several targeted future directions are warranted—all of which build on the foundation laid by Wang *et al*. and integrate insights from broader HMG family and GBM immunotherapy research:

Experimental validation of HMGB3's functional role

Primarily, *in vitro* and *in vivo* experimental validation is indispensable to confirm HMGB3's causal involvement in GBM—particularly in comparison to other HMG family members. For *in vitro* assays, CRISPR-Cas9 or RNAi-mediated HMGB3 silencing in GBM cell lines (e.g., U87MG, U251) could be performed, with subsequent analysis of TME-associated phenotypes including immune cell infiltration, MHC expression, and cytokine secretion. *In vivo* studies may adopt orthotopic GBM xenograft models to test the ability of HMGB3 silencing to enhance the therapeutic efficacy of ICB agents or standard chemotherapy (e.g., temozolomide, the first-line treatment for GBM) [9]. Such experiments would not only validate HMGB3 as a promising therapeutic target but also provide robust preclinical data to justify the development of clinical trials.

Expansion to larger, diverse cohorts

To address sample size limitations, future studies should analyze HMGB3 expression in larger, multi-center GBM cohorts—ideally including longitudinal data (e.g., pre- and post-treatment samples) and detailed clinical annotations (e.g., molecular subtype, treatment regimen, OS/DSS). This strategy would enable: (1) corroboration of HMGB3's prognostic significance for OS and DSS; (2) investigation of HMGB3 expression profiles across GBM molecular subtypes; (3) evaluation of HMGB3's predictive performance for treatment response in real-world clinical settings. Additionally, adopting continuous or quartile-derived HMGB3 expression thresholds rather than binary high/low classification would provide a more refined insight into HMGB3's clinical implications.

Deep mechanistic exploration of HMGB3 signaling

To fill gaps in mechanistic understanding, future research should identify HMGB3's upstream regulators and downstream effectors in GBM. For example, chromatin immunoprecipitation sequencing (ChIP-seq) could identify transcription factors that bind to the HMGB3 promoter, while co-immunoprecipitation coupled with mass spectrometry could reveal HMGB3-interacting proteins. Additionally, single-cell ATAC-seq (assay for transposase-accessible chromatin) could link HMGB3 expression to epigenetic changes in the TME, such as chromatin accessibility in immune cells. These studies would uncover novel signaling pathways regulated by HMGB3, providing opportunities for combination therapies.

Development of HMGB3-targeted therapeutics

Finally, the study's identification of drugs negatively correlated with HMGB3 (e.g., Navitoclax, Axitinib) provides a starting point for repurposing trials. Future work could test these drugs in HMGB3-high GBM patient-derived xenografts (PDXs)—a model that better recapitulates human GBM heterogeneity than cell line xenografts. Additionally, the development of HMGB3-specific inhibitors (e.g., small molecules, neutralizing antibodies) would enable direct targeting of HMGB3, complementing repurposing strategies. Clinical trials could then evaluate these agents alone or in combination with ICB, using HMGB3 expression as a stratification biomarker to ensure patient selection.

Conclusion

Wang *et al.*'s study makes a valuable contribution to GBM research by identifying HMGB3 as a promising immunotherapeutic target and predictive biomarker. Its integrated multi-omics approach, systematic functional analysis, and clinical translational focus are notable strengths that advance our understanding of GBM immunosuppression. However, the study's reliance on bioinformatics, small sample size, incomplete mechanistic characterization, and lack of OS correlation are important limitations that must be addressed. With experimental validation, larger cohorts, deeper mechanistic research, and the development of HMGB3-targeted therapies, HMGB3 has the potential to become a transformative biomarker and therapeutic target for GBM—offering new hope for patients with this devastating disease. As the field of neuro-oncology evolves, experimental validation of computational discoveries remains indispensable to bridge the gap between *in silico* predictions and clinical application. Only through rigorous *in vitro* and *in vivo* verification can bioinformatics-derived targets like HMGB3 be reliably translated into actionable therapies, addressing the unmet clinical needs of GBM patients. This study will serve as a critical foundation for future research that prioritizes the synergy of computational biology and experimental validation, driving impactful progress in neuro-oncology immunotherapy.

Author Contributions

XL-L: Conceptualization, Writing–original draft, Writing-review & editing, Funding acquisition. FY-M: Writing–review & editing, Validation. XY-L: Writing–review & editing, Validation. JG: Writing–review & editing, Funding acquisition. CG-L: Writing-original draft, Writing-review & editing, Validation, Conceptualization, Funding acquisition.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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