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RESEARCH

Causal Association Between Sleep Deprivation and Glioblastoma Risk: Insights from Multi-Omics Analysis

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Abstract

Emerging evidence suggests that sleep deprivation may contribute to cancer risk. However, the genetic association between sleep deprivation and glioblastoma (GBM) remains unexplored. This study aimed to investigate the causal relationship between sleep traits and GBM using genome-wide association study (GWAS) data of sleep duration, sleeplessness, GBM, and immune cell traits from the UK Biobank and FinnGen databases. Mendelian randomization (MR) analyses were conducted to assess potential causal links between sleep traits and GBM risk. Mediation analysis was performed to identify immune mediators affected by sleep duration that might influence GBM development. Single-nucleus RNA sequencing (snRNA-seq) was utilized to examine cellular subpopulation changes in brain tissue from sleep-deprived (*SD*) and ad libitum sleep mice. Additionally, a mouse model of sleep deprivation was established for transcriptomic analysis. We found a significant causal association between reduced sleep duration and increased GBM risk (*IVW OR* = 6.000×10^{-5}, $P = 0.003$, Bonferroni $P = 0.025$). Sleeplessness also emerged as a potential risk factor for GBM (*OR-IVW* = 20.221, $P = 0.038$). Mediation analysis identified CD80 expression on plasmacytoid dendritic cells (pDCs) as a mediator in the association between sleep duration and GBM, with a mediation effect of 0.256. SnRNA-seq confirmed significant alterations in CD80 + pDCs in sleep-deprived mice. Transcriptomic analysis of SD mice demonstrated upregulation of GBM-related markers (Egfr, Tert, and Mgmt) and associated signaling pathways. These findings suggest a potential causal link between insufficient sleep and increased GBM risk, highlighting the importance of sleep management for GBM patients.

Keywords Glioblastoma · Sleep duration · Immunity · Mendelian randomization · Pathogenesis

Introduction

Glioblastoma (GBM), a challenging entity in the realm of neuro-oncology, presents as a highly aggressive malignancy characterized by rapid progression and a complex pathophysiological landscape (Louis et al. 2021). The quest to unravel the etiological underpinnings of GBM is beset by a multifaceted interplay of genetic, environmental, and biological determinants (Sesé et al. 2021; Kumari et al. 2023). Despite the valuable contributions of traditional epidemiological studies in shedding light on GBM's risk factors (Grochans et al. 2022; Pellerino et al. 2022), these approaches often encounter limitations in clearly delineating the direct causal pathways leading to the disease (Molinaro et al. 2019).

The role of sleep, a vital physiological process, has increasingly come to the forefront in oncological research (Chen et al. 2018; Walker and Borniger 2019). There is a burgeoning interest in exploring the potential association between sleep deprivation and heightened cancer risk, including GBM (van Kooten et al. 2019). Despite the recognized importance of sleep in cancer development, the specific causal linkage between sleep duration and the incidence of GBM has not been thoroughly examined. Traditional observational studies could identify associations between variables, but they often struggle to establish causality due to confounding factors and reverse causation

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(Colnet et al. 2024). For example, an observed association between sleep patterns and GBM could be influenced by an unmeasured confounder, or GBM itself could alter sleep patterns. These are prone to confounding because many factors (e.g., lifestyle and comorbidities) can influence both sleep patterns and GBM risk, complicating the interpretation of results. However, Mendelian randomization (MR) uses genetic variants as instrumental variables (IVs) to infer causal relationships (Burgess et al. 2017). Since genetic variants are randomly assorted at conception and generally not influenced by confounders or the disease process, MR can provide stronger evidence for causality (Burgess and Thompson 2015). For instance, genetic variants associated with sleep duration can be used to assess whether sleep duration causally affects GBM risk. By using genetic variants that are robustly associated with the exposure (sleep duration) but not directly associated with confounders, MR reduces the bias from confounding. In addition, traditional observational studies often require large, well-characterized cohorts with detailed phenotypic data, which can be resource-intensive to collect. While MR could leverage existing large-scale genome-wide association study (GWAS) data, enhancing statistical power and feasibility. Genetic consortia and biobanks provide a wealth of data that can be used for MR analyses, facilitating studies on the genetic determinants of sleep duration and their relationship with GBM. By leveraging genetic variants as instrumental variables, MR offers a more reliable approach to understanding the potential causal role of sleep in GBM etiology, ultimately contributing to more informed public health strategies and therapeutic interventions. Moreover, disruptions in normal sleep patterns are known to impact immune responses (Besedovsky et al. 2012; Jin et al. 2023), an aspect of critical relevance to GBM pathogenesis (Pombo Antunes et al. 2020). This is especially pertinent given the distinct immune microenvironment of the brain, where immune interactions play a complex role in disease progression. Unraveling the relationship between sleep, immune function, and GBM could provide key insights into the mechanisms driving this aggressive cancer.

Consequently, the primary objective of our research is to explore the association between sleep duration and the development of GBM. We hypothesize that sleep duration exerts its influence on GBM development and progression through modulating the functionality of human immunity. This study employs MR analysis, single cell and transcriptomic data to probe into the causal relationship between sleep duration and GBM. An integral part of this analysis includes exploring which immune phenotypes are potentially affected by variations in sleep duration and how these changes might serve as mediators in the pathogenesis and progression of GBM.

Material and Methods

GWAS Data Sources of Sleep-Related Traits and GBM

For this study, data on "sleep duration" and "sleeplessness/insomnia" acquired from the IEU Open GWAS database (ebi-a-GCST003839 and ukb-a- 13), involving a cohort of 128,266 and 336,965 individuals of European from the initial UK Biobank genetic data release, accessible at UK Biobank (Jones et al. 2016). The criteria for British descent included self-identification as white British and ancestral Caucasian classification confirmed through principal component analyses, details of which are available at UK Biobank. Sleep duration cohort consisted of 120,286 individuals identified as unrelated and an additional 7980 individuals categorized as first- to third-degree relatives. The GWAS dataset for GBM was procured from the FinnGen R12 database. This particular dataset encompassed a comprehensive GWAS conducted on a European cohort of 406 individuals, including a control group of 378,749 individuals. This analysis meticulously assessed approximately 16,380,303 genetic variants, each subjected to stringent quality control protocols and advanced imputation techniques to ensure the integrity and accuracy of the findings.

Data Collection of Immune Trait GWAS

In our study, we utilized GWAS summary statistics related to immune traits, which were obtained from the GWAS Catalog, specifically focusing on accession numbers ranging from GCST0001391 to GCST0002121 (Orrù et al. 2020; Wang et al. 2023). This dataset covered a wide spectrum of 731 immune traits, which were systematically categorized into groups including absolute cell counts, median fluorescence intensities (reflecting surface antigen levels), morphological characteristics, and relative cell counts. These traits encompassed a variety of immune cell types, such as B cells, T cells, and monocytes. The GWAS data were obtained from a European cohort consisting of 3757 participants, with careful measures taken to ensure there was no overlap with other cohorts.

Two-sample Mendelian Randomization Analysis

To enhance the accuracy of our MR findings, we included SNPs with a MAF above 1% to focus on common variants and mitigate the risk of false associations caused by rare variants. A rigorous threshold of P value $< 1 \times 10^{-8}$ was set for SNP-gene associations to ensure the reliability of the identified exposures. We applied a minimum F-statistic of 10 to exclude weak instrumental variables that could

introduce bias into the MR estimates. LD pruning was conducted with an r^2 threshold below 0.1 to reduce confounding effects linked to LD, following recommendations from the 1000 Genomes Project's European cohort. These steps were crucial for minimizing the influence of weak instruments and bolstering the robustness of our genetic associations. All statistical analyses were performed in R using the "TwoSampleMR" package, applying methodologies such as inverse variance weighting (IVW), weighted median, mode-based estimations, MR Egger, simple mode, and weighted mode, to explore causal relationships between exposures and outcomes. Heterogeneity among instrumental variables was evaluated using Cochran's Q statistic and corresponding *P*-values. In cases where significant heterogeneity was detected, a random-effects model was used for the IVW analysis. The MR-Egger method was employed to check for horizontal pleiotropy, with a non-zero intercept serving as an indicator. Additionally, MR-PRESSO was utilized to identify and exclude horizontal pleiotropic outliers, further ensuring the validity of our results.

Bayesian Weighted Mendelian Randomization Analysis

In our study, we applied Bayesian Weighted MR analysis to verify the potential causal relationships between sleep patterns and GBM. This approach integrates a probabilistic framework, weighting each genetic instrument based on its reliability, and uses posterior probabilities to enhance the estimation of causal effects (Zhao et al. 2020). We used Bayesian weighted median methods, which are particularly effective when some instruments might be invalid or exhibit pleiotropy. Sensitivity analyses, including Bayesian MR-Egger regression, were conducted to ensure the robustness of our findings. This method allows us to provide more precise and credible estimates of the causal links.

Mediation Analysis

To compute the mediating effect, we used the formula Beta = Beta(XZ) × Beta(ZY) (Paul et al. 2024). To determine the proportion of the mediating effect within the overall effect, we calculated $R = (\text{Beta}/\text{Beta}(XY)) \times 100\%$ (Cao et al. 2023). Once adjustments were made for potential confounders, we regarded the influence of the exposure variable on the outcome variable as a direct effect, which is defined as Direct Effect = Beta(XY) – Beta (Cao et al. 2023; He et al. 2024).

Single-Nucleus RNA Sequencing Analysis

The GSE214337 dataset (Kim et al. 2022), containing single-nucleus RNA sequencing (snRNA-seq) data from the primary motor cortex of one sleep-deprived and one ad libitum sleep mouse, was thoroughly analyzed. Data preprocessing was carried out in R using the Seurat package (v.4), starting with the import of a filtered feature-barcode matrix. To ensure data quality, genes expressed in fewer than five cells and nuclei with fewer than 200 detected genes were excluded. Additional quality control measures included filtering out nuclei with fewer than 1500 or more than 8000 detected genes, as well as those with mitochondrial gene percentages above 2% or any single gene accounting for more than 20% of total expression. Genes with at least ten counts were retained, while mitochondrial genes and Malat1 were excluded from further analysis. The dataset was normalized using the LogNormalize method. Subsequent analysis involved an initial clustering step to remove striatal neurons, followed by secondary clustering for accurate cell type identification. Principal component analysis (PCA) was conducted on the top 20 principal components, with dimensionality reduction achieved using the TSEN algorithm across 1 to 10 dimensions. Clustering was performed using the FindNeighbors function based on the top 10 dimensions, and clusters were defined with a resolution of 0.4 using the FindClusters function. Cell types within each cluster are identified using specific marker genes (Kim et al. 2022), as shown in Table S1. Comparative analysis of cell type proportions between the sleep-deprived and ad libitum sleep samples was conducted, followed by gene set enrichment analysis using the "irGSEA" package. Finally, previously identified genes were mapped onto specific cell subpopulations to observe their expression patterns.

Animal Model and Sleep Deprivation Protocol

To investigate the effects of acute sleep deprivation on brain transcriptomes, two pairs of male and female mice were used. Mice were randomly assigned to either the sleep deprivation group or the control group, ensuring one male and one female per condition. Following fear conditioning, mice in the sleep deprivation group were subjected to 6 h of sleep deprivation (Gentry et al. 2022) using gentle handling techniques to maintain wakefulness while minimizing stress. Control mice underwent identical fear conditioning but were allowed to sleep ad libitum for 6 h following training.

Sample Collection and RNA Extraction

At the end of the 6-h experimental period, whole-brain tissues were rapidly extracted from all mice and immediately flash-frozen in liquid nitrogen to preserve RNA integrity. Total RNA was isolated from the entire brain using a standardized extraction protocol. RNA quality and concentration were assessed using an Agilent Bioanalyzer to ensure high-quality input for sequencing.

Sleep-deprived Mouse Brain Tissue Transcriptomic Data Analysis

The raw data underwent preprocessing, including normalization and quality control measures, to ensure comparability across samples. Differential expression analysis was conducted through "limma" package to identify genes whose expression levels significantly differed between the sleep-deprived and control groups. Criteria for differential expression included a statistical significance threshold (the abs of log2 fold-change > 1 and adjustable *P*-value < 0.05). Gene set enrichment analysis (GSEA) was employed to investigate the enrichment of GBM-related pathways among the DEGs. The analysis leveraged the "HP_GLIOBLASTOMA_MULTIFORME" and "WP_GLIOBLASTOMA_SIGNALING_PATHWAYS" gene sets from the GSEA database to assess the association of DEGs with GBM. To quantify the activity levels of GBM-related signaling pathways in individual samples, ssGSEA was conducted. This analysis provided a score reflecting the degree of upregulation or downregulation of the pathways in the brain tissues of the sleep-deprived and control groups.

Statistical Analysis

All data were analyzed using R software. Differences between two groups were assessed using paired or unpaired Student's *t*-test, while comparisons across three or more groups were performed using one-way analysis of variance (ANOVA). Statistical significance was defined as a *P*-value less than 0.05. To account for multiple testing in examining the associations between GBM and sleep duration as well as sleeplessness, we applied a Bonferroni-corrected significance threshold of $P < 0.025$ ($\alpha = 0.05/2$) to evaluate the results. When the *P*-value was between the Bonferroni-corrected value and 0.05, suggestive evidence of association was considered, and further confirmation was required.

Results

MR Study Design

MR analysis employs genetic variants as instrumental variables to assess causal relationships between exposures and outcomes (Burgess et al. 2017). This approach is grounded in three critical assumptions (Fig. 1A). First, the genetic variants chosen as instrumental variables must have a strong association with the exposure of interest. Second, these variants should be independent of any confounding factors. Third, the genetic variants should influence the outcome solely through the exposure under investigation, without involving alternative biological pathways (Burgess and Thompson 2015). As Fig. 1B has shown, we investigate the overall causal relationship between sleep duration and GBM initially, followed by an analysis of immune cells as potential exposome factors for GBM. Subsequently, we identified immunophenotypes potentially affected by sleep duration as exposure factors.

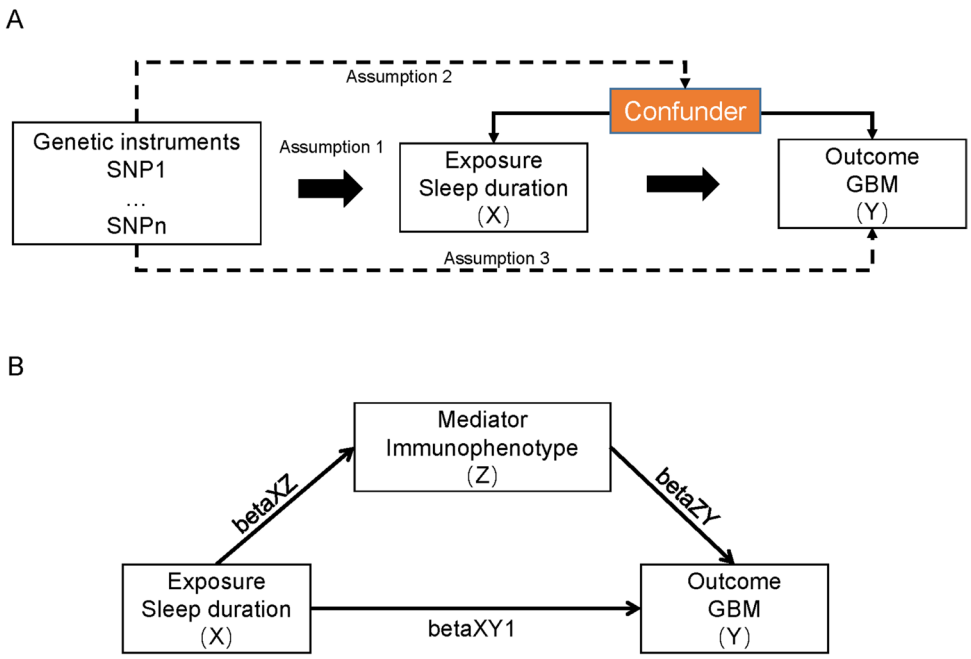

Fig. 1 Study design of this Mendelian randomization (MR) research. **A** Three assumptions of MR study. **B** Our MR study design. SNP, single-nucleotide polymorphism

Mendelian Randomization Analysis of Sleep Duration and Sleeplessness Impact on GBM

Using a stringent *P*-value threshold ($P < 5 \times 10^{-8}$), we exclude SNPs prone to LD to ensure the selection of independent variants, as illustrated in Figure S1A. Three independent SNPs were identified and utilized as instrumental variables to assess the causal relationship between sleep duration and GBM (Figure S1B). The MR analysis results for the effect of sleep duration on GBM are summarized in Fig. 2. Both the IVW and WM methods demonstrated a significant inverse association between sleep duration and the risk of GBM (*IVW OR* = 6.000×10^{-5}, 95% *CI* = [5.946×10^{-8}, 3.438×10^{-2}], $P = 0.003$; *WM OR* = 2.226×10^{-4}, 95% *CI* = [1.380×10^{-7}, 3.710×10^{-1}], and $P = 0.003$, Bonferroni $P = 0.025$).

In the MR analysis evaluating the impact of sleeplessness on GBM (Fig. 2), sleeplessness emerged as a significant positive risk factor for the development of GBM (*IVW OR* = 20.221, 95% *CI* = [1.927, 440.892], $P = 0.038$, Bonferroni $P = 0.025$). The MR analyses showed no evidence of heterogeneity, supporting the robustness of the findings for both sleep duration and sleeplessness in relation to GBM. BWMR further corroborated these results. Detailed results from the two-sample MR analysis can be found in Table S2 and Figures S1–2. Heterogeneity testing reveals no significant horizontal pleiotropy, and the "leave-one-out" sensitivity analysis indicates minimal fluctuations in the error lines, as shown in Figures S1C, D, and E and S2C and D.

Reverse Mendelian Randomization of GBM on Sleep Duration

In the reverse two-sample MR analysis examining the potential relationship between GBM and sleep duration as well as sleeplessness, a less stringent threshold of 1×10^{-5} was applied to allow for the inclusion of a wider range of instrumental variables. This approach identified eight SNPs for analysis. Despite utilizing the IVW method alongside four other MR techniques, no significant causal relationship was observed between GBM and sleep duration or sleeplessness, with all methods yielding *P*-values greater than 0.05 (Fig. 3 and Supplementary Table S3). The analysis revealed no evidence of heterogeneity or horizontal pleiotropy (Table S3). The findings consistently indicated a lack of causal association between GBM (as exposure) and sleep duration or sleeplessness.

Exploration of the Causal Effect Between Immunophenotypes and GBM

To investigate the immunological factors contributing to GBM susceptibility, we conducted a two-sample MR analysis, employing the IVW method as the primary analytic tool. From an analysis of 731 immune cell types, we identified 24 immunophenotypes as potential causal factors for GBM, each meeting a significance threshold of $P < 0.05$ (Fig. 4). These immunophenotypes encompass a diverse range of immune subsets, including 4 B cell phenotypes, 6 phenotypes within the TBNK (T cells, B cells, and natural killer cells) cluster, 3 T cell maturation stages, 5 myeloid cell-related phenotypes, 2 linked to regulatory T (Treg) cells, and 4 associated with conventional dendritic cells (cDC), as detailed in Supplementary Table 4. Figure 4 provides an overview of the MR results for these 24 immunophenotypes, including *P*-values, odds ratios (*OR*s) with 95% confidence intervals, and findings from heterogeneity and pleiotropy assessments.

Among the findings, the DP (CD4 + CD8 +) %leukocyte phenotype was significantly associated with an increased

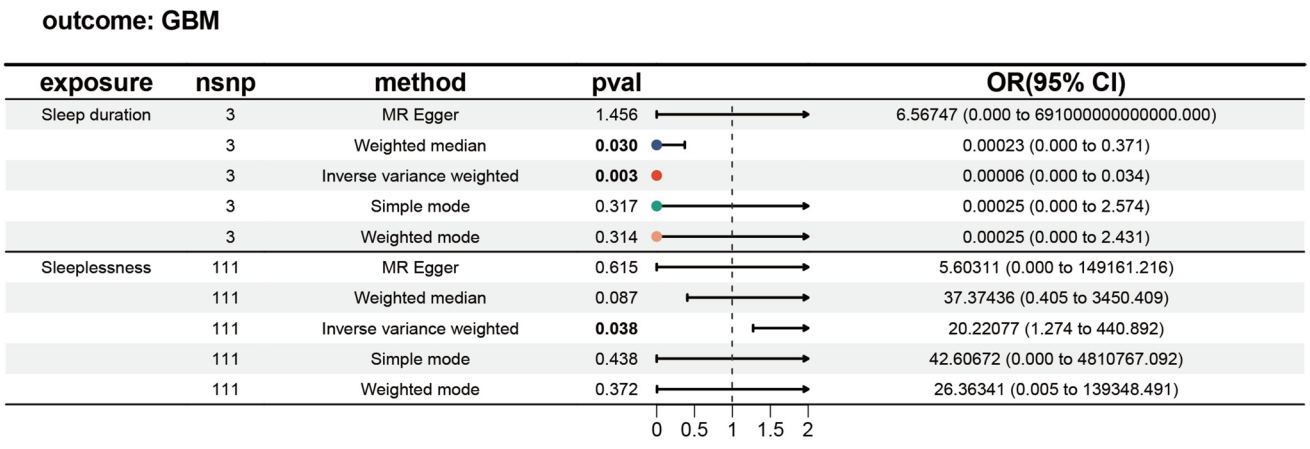

Fig. 2 Mendelian randomization analysis of sleep duration/sleeplessness effect on GBM. Forest plot summarizing the MR results from various analytical methods assessing the causal effect of sleep duration and sleeplessness on GBM

exposure: GBM

outcome	nsnp	method	pval	OR(95% CI)
Sleep duration	14	MR Egger	0.093	1.14016 (0.139 to 1.141)
	14	Weighted median	0.077	1.14017 (0.140 to 1.141)
	14	Inverse variance weighted	0.140	1.14000 (0.140 to 1.140)
	14	Simple mode	0.082	1.14020 (0.140 to 1.141)
	14	Weighted mode	0.080	1.14018 (0.140 to 1.141)
Sleeplessness	14	MR Egger	0.436	1.27161 (0.570 to 1.573)
	14	Weighted median	0.424	0.97127 (0.570 to 1.257)
	14	Inverse variance weighted	0.370	0.97126 (0.571 to 1.257)
	14	Simple mode	0.329	0.97105 (0.570 to 1.272)
	14	Weighted mode	0.453	0.97129 (0.570 to 1.257)

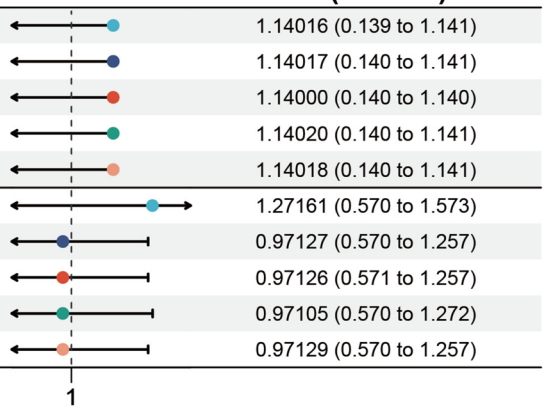

Fig. 3 Analysis of GBM Impact on sleep duration and sleeplessness via reverse MR. Forest plot summarizing the lack of significant causal relationship between GBM and sleep duration and sleeplessness across multiple MR methods, with *p*-values exceeding 0.05

outcome: GBM

exposure	nsnp	method	pval	OR(95% CI)
CD80 on plasmacytoid DC	19	Inverse variance weighted	**0.037**	0.89803 (0.794 to 0.908)
HVEM on naive CD4+	16	Inverse variance weighted	**0.041**	0.73324 (0.614 to 0.875)
CD3 on CD39+ resting Treg	20	Inverse variance weighted	**0.001**	0.70329 (0.569 to 0.869)
CD66b on CD66b++ myeloid cell	25	Inverse variance weighted	**0.003**	0.76865 (0.649 to 0.910)
CD38 on PB/PC	15	Inverse variance weighted	**0.013**	0.67114 (0.498 to 0.905)
CD20 on CD20− CD38−	15	Inverse variance weighted	**0.023**	0.53581 (0.395 to 0.726)
CD19 on IgD+ CD38dim	24	Inverse variance weighted	**0.021**	1.28544 (1.147 to 1.440)
CD19 on IgD+ CD24−	25	Inverse variance weighted	**0.031**	1.27440 (1.137 to 1.428)
Granulocyte AC	24	Inverse variance weighted	**0.019**	0.83187 (0.704 to 0.983)
CD8dim %leukocyte	18	Inverse variance weighted	**0.024**	0.75679 (0.640 to 0.894)
DP (CD4+CD8+) %leukocyte	21	Inverse variance weighted	**0.022**	1.57570 (1.148 to 2.162)
CD11c on monocyte	17	Inverse variance weighted	**0.021**	0.70138 (0.609 to 0.970)
Lymphocyte AC	19	Inverse variance weighted	**0.021**	0.80371 (0.680 to 0.950)
EM DN (CD4−CD8−) %DN	26	Inverse variance weighted	**0.021**	0.79783 (0.664 to 0.959)
CD33− HLA DR− AC	21	Inverse variance weighted	**0.026**	1.40165 (1.140 to 1.723)
CD33br HLA DR+ AC	28	Inverse variance weighted	**0.003**	0.82614 (0.750 to 0.909)
Myeloid DC AC	23	Inverse variance weighted	**0.039**	1.29409 (1.104 to 1.518)
SSC−A on CD8br	17	Inverse variance weighted	**0.030**	1.27924 (1.033 to 1.585)
CD45 on CD33− HLA DR−	14	Inverse variance weighted	**0.048**	1.32676 (1.015 to 1.735)
CD45 on CD33br HLA DR+ CD14dim	15	Inverse variance weighted	**0.045**	1.34984 (1.102 to 1.653)
FSC−A on NKT	20	Inverse variance weighted	**0.021**	0.62314 (0.464 to 0.932)
CD127 on T cell	18	Inverse variance weighted	**0.031**	0.69700 (0.508 to 0.956)
CD86 on CD62L+ myeloid DC	21	Inverse variance weighted	**0.009**	1.46981 (1.196 to 1.806)
HVEM on CM CD8br	17	Inverse variance weighted	**0.013**	0.71576 (0.620 to 0.826)

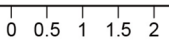

Fig. 4 MR analysis of 731 immunophenotypes on GBM. Forest plot summarizing the causal effects of various immunophenotypes on the risk of GBM as mainly analyzed by the IVW method. *OR* with corresponding 95% confidence intervals are presented, alongside *p*-values assessing the statistical significance of each association

risk of GBM (*OR* 1.576, 95% *CI* 1.148–2.162, $P = 0.022$). On the other hand, CD38 expression on PB/PC cells emerged as the most significant protective factor against GBM (*OR* 0.671, 95% *CI* 0.498–0.905, $P = 0.013$). Additionally, CD3 expression on CD39 + resting Treg cells was identified as having a significant protective effect (*OR* 0.703,

95% *CI* 0.569–0.869, *P* = 0.001). Among the immunophenotypes associated with promoting GBM development, CD45 expression on CD33bright HLA-DR + CD14 dim cells had the lowest *P*-value (*OR* 1.350, 95% *CI* 1.1025–1.653, *P* = 0.045). Rigorous sensitivity analyses and the application of MR-Egger and IVW methods for pleiotropy assessment confirmed the absence of horizontal pleiotropy, reinforcing the validity of these associations (Table S4). These findings suggest that the identified immunophenotypes play a crucial role in modulating GBM risk.

However, in the reverse MR analysis, where GBM was considered as the exposure influencing various immunophenotypes, no significant changes were observed in the immunophenotypes implicated in GBM development (Table S5).

Causal Effect of Sleep Duration on Immune Phenotypes Affecting GBM

To explore the causal connections between sleep duration and immune-related phenotypes and determine whether immune cells serve as mediators through which sleep duration influences the incidence and progression of GBM, we utilized sleep duration as the exposure variable and employed the IVW method as the primary analytical approach for MR. The analysis revealed significant effects of sleep duration on specific immunophenotypes (Fig. 5). Notably, sleep duration significantly impacted CD20 on CD20 − CD38 − (TBNK cells, IVW: *OR* 0.271, 95% *CI* 0.090–0.817, *P*-value 0.031; WM: *OR* 0.243, 95% *CI* 0.071–0.833, *P*-value 0.034), granulocyte AC (B cells, IVW: *OR* 1.510, 95% *CI* 1.490–3.751, *P*-value 0.007; WM: *OR* 1.487, 95% *CI* 1.290–4.307, *P*-value 0.029), and CD80 on plasmacytoid dendritic cells (pDCs, IVW: *OR* 5.220, 95% *CI* 1.480–18.397, *P*-value 0.015; WM: *OR* 6.300, 95% *CI* 1.430–27.771, *P*-value 0.020). Additionally, funnel plots, scatter plots, and leave-one-out sensitivity analyses indicated consistency across various MR methods, with no evidence of heterogeneity or horizontal pleiotropy affecting the results (Figure S3A-I). Detailed analytical outcomes are provided in Table S6.

Mediating Effects of Immune Phenotypes on Sleep Duration-GBM Causal Pathway

A mediation analysis was performed to determine whether the effect of sleep duration on GBM was mediated through specific immunophenotypes. Among the three immunophenotypes examined (as depicted in Figs. 4 and 5), only CD80 on plasmacytoid dendritic cells (pDCs) emerged as a potential mediator in the pathway through which sleep duration impacts GBM. The significant positive impact of sleep duration on CD80 on pDCs is noteworthy, as CD80 on pDCs emerges as a significant protective factor in GBM. This aligns with the consistent observation of sleep duration exhibiting a negative causal association with GBM. While sleep duration does influence two other immune cell types, their associations with GBM do not align, indicating that they may not be mediating factors in the negative impact of sleep duration on GBM. The analytical results delineated the total effect of sleep duration on GBM as Beta(XY) = − 15.764, with the effect of sleep duration on the mediator (CD80 on pDCs) being Beta(XZ) = 1.535, and the effect of the mediator on GBM as Beta(ZY) = − 0.169. The mediation effect, calculated as the product of Beta(XZ)

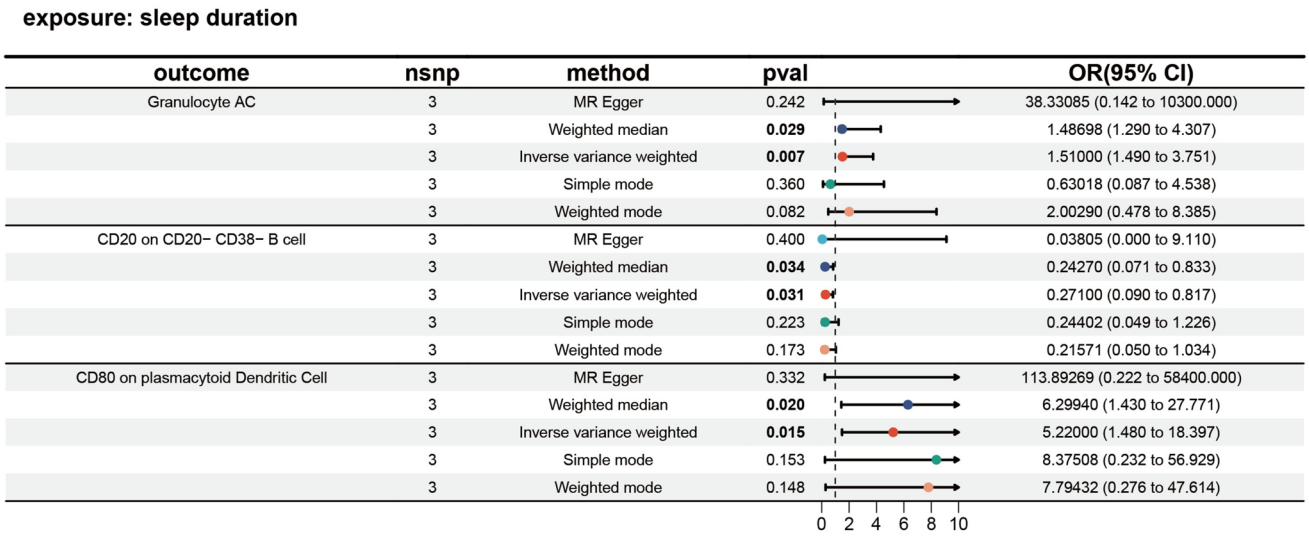

Fig. 5 Analysis of the impact of sleep duration on immunophenotypes related to GBM. The forest plot illustrates the significant effects of sleep duration on selected immunophenotypes related to GBM. Each row represents a different immunophenotype with *OR* and 95% *CI* from both IVW and WM analyses

and Beta(ZY), was −0.259. This translates to a mediation proportion of $R = 1.642\%$, indicating the fraction of the total effect mediated by CD80 on pDCs. The direct effect, which is the total effect minus the mediation effect, was Beta(XY) − Beta(XZ) × Beta(ZY) = − 15.505. These findings suggest that the expression of CD80 on plasmacytoid dendritic cells may serve as a mediator in the causal relationship between sleep duration and GBM.

Transcriptomic Alterations and Glioblastoma Signaling Enhancement Due to Sleep Deprivation

Through comparative analysis of sleep-deprived and control mice, we identified 938 differentially expressed genes exhibiting significant co-expression correlations (Figure S4A), predominantly participating in ribosomal function-related pathways (Figure S4B and C). Notably, an upregulation in the expression of molecules such as Egfr, Tert, and Mgmt, closely associated with GBM, was observed in the brain tissues of sleep-deprived mice (Fig. 6A). For the GSEA, we focused on pathways associated with GBM to further investigate the impact of sleep deprivation on oncogenic signaling. Two specific gene sets, "HP_GLIOBLASTOMA_MULTIFORME" and "WP_GLIOBLASTOMA_SIGNALING_PATHWAYS," were selected from the GSEA database. Subsequently, single-sample GSEA was performed to quantify the activity levels of GBM-related pathways in brain tissue samples from both sleep-deprived and control mice. Our findings indicated a significant upregulation of GBM-related signaling pathways in the brain tissues of the sleep-deprived cohort (Fig. 6B).

Comparison of Single-Nucleus Transcriptomic Profiles between Sleep-Deprived and Ad Libitum Sleep Mice

The single-nucleus RNA sequencing (snRNA-seq) analysis of the primary motor cortex from sleep-deprived and ad libitum sleep mice revealed significant alterations in cell type proportions. CD80 on pDCs was chosen as a focal point because it emerged as a crucial mediator in the causal pathway linking sleep duration to GBM, based on our MR mediation analysis. Figure 7A and B present t-SNE plots that demonstrate the clustering of various cell populations, with a specific focus on the CD80 + pDCs. In the sleep-deprived condition, a marked reduction in the proportion of CD80

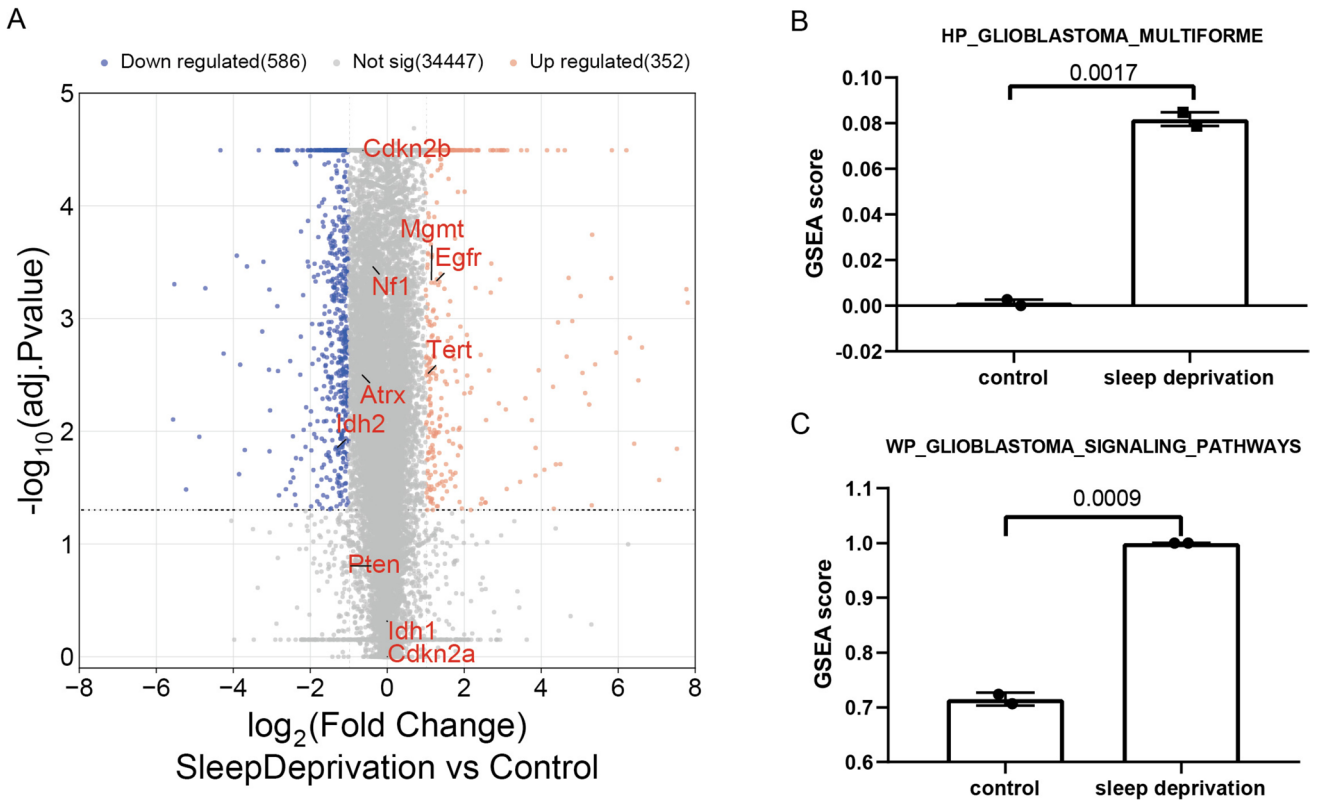

Fig. 6 Differential expression and pathway analysis among sleep deprivation and control mice brain tissue. **A** Volcano plot illustrating the differential gene expression between sleep-deprived and control mice, highlighting key GBM-associated markers. **B** and **C** Bar graph showing GSEA scores for the "HP_GLIOBLASTOMA_MULTIFORME" and "WP_GLIOBLASTOMA_SIGNALING_PATHWAYS" among control and sleep deprivation mice brain tissue

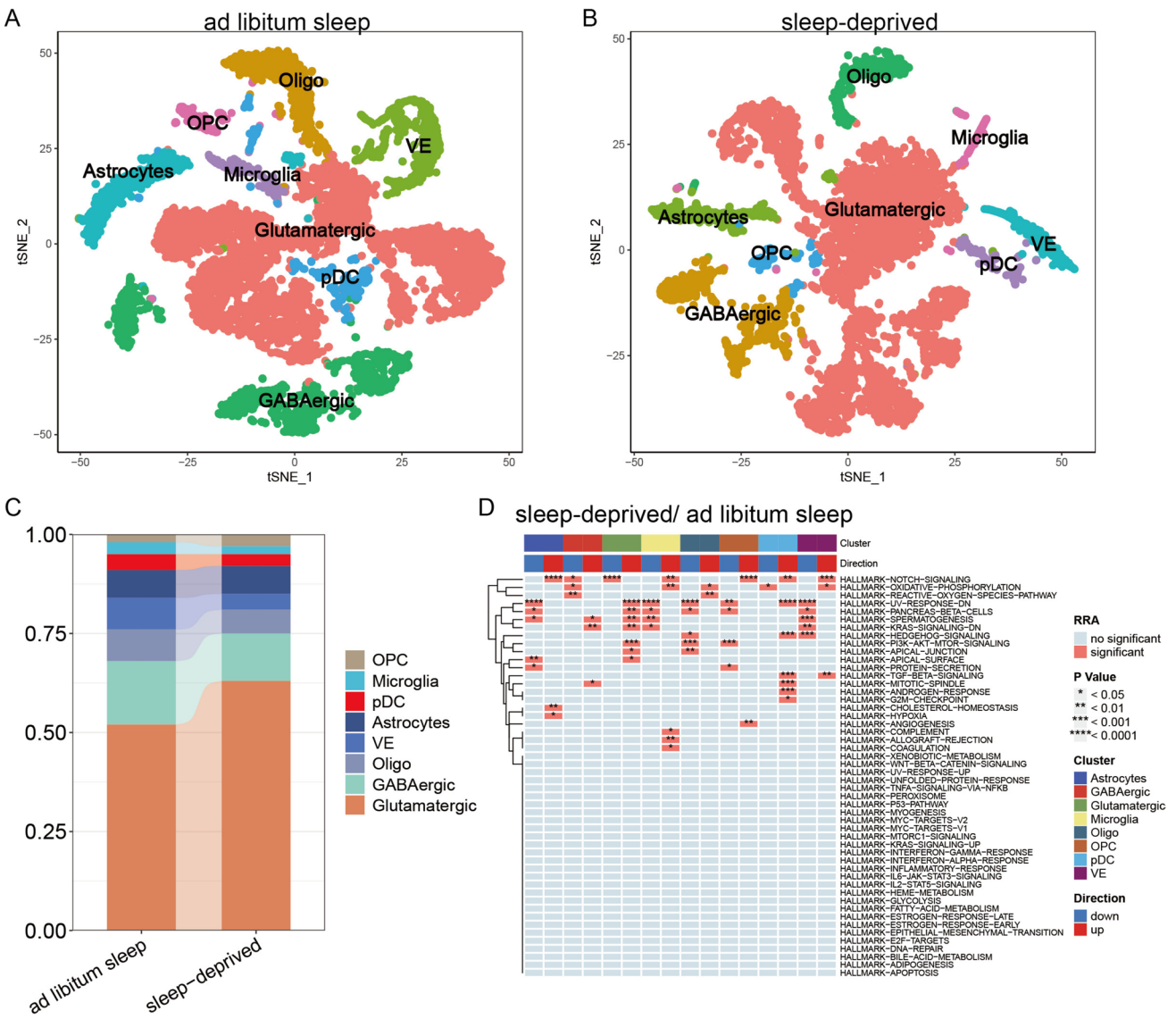

Fig. 7 Single-nucleus transcriptomic analysis of the primary motor cortex in sleep-deprived and ad libitum sleep mice. **A** and **B** t-SNE plots illustrating the distribution and clustering of major cell types, including glutamatergic neurons, GABAergic neurons, astrocytes, oligodendrocytes (Oligo), oligodendrocyte progenitor cells (OPC), microglia, vascular endothelial cells (VE), and plasmacytoid dendritic cells (pDCs), with a specific focus on CD80 + pDCs in the ad libitum sleep (**A**) and sleep-deprived (**B**) conditions. **C** Stacked bar plot comparing the relative proportions of each cell type. **D** Heatmap of gene set enrichment analysis results. Pathways are categorized by their direction of regulation (up or down) and their statistical significance, with *p*-value thresholds denoted by varying shades of red and blue

+ pDCs is observed compared to the ad libitum sleep controls. This reduction is quantitatively confirmed in Fig. 7C, where the relative abundance of CD80 + pDCs shows a significant decline in sleep-deprived samples. Pathways such as "HALLMARK_NOTCH_SIGNALING," "HALLMARK_TNFA_SIGNALING_VIA_NFKB," and "HALLMARK_APOPTOSIS" are prominently activated. This upregulation in pDCs suggests that sleep deprivation may induce a pro-tumorigenic environment by altering the signaling landscape within these immune cells, potentially contributing to an increased risk of tumorigenesis in the brain.

Reduction of CD80 Expression in pDCs and Upregulation of EGFR in Sleep-Deprived Mice

The analysis of single-cell RNA sequencing data revealed significant alterations in the expression of key genes within the primary motor cortex of sleep-deprived versus ad libitum sleep mice, particularly focusing on CD80 and EGFR. Figure 8 presents the t-SNE plots depicting the expression patterns of CD80, Mgmt, Egfr, Tert, Cdkn2b, and Gapdh across different cell types. In ad libitum sleep mice (Fig. 8A), CD80 expression is distinctly localized within a specific

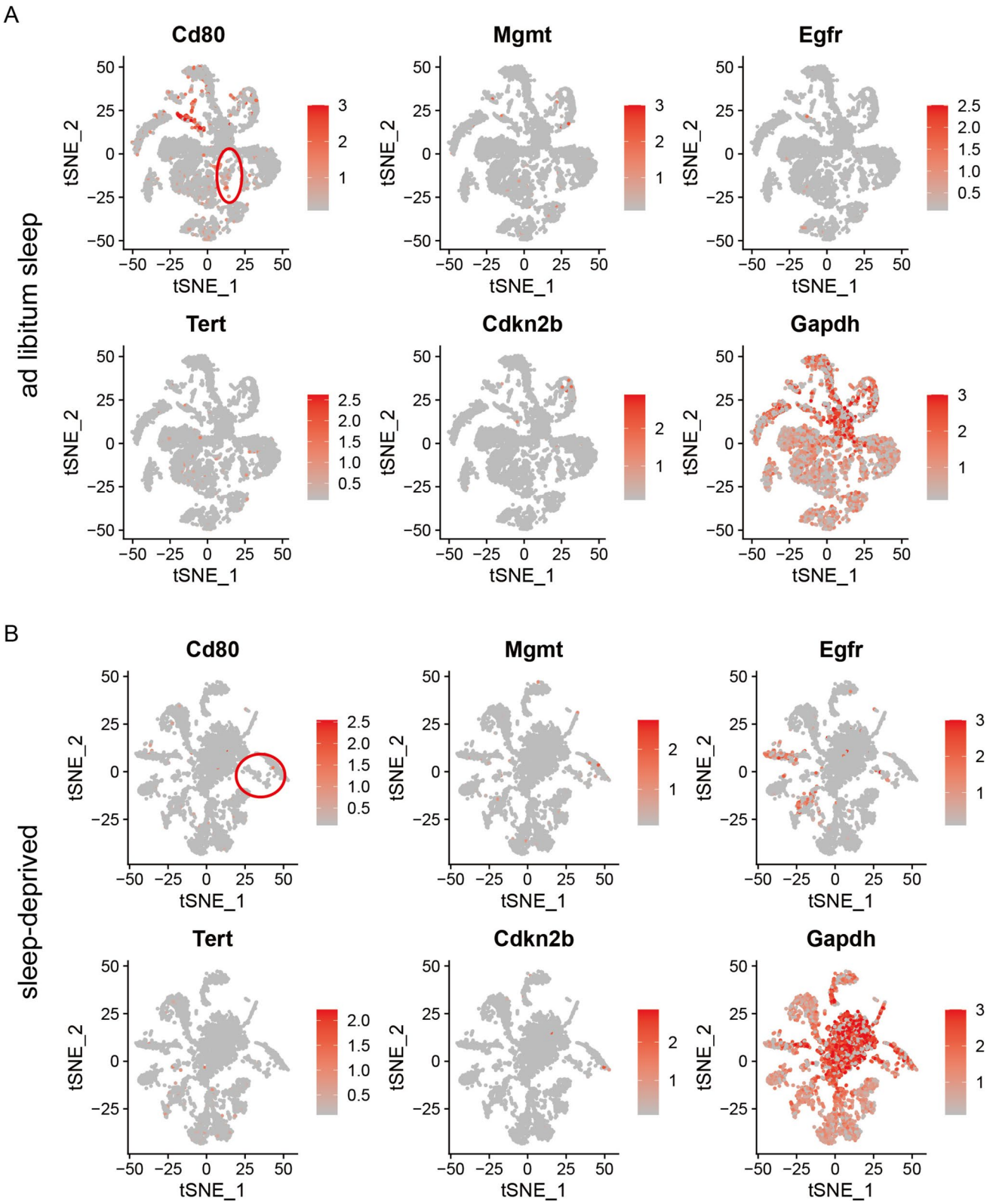

Fig. 8 t-SNE visualization of gene expression in the primary motor cortex of sleep-deprived and ad libitum sleep mice. **A** Expression patterns of Cd80, Mgmt, Egfr, Tert, Cdkn2b, and Gapdh in ad libitum sleep mice. **B** Expression patterns of the same genes in sleep-deprived mice

cluster corresponding to CD80 + plasmacytoid dendritic cells (pDCs), as indicated by the red circled area. However, in the sleep-deprived condition (Fig. 8B), there is a marked reduction in CD80 expression within this same cluster, indicating that sleep deprivation significantly diminishes the presence of CD80 in pDCs. Conversely, EGFR, a critical marker associated with glioblastoma multiforme (GBM), exhibits an increase in expression in the sleep-deprived samples compared to the ad libitum sleep condition.

Discussion

Although traditional research often employs multicentric and multi-dataset approaches to investigate risk factors for certain diseases (Samanic et al. 2021; Huang et al. 2021.), it is important to acknowledge that inherent biases in results cannot be entirely overlooked due to several objective reasons. For instance, in studies exploring the impact of sleep on GBM patients, one must consider potential confounders that could skew the findings. Factors such as the use of steroids, the anxiety associated with a new diagnosis of a fatal brain cancer, and the amount of time spent in a hospital environment can all significantly influence sleep duration in these patients. However, MR analysis is adept at bypassing these clinical confounders, providing a more reliable estimation of the causal relationships between exposure factors and outcomes (Sanderson et al. 2022). In this study, we elucidate the potential genetic interplay between sleep duration/sleeplessness and the pathogenesis of GBM through a MR analysis. As Fig. 9 described, our analysis delineates an underlying pathway where sleep duration (X) ostensibly exerts an influence on GBM development (Y), quantified by the regression coefficient betaXY1. Concurrently, we investigate the ramifications of sleep duration on immunophenotypic variations (Z), captured by the parameter betaXZ. Further scrutiny reveals that these immunophenotypes, in turn, bear important effects on GBM, indicated by betaZY. These

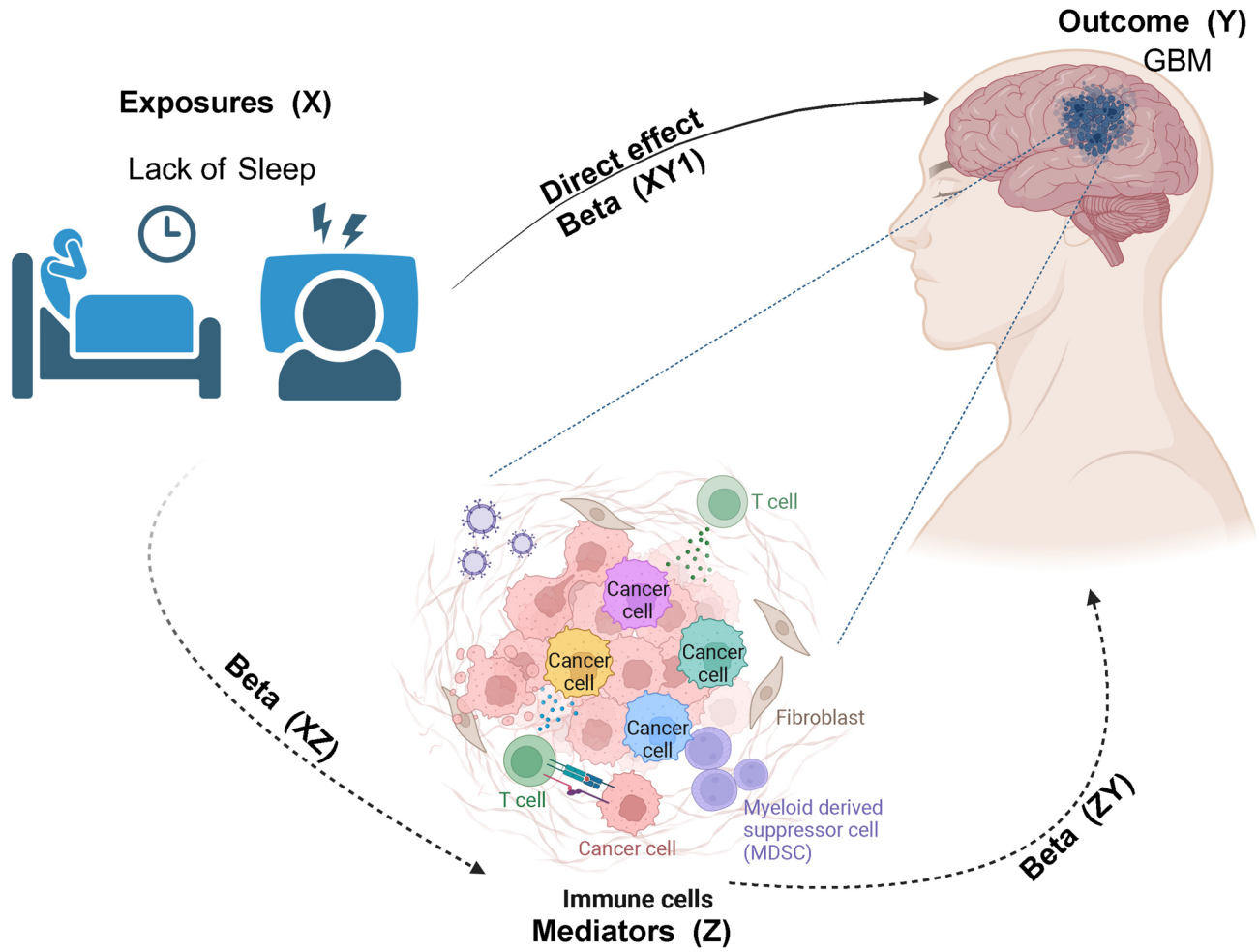

Fig. 9 Schematic representation of the mediation and single-nucleus analysis exploring the relationships between sleep, immunophenotypes, and GBM

findings are further substantiated by our sn-RNA sequencing analysis of the cortex from sleep-deprived mice. This analysis demonstrated a significant reduction in the expression of CD80 on pDCs in sleep-deprived conditions. Additionally, there was a notable upregulation of GBM-related markers such as Egfr and Tert, as well as the activation of GBM-associated signaling pathways, in the brain tissues of sleep-deprived mice. These results suggest that sleep deprivation may contribute to a tumorigenic microenvironment by modulating the immune landscape, particularly through the downregulation of CD80 on pDCs and the upregulation of oncogenic pathways.

Circadian rhythms, the endogenous oscillators of the human body, are instrumental in regulating myriad physiological functions, including the cell cycle (Farshadi et al. 2020) and apoptosis (Yang et al. 2020). Sleep quality is intimately linked to immune competence—an essential defense against neoplastic processes (Besedovsky et al. 2012; Scheiermann et al. 2013; Garbarino et al. 2021). Insufficient sleep may compromise this protective mechanism, indirectly augmenting the risk and progression of tumors such as GBM (Noorvash et al. 2022). Concurrently, inadequate sleep is known to elevate systemic inflammation, a condition recognized as a contributor to carcinogenesis (Mullington et al. 2010), including GBM (Jin et al. 2023). However, research incorporating cohorts from the UK Biobank and two pioneering longitudinal studies (Samanic et al. 2021), which demonstrated no significant connection between sleep duration and glioma risk, including GBM, stands in contrast to our findings. This divergence may stem from methodological variances, demographic disparities, or differences in sleep duration assessments. An additional noteworthy aspect of this study (Samanic et al. 2021) is its exclusive reliance on surveys conducted among GBM patients without the inclusion of a control group, such as individuals in good health. Consequently, the baseline conditions of GBM patients may have undergone alterations, rendering the use of a 7-h threshold for differentiation seemingly inappropriate. However, viewed from an alternative perspective, this observation aligns with our own research findings, as our study results similarly indicate that GBM may not significant influence the sleep duration of individuals.

Moreover, we identified 24 immunophenotypes causally linked with GBM. Notably, the DP (CD4 + CD8 +) leukocyte phenotype was associated with an elevated risk of GBM, while the expression of CD38 on PB/PC cells emerged as a significant protective factor. Typically, CD4 + T cells and CD8 + T cells play distinct roles in immune responses (Chien et al. 1996) — CD4 + T cells primarily act as "helper" cells, regulating immune responses (Tubo and Jenkins 2014), while CD8 + T cells function as "cytotoxic" cells, directly targeting infected or aberrant cells (Bevan 2004), including cancer cells. DP T cells, which express both CD4 and CD8 molecules, are an uncommon occurrence in mature T cells (Parel and Chizzolini 2004). The specific role of DP T cells in GBM remains unclear, with their potential involvement in the immune response to GBM warranting further investigation. CD38 is a surface molecule broadly expressed on various immune cells, including peripheral blood (PB) and plasma cells (PC) (Lund et al. 1998). The role of CD38 in GBM is currently limited in existing research. Theoretically, CD38 could influence the function of immune cells within the GBM tumor microenvironment, potentially relating to tumor growth, spread, or immune evasion (Manna et al. 2020). Although the precise role of CD38 in GBM is not fully understood, its widespread expression in the immune system holds potential as a focus for future research, especially in exploring novel immunotherapeutic strategies. Among these immunophenotypes, our study identified 3 immunophenotypes potentially impacted by sleep duration that influence GBM onset, including CD20 on CD20 − CD38 cells, granulocyte AC cells, and CD80 on plasmacytoid dendritic cells. CD20 is typically expressed on B cells but may be absent or expressed at lower levels on certain cells, such as CD20 − CD38 cells (Rougé et al. 2020; Pavlasova and Mraz 2020). In GBM, B cells and other immune cells can influence the tumor microenvironment and immune response (Lee-Chang et al. 2019; Pombo Antunes et al. 2020). CD20 − CD38 cells may be part of this process, but their exact role remains unclear. The expression of CD38 on granulocytes might relate to immune responses, inflammatory reactions, or tumor growth (Kageyama and Katayama 2020; Tommy Gambles et al. 2022). Granulocytes may indirectly affect GBM progression by influencing immune responses or promoting inflammation within the tumor microenvironment (Drill et al. 2020; Blank et al. 2021; Krishnan et al. 2021). Plasmacytoid dendritic cells (pDCs) are a unique subtype of dendritic cells, crucial for their role in regulating immune responses (Ye et al. 2020; Ziegler-Heitbrock et al. 2023). CD80 is a co-stimulatory molecule typically expressed on dendritic cells, vital for T cell activation (Trzupek et al. 2020; Kennedy et al. 2022). In GBM, pDCs may regulate T cell responses by expressing CD80, thereby affecting tumor immune evasion or anti-tumor immune responses (Ahmed et al. 2023). The expression and function of CD80 could play a significant role in developing immunotherapeutic strategies against GBM, particularly in enhancing T cell-mediated anti-tumor reactions (Liu et al. 2021). This study suggests that sleep duration may inhibit GBM onset and progression primarily by promoting CD80 on plasma-like dendritic cells. Theoretically, sleep may indirectly affect dendritic cell activity by influencing the overall functionality of the immune system, with possible mechanisms including the regulation of hormone levels,

inflammatory responses, and cytokines (Garbarino et al. 2021). These factors could impact the function of immune cells, including pDCs (Dimitrov et al. 2007).

The findings of this study underscore the critical importance of incorporating sleep management into the perioperative care for glioma patients. Historically, sleep management has not been emphasized in the clinical guidelines for glioma care (Weller et al. 2024). However, sleep deprivation may significantly impair the systemic immune function, particularly affecting pDCs, and thereby influence the progression of GBM. In brain tissues of sleep-deprived mice, we observed significant upregulation of markers associated with GBM development, such as cdk4, egfr, and mgmt, as well as key tumor signaling pathways. These observations highlight the need to prioritize sleep pattern management as an important indicator in the care of GBM patients. Adequate sleep may serve as a preventative measure against the onset of GBM. Postoperative sleep sufficiency, anxiety reduction, and the implementation of sleep therapies could be effective strategies in preventing tumor recurrence.

However, this study's findings are based on MR analysis, which, although effective for inferring causality by reducing confounding and reverse causation, lack validation through experimental or clinical research. The absence of corroborative clinical samples or animal studies limits the direct applicability of the results to clinical contexts. Future research, including longitudinal and prospective clinical studies, is essential to confirm the genetic association between sleep duration and GBM and to explore its practical implications in GBM risk and progression. Additionally, this study's another limitations include the differences between sleep traits (sleep duration, sleeplessness, and experimental sleep deprivation), which may affect the generalizability and interpretation of our findings on sleep's role in GBM pathogenesis.

Conclusions

This study identified a significant genetic association between sleep duration and GBM, with CD80 expression on plasmacytoid dendritic cells as a key mediator. These findings offer insights into sleep's role in GBM pathogenesis, highlighting the need for sleep management in GBM prevention and treatment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12031-025-02354-3.

Author Contributions R.D., Mu.M., and A.Y. contributed to the conception and design of this study. S.D. and X.C. contributed to the analysis and interpretation of data, T.X., W.S. collaborated closely with Ma.M. in project oversight. All authors read and approved the final manuscript.

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Data Availability The datasets generated and/or analyzed during the current study are available in the NHGRI-EBI GWAS Catalog at https://www.ebi.ac.uk/gwas/. Transcriptomic data from sleep-deprived mice are available upon reasonable request from the corresponding author.

Declarations

Ethics Approval and Consent to Participate Ethical review and approval can be accessed in the original studies. Informed consent was obtained from all subjects in the original genome-wide association studies. In this MR study, only summary-level statistics were used. No identifiable private information was contained in the GWAS datasets.

Consent for Publication Not applicable.

Competing interests The authors declare no competing interests.

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