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Original Article

Low expression of polo-like kinase 2 predicts high postoperative seizure recurrence in glioma-related epilepsy

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Abstract: Objective: To investigate the association between tumor expression of polo-like kinase 2 (PLK2) and postoperative seizure recurrence in glioma-related epilepsy (GRE) patients, and to explore mechanisms related to synaptic function and inflammation. Methods: We retrospectively analyzed a cohort of 189 adult patients with supratentorial glioma and preoperative seizures. Among them, 98 were classified as PLK2-low and 91 as PLK2-high based on the histoscore. PLK2 expression in tumor tissues was quantified using immunohistochemistry (IHC) and quantitative real-time PCR (qRT-PCR), while serum PLK2 levels were measured by enzyme-linked immunosorbent assay (ELISA). Interictal discharges, residual tumor volume, peritumoral metabolism, synaptic protein expression, and cytokine profiles were also assessed. Cox regression analysis was conducted to identify independent predictors of postoperative seizure recurrence. Results: Tumors with low PLK2 expression exhibited significantly reduced IHC H-scores, protein and mRNA levels, and serum concentrations (all $P < 0.05$). Patients in the PLK2-low group showed higher interictal spike rates, larger residual tumor volume, and elevated peritumoral standardized uptake values (all $P < 0.05$). Expression of synaptic markers PSD-95 and synaptophysin was decreased, while levels of interleukin-1β, tumor necrosis factor-α, and interleukin-6 were significantly elevated (all $P < 0.05$). Postoperative seizure recurrence was more frequent in the PLK2-low group, and low PLK2 expression remained an independent predictor in multivariable Cox regression analysis. Conclusion: Low PLK2 expression is strongly associated with postoperative seizure recurrence, synaptic dysfunction, and neuroinflammation, suggesting its use as a predictive biomarker and therapeutic target in GRE.

Keywords: Polo-like kinase 2, glioma-related epilepsy, seizure recurrence, synaptic plasticity, neuroinflammation, biomarker

Introduction

Glioma-related epilepsy (GRE) is a common neurological complication in patients with diffuse glioma, significantly affecting quality of life and prognosis. Seizures may present as the initial symptom of glioma or develop postoperatively despite maximal safe resection [1]. Preoperative seizures occur in 40% to 90% of glioma cases, with higher rates in low-grade gliomas (80-90%) and lower rates in high-grade gliomas (40-60%) [2, 3]. Despite gross total resection, approximately 30% of GRE patients experience seizure recurrence, emphasizing the need for reliable prognostic biomarkers [4].

The pathophysiology of GRE is multifactorial, involving tumor-intrinsic factors, peritumoral microenvironment changes, and network-level disturbances. At the cellular level, glioma cells disrupt ionic homeostasis and neurotransmitter cycling through aberrant expression of glutamate transporters, GABA-A receptor subunits, and voltage-gated ion channels, inducing a hyperexcitable peritumoral cortex [5]. Simultaneously, tumor-driven extracellular matrix remodeling and neuroinflammation promote astrocyte activation and blood-brain barrier disruption, further enhancing epileptogenesis [6]. At the systems level, magnetoencephalography and functional MRI connectome analy-

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ses have shown that disrupted structural and functional connectivity correlates with seizure risk in glioma patients [7].

Genetic alterations characteristic of gliomas also contribute to epileptogenesis. Mutations in IDH1/2, commonly found in World Health Organization (WHO) grade II-III tumors, lead to the accumulation of the oncometabolite D-2-hydroxyglutarate, which mimics glutamate at N-methyl-D-aspartate (NMDA) receptors, increasing neuronal excitability. Patients with IDH-mutant gliomas have significantly higher rates of preoperative seizures compared to those with wild-type tumors [8]. Furthermore, the IDH1R132H mutation has been linked to both preoperative seizures and postoperative seizure recurrence, serving as an independent risk factor in multivariable analysis [9].

Polo-like kinase 2 (PLK2) is a serine/threonine kinase involved in both cell-cycle regulation and synaptic homeostasis. In neurons. PLK2 is activity-induced and regulates synaptic scaling by phosphorylating synaptic proteins such as SPAR, thus stabilizing excitatory synaptic strength during heightened activity [10]. In cancer biology, PLK2's role is context-dependent, with oncogenic and chemoresistance-associated functions reported in colorectal and breast cancers [11, 12]. Transcriptomic analyses integrating Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), and Chinese Glioma Genome Atlas (CGGA) datasets consistently show PLK2 downregulation in glioblastoma compared to normal brain tissue [13]. *In vitro*, PLK2 overexpression in U87MG and U251 cell lines inhibits proliferation, migration, and survival while inducing G0/G1 cell-cycle arrest and apoptosis; *in vivo*, PLK2 overexpression significantly suppresses glioblastoma xenograft growth [14, 15]. Given the high burden of postoperative seizure recurrence in GRE, and the dual roles of PLK2 in synaptic regulation and glioma suppression, PLK2 represents a promising candidate biomarker for GRE outcomes. Traditional clinical predictors - such as younger age, temporal lobe involvement, extent of resection, and preoperative seizure history - have limited predictive value when used alone [4, 16]. Incorporating molecular markers like PLK2 expression may enhance risk stratification, enable personalized antiepileptic therapy, and guide postoperative surveillance.

In this retrospective cohort study of 189 adult patients with histologically confirmed supratentorial gliomas and preoperative epilepsy, tumor PLK2 expression was quantified using immunohistochemistry (IHC) and quantitative real-time PCR (qRT-PCR). These molecular measures were correlated with postoperative seizure recurrence during a minimum 12-month follow-up, alongside clinicopathologic variables, including patient age, tumor grade and location, extent of resection, IDH1/2 mutation status, and antiepileptic drug regimen. The specific aims were to: 1) stratify postoperative seizure risk by PLK2 expression level; 2) assess the prognostic value of PLK2 relative to established clinical predictors using multivariable Cox regression and receiver operating characteristic (ROC) analyses; and 3) explore mechanistic associations between PLK2-mediated pathways - such as synaptic protein regulation and neuroinflammation - and glioma-related hyperexcitability. By elucidating the relationship between PLK2 expression and GRE outcomes, this study aims to establish PLK2 as a mechanism-based biomarker to improve postoperative seizure control and patient quality of life.

Patients and methods

Study design and patient cohort

A retrospective cohort study was conducted at the General Hospital of Northern Theater Command, enrolling 189 adult patients (≥ 18 years) with histologically confirmed supratentorial gliomas and preoperative epilepsy, treated between January 2015 and December 2023. Among the 189 eligible patients, 98 were classified as PLK2-low and 91 as PLK2-high based on the H-score. Inclusion criteria were: 1) patient underwent maximal safe resection with at least 12 months of follow-up; 2) age ≥ 18 years; 3) histopathologically confirmed supratentorial glioma (WHO grade II-IV, 2016/2021 WHO classification); 4) documented history of preoperative epilepsy (≥ 1 unprovoked seizure before surgery). Exclusion criteria included: 1) patients with infratentorial gliomas or non-glioma tumors; 2) seizures attributable to acute perioperative complications (e.g., within 1 month after surgery); 3) history of other neurological disorders that may predispose to seizures (e.g., stroke, traumatic brain injury, CNS infection, metabolic encephalopathy); 4) prior intra-

cranial surgery, radiotherapy, or chemotherapy before the glioma resection; 5) incomplete clinical data or loss to follow-up within 12 months. The primary endpoint was postoperative seizure recurrence, defined as any unprovoked seizure occurring ≥ 1 month after surgery. The study protocol was approved by the institutional review board, with informed consent waived due to the retrospective and de-identified design.

Clinical data collection

Demographic and clinical variables - including age, sex, Karnofsky Performance Status, seizure semiology and frequency, tumor location and maximum diameter on preoperative MRI, WHO grade (2016/2021 criteria), and extent of resection (gross total vs. subtotal, determined by MRI within 72 hours) - were extracted from medical records and independently verified by two investigators.

Neurophysiological and imaging assessments

Routine 30-minute scalp EEG (10-20 system) was performed pre- and postoperatively, and in 112 patients, 24-hour video-EEG was used to record ictal events and quantify interictal spike frequency [4]. All patients underwent 3.0-T MRI, including T1 (pre-/post-contrast), T2-FLAIR, and diffusion-weighted sequences. Tumor and residual volumes were segmented on contrast-enhanced T1 images using semi-automated software. In 48 cases, postoperative ^{18}F-FDG PET/MRI fusion was performed within one month to measure peritumoral standardized uptake value mean (PET-SUV).

Tissue handling and preparation

Archival formalin-fixed, paraffin-embedded (FFPE) tumor blocks were sectioned at 4 μm for IHC, while adjacent blocks were used for DNA and RNA extraction. Matched snap-frozen specimens were stored at -80°C for protein assays and cytokine profiling.

IHC

FFPE glioma sections (4 μm) were deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval in citrate buffer (pH 6.0) at 95°C for 20 minutes. Endogenous peroxidase activity was blocked with 3% H_2O_2, and sections were incubated with 5% normal goat serum for 30 minutes. Slides were then incubated overnight at 4°C with rabbit anti-PLK2 (Abcam, ab137539; 1:200), followed by biotinylated goat anti-rabbit secondary antibody and streptavidin-HRP (Vector Laboratories). Signal was visualized with DAB chromogen, and nuclei were counterstained with hematoxylin. Two neuropathologists, blinded to clinical data, independently scored staining using the H-score method (range 0-300), calculated as $\Sigma(Pi \times i)$, where i = 0-3 indicates staining intensity and Pi is the percentage of cells at that intensity. Tumors were classified as PLK2-low (H-score < 140) or PLK2-high (H-score ≥ 140).

qRT-PCR

Total RNA was extracted from snap-frozen tissue using the RNeasy Mini Kit (Qiagen) and treated with DNase I. cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real-time PCR was performed using SYBR Green Master Mix (Applied Biosystems) on a QuantStudio 5 system with the following primers: PLK2 forward 5'-AGCTCAGGAGGAGGACGATG-3', reverse 5'-TGGCATCTTCAGGTTCTTCC-3'; GAPDH forward 5'-GAAGGTGAAGGTCGGAGTC-3', reverse 5'-GAAGATGGTGATGGGATTTC-3'. Cycling conditions were 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Relative PLK2 expression was calculated by the $2^{-\Delta\Delta Ct}$ method with GAPDH as the internal reference.

Enzyme-linked immune sorbent analysis (ELISA)

Preoperative serum was collected by centrifugation at 2,000 g for 10 minutes and stored at -80°C. Human PLK2 concentrations were measured in duplicate using the Quantikine ELISA Kit (R&D Systems, DY3838-05) according to the manufacturer's instructions. Optical density was recorded at 450 nm, and concentrations were interpolated from a five-parameter logistic standard curve. Tumor lysates (1 mg/mL) were analyzed with the Bio-Plex Pro Human Panel on a Luminex 200 system to quantify interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6).

Table 1. Clinical characteristics and surgical outcomes

Data	PLK2-low (n = 98)	PLK2-high (n = 91)	t or χ^2 value	*p* value
Age (years, mean ± SD)	48.1 ± 12.9	46.3 ± 14.1	0.881	0.381
Male sex (%)	53 (54.08%)	49 (53.84%)	0.002	0.964
Preop KPS (median, IQR)	80 (70-90)	80 (70-90)	0.025	0.875
WHO grade II (%)	33 (33.67%)	31 (34.06%)	0.005	0.943
WHO grade III (%)	30 (30.61%)	28 (30.8%)	0.000	0.982
WHO grade IV (%)	35 (35.71%)	32 (35.16%)	0.011	0.916
Gross total resection (%)	69 (70.41%)	73 (80.22%)	2.52	0.112
Seizure recurrence (%)	43 (43.88%)	18 (19.78%)	12.22	< 0.001

PLK2, Polo-like kinase 2.

Subgroup and stratified analysis

To assess the combined effect of PLK2 expression and clinical variables on seizure recurrence risk, subgroup analyses were performed. Patients were stratified by PLK2 status (low vs. high, using an H-score cutoff of 140), extent of resection (gross total vs. subtotal), and tumor location (temporal vs. non-temporal).

Statistical analysis

Continuous variables were expressed as mean ± SD, tested for normality using the Shapiro-Wilk test, and compared with t-tests or Mann-Whitney U tests. Categorical variables were expressed as frequency and percentage and analyzed using χ^2 or Fisher's exact tests. To assess the combined effects of PLK2 expression and clinical factors on seizure recurrence risk, subgroup analyses were performed, stratifying patients by PLK2 status, extent of resection, and tumor location (temporal vs. non-temporal). The predictive performance of the PLK2 IHC H-score was evaluated using receiver operating characteristic (ROC) analysis, with area under the curve (AUC), sensitivity, specificity, and the Youden index calculated to determine the optimal threshold. Seizure-free survival was estimated using Kaplan-Meier curves and compared by log-rank tests across PLK2 expression groups, extent of resection, and tumor location. Cox proportional hazards models were applied for univariate and multivariate analyses, adjusting for clinical and molecular variables. Variables with $P < 0.10$ in univariable analysis were entered into multivariable models, and the proportional hazards assumption was verified using Schoenfeld residuals. All analyses were performed using R version 4.2 (packages: survival, pROC) and SPSS version 26, with two-tailed $P < 0.05$ considered significant.

Results

Patient characteristics and surgical outcomes

Tumor grades were evenly distributed between the groups (all $P > 0.9$). The rates of gross total resection were similar between the PLK2-low and PLK2-high groups ($P = 0.112$). During a median follow-up of 18.6 months, postoperative seizure recurrence was significantly more frequent in the PLK2-low group compared to the PLK2-high group ($P < 0.001$), indicating a strong unadjusted association between low PLK2 expression and seizure relapse (**Table 1**).

Neurophysiological and imaging findings

PLK2-low patients exhibited significantly greater cortical hyperexcitability and residual disease, including higher interictal EEG spike rates ($P < 0.001$) and larger postoperative residual tumor volumes ($P < 0.001$). Among the 48 patients who underwent PET/MRI fusion, peritumoral PET-SUV was significantly higher in the PLK2-low group compared to the PLK2-high group, indicating increased metabolic activity in low-PLK2 tumors (**Table 2**).

PLK2 expression levels

PLK2 expression was semi-quantified using the H-score system. The mean H-score was 98.32 ± 21.71 in the PLK2-low group and significantly higher in the PLK2-high group ($P < 0.001$). Tumors with high PLK2 expression exhibited diffuse moderate-to-strong cytoplasmic staining, while PLK2-low tumors showed faint or

Table 2. Neurophysiological and imaging metrics

Data	PLK2-low (n = 98)	PLK2-high (n = 91)	t value	*p* value
Interictal spikes (spikes/hour, mean ± SD)	7.81 ± 2.92	3.13 ± 1.24	14.61	< 0.001
Residual tumor volume (cm^3, mean ± SD)	4.18 ± 1.53	2.08 ± 1.03	11.02	< 0.001
PET-SUV (n = 48, mean ± SD)	2.92 ± 0.71	1.81 ± 0.52	7.63	< 0.001

PLK2, Polo-like kinase 2; PET-SUV, peritumoral standardized uptake value mean.

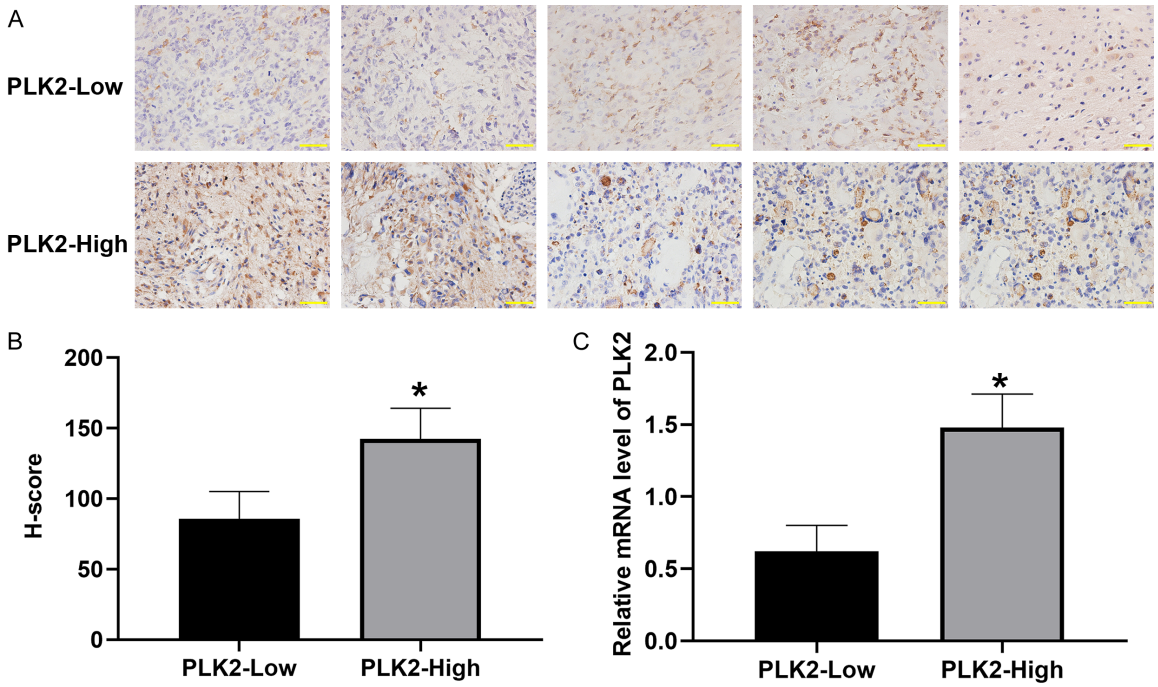

Figure 1. Polo-like kinase 2 (PLK2) expression levels detected by immunohistochemistry (IHC) and quantitative real-time PCR (RT-qPCR). A. Representative images of IHC detection of PLK2 levels in five patients in each group. B. Quantitative analysis results of IHC H-score. C. Quantitative analysis results of IHC RT-qPCR detection of PLK2 mRNA levels. "*" $P < 0.05$ vs. PLK2-Low group.

focal staining. PLK2-low tumors also demonstrated pronounced alterations in synaptic integrity and inflammatory signaling, both of which contribute to glioma-associated epileptogenesis. Quantitative immunohistochemistry revealed significantly reduced expression of synaptic markers in the PLK2-low group compared to the PLK2-high group (H-scores: 85.61 ± 19.43 vs. 142.32 ± 21.66, P < 0.001) (**Figure 1A**, **1B**). Morphologically, synaptophysin staining appeared patchy and disrupted, while PSD-95 localization was disorganized, indicating synaptic destabilization. Consistent with these findings, qRT-PCR showed significantly lower PLK2 mRNA expression in the PLK2-low group (0.62 ± 0.18) compared to the PLK2-high group (1.48 ± 0.23, P < 0.001) (**Figure 1C**).

Inflammatory profiles

Cytokine profiling of tumor lysates revealed a markedly pro-inflammatory microenvironment in PLK2-low tumors, with significantly elevated levels of IL-1β, TNF-α, and IL-6 compared to PLK2-high tumors (all P < 0.001). These cytokines are known to promote neuronal hyperexcitability by enhancing glutamatergic transmission, disrupting the blood-brain barrier, and recruiting immune cells (**Table 3**).

Predictive potential of findings

Univariate Cox regression identified PLK2-low status (P < 0.001), subtotal resection (P = 0.001), temporal lobe location (P = 0.020), interictal spikes > 5/hour (P = 0.003), and IL-1β

Table 3. Serum PLK2 and inflammatory cytokines levels in different groups

Data	PLK2-low (n = 98)	PLK2-high (n = 91)	t value	*p* value
Serum PLK2 (ng/mL, mean ± SD)	0.94 ± 0.35	1.62 ± 0.48	11.06	< 0.001
IL-1β (pg/mg tissue, mean ± SD)	45.23 ± 11.62	21.4 ± 7.88	16.02	< 0.001
TNF-α (pg/mg tissue, mean ± SD)	62.54 ± 14.76	26.72 ± 8.43	19.96	< 0.001
IL-6 (pg/mg tissue, mean ± SD)	55.11 ± 13.23	23.93 ± 6.52	19.63	< 0.001

PLK2, Polo-like kinase 2.

Table 4. Regression analysis of risk factors associated with GRE

Data	Univariate		Multivariate	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
PLK2-low (H-score < 140)	2.70 (1.70-4.30)	< 0.001	2.45 (1.47-4.10)	0.001
Subtotal resection	2.20 (1.36-3.57)	0.001	1.92 (1.15-3.20)	0.013
Temporal lobe location	1.75 (1.09-2.81)	0.022	1.60 (1.01-2.55)	0.046
Interictal spikes > 5/hour	1.90 (1.25-2.89)	0.003	NA	NA
IL-1β > 30 pg/mg	2.15 (1.40-3.30)	0.001	NA	NA

PLK2, Polo-like kinase 2.

Table 5. Seizure recurrence rates stratified by combined risk factors

Group	n	Seizure Recurrence (%)
PLK2-low + Subtotal Resection	23	65.20%
PLK2-low + Gross Resection	75	38.70%
PLK2-high + Subtotal Resection	13	30.80%
PLK2-high + Gross Resection	78	15.40%
PLK2-low + Temporal Tumor	49	51.00%
PLK2-high + Temporal Tumor	35	22.90%

PLK2, Polo-like kinase 2.

> 30 pg/mg (P = 0.001) as significant predictors of seizure recurrence (**Table 4**). In multivariable analysis, PLK2-low status (P = 0.001), subtotal resection (P = 0.013), and temporal lobe location (P = 0.046) remained independent risk factors. The final model demonstrated good discriminative performance, with a Harrell's C-index of 0.78 (**Table 4**).

Stratified seizure risk analysis based on PLK2 and clinical variables

To evaluate interactions between PLK2 expression and clinical risk factors, stratified subgroup analyses were performed based on PLK2 status, extent of resection, and tumor location (**Table 5**). Seizure recurrence was highest among patients with PLK2-low tumors who underwent subtotal resection, highlighting the additive risk of molecular and surgical factors. In contrast, patients with PLK2-high tumors and gross total resection had the lowest recurrence rates, emphasizing the benefit of complete tumor removal in favorable molecular contexts. Notably, PLK2-high patients still demonstrated substantial recurrence when resection was incomplete, underscoring the critical role of surgical extent. Temporal lobe tumors, a known epileptogenic risk factor, exhibited amplified recurrence risk when combined with low PLK2 expression, suggesting that PLK2 status modifies the seizure potential of anatomically vulnerable regions. Overall, these analyses indicated that low PLK2 expression synergizes with adverse surgical and anatomic features to elevate recurrence risk, whereas high PLK2 expression appears protective even in the presence of other risk factors. This stratified approach enhances the clinical relevance of PLK2 by identifying patient subsets most vulnerable to seizure recurrence and may inform personalized postoperative management.

Predictive value of different factors in the recurrence of GRE

To assess predictive performance, ROC curve analysis was performed using the PLK2 IHC H-score as a continuous variable. The area under the curve (AUC) was 0.63, with an optimal cutoff of 112.3 determined by the Youden Index, yielding 70.5% sensitivity and 75.8%

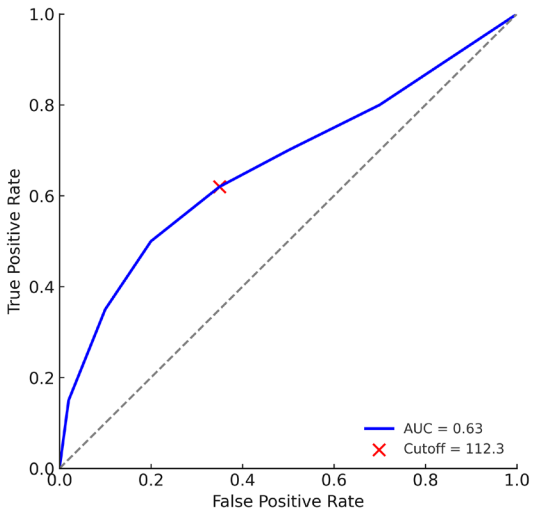

Figure 2. Receiver-operating characteristic (ROC) curve for Polo-like kinase 2 (PLK2) H-score predicting postoperative seizure recurrence. ROC curve analysis was performed to evaluate the predictive accuracy of PLK2 immunohistochemistry (IHC) H-scores for postoperative seizure recurrence in 189 glioma patients with preoperative epilepsy. The red dot marks the optimal discrimination point. This cutoff was used to define PLK2-low and PLK2-high groups throughout the study.

specificity (**Figure 2**). Kaplan-Meier analyses further demonstrated the prognostic value of PLK2: patients with PLK2-low expression had significantly shorter seizure-free survival compared to those with high expression (log-rank $P < 0.001$) (**Figure 3A**). Subtotal resection was associated with a higher risk of seizure recurrence than gross total resection ($P = 0.004$) (**Figure 3B**). Similarly, temporal lobe tumors exhibited poorer seizure control compared to non-temporal gliomas ($P = 0.013$) (**Figure 3C**). Together, these findings support the prognostic and mechanistic significance of PLK2 in GRE, showing that PLK2 functions as an independent molecular predictor while also interacting with surgical extent and tumor location to determine seizure outcome.

Discussion

Postoperative seizure recurrence remains a major clinical challenge in GRE, affecting up to 30% of patients despite gross total resection and antiseizure medication (ASM) therapy [1, 2]. The mechanisms underlying seizure persistence are multifactorial, involving tumor-intrinsic changes, peritumoral cortical hyperexcitability, and systemic immune responses [17]. While clinical factors such as temporal lobe involvement, extent of resection, and IDH1 mutation status have been proposed as predictors [18, 19], molecular determinants remain poorly defined. In this retrospective cohort study, we identified PLK2, a kinase involved in neuronal homeostasis, as a key modulator of epileptogenic risk in glioma patients. By integrating histopathologic, molecular, neurophysiological, and clinical data from 189 patients, we demonstrated that low PLK2 expression is independently associated with an increased risk of postoperative seizure recurrence. Patients with low PLK2 expression not only had higher interictal spike rates and shorter seizure-free survival but also exhibited marked synaptic degeneration and inflammatory activation - two critical drivers of epileptogenesis. These findings suggest that PLK2 may serve as both a predictive biomarker and a mechanistic regulator in GRE.

PLK2 is a serine/threonine kinase enriched in the central nervous system, where it regulates synaptic scaling, activity-dependent plasticity, and neurotransmitter receptor trafficking [10, 20]. Experimental studies show that PLK2 is upregulated in response to sustained neuronal activity and reduces excitatory synaptic strength by promoting AMPA receptor internalization and degradation [21]. Consistent with this role, tumors with low PLK2 expression in our cohort exhibited markedly reduced levels of synaptic markers PSD-95 and synaptophysin, suggesting impaired synaptic integrity and plasticity. These synaptic deficits may make peritumoral neurons more vulnerable to hyperexcitability and seizure generation. Additionally, PLK2 appears to intersect with inflammatory pathways, further amplifying epileptogenic potential. We observed significantly elevated IL-1β, TNF-α, and IL-6 in PLK2-low tumors. These cytokines are known to enhance NMDA receptor activity, disrupt astrocytic glutamate transport, and increase neuronal firing [22, 23]. IL-1β activates p38 MAPK signaling in neurons, lowering the seizure threshold and promoting hippocampal excitability [24], while TNF-α facilitates AMPA receptor surface expression and inhibits GABAergic transmission, shifting the excitatory/inhibitory balance toward hyperexcitability [25]. Thus, the pro-inflammatory microenvironment in PLK2-low tumors represents a

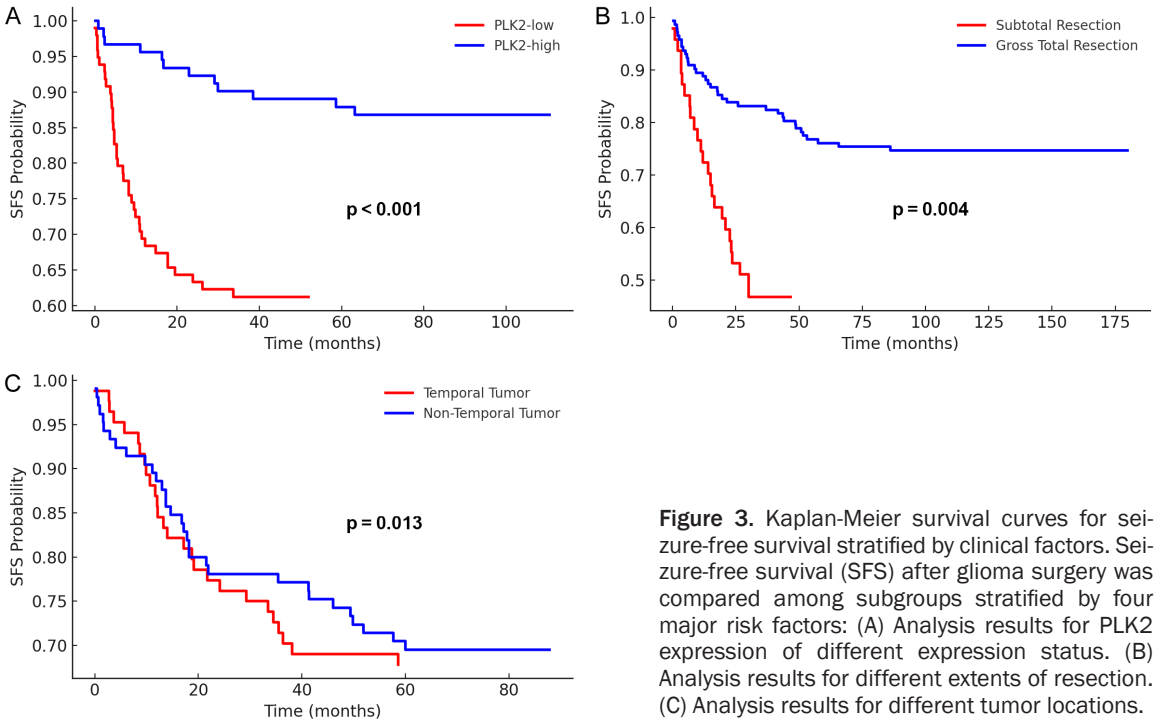

Figure 3. Kaplan-Meier survival curves for seizure-free survival stratified by clinical factors. Seizure-free survival (SFS) after glioma surgery was compared among subgroups stratified by four major risk factors: (A) Analysis results for PLK2 expression of different expression status. (B) Analysis results for different extents of resection. (C) Analysis results for different tumor locations.

second convergent mechanism contributing to GRE recurrence.

Our findings also suggest that PLK2 may function as a tumor suppressor in glioma. PLK2 expression was inversely correlated with residual tumor volume and peritumoral metabolic activity on PET/MRI. Prior studies have shown that PLK2 is downregulated in high-grade gliomas and that its overexpression suppresses proliferation, migration, and stemness in glioblastoma cell lines [13]. *In vivo*, PLK2-overexpressing glioblastoma xenografts exhibit slower growth and reduced angiogenesis [15]. While seizure control was the primary outcome of our study, the inverse association between PLK2 and tumor aggressiveness raises the possibility that PLK2 exerts dual roles in regulating both oncogenicity and neural excitability. Importantly, the prognostic value of PLK2 was validated in multivariable models: even after adjusting for established clinical predictors such as extent of resection, temporal lobe location, and IDH1/2 mutation status, PLK2 remained an independent predictor of seizure recurrence. These findings support the incorporation of PLK2 IHC assessment into standard histopathologic evaluation of gliomas with regard to epilepsy. Furthermore, the concordance between PLK2 levels measured by IHC and qRT-PCR indicates that PLK2 expression can be reliably quantified across platforms, enhancing its translational feasibility.

Several clinical implications arise from these findings. First, PLK2 may serve as a biomarker to stratify postoperative seizure risk, with patients exhibiting low expression possibly benefiting from intensified ASM regimens, closer EEG surveillance, or adjunctive anti-inflammatory therapies. Second, therapeutic strategies targeting PLK2 signaling - through upregulation or pharmacologic mimetics - may represent a novel approach to prevent seizure recurrence in GRE. Small-molecule modulators of PLK family kinases are already in development for other central nervous system disorders and could be repurposed for this indication [26]. Finally, our results reinforce the view that seizures in glioma are not merely secondary to mass effect or edema, but are driven by active molecular signaling between tumor cells and neural networks.

The current study has several limitations. Its retrospective, single-center design may introduce selection bias, and although PLK2 status was strongly associated with outcomes, causality cannot be established. Functional validation *in vitro* or in animal models was not per-

formed, and some measures, such as EEG spike rates, were available in only a subset of patients. Future prospective, multicenter studies are needed to validate the predictive value of PLK2 and to elucidate its mechanistic role using electrophysiological and pharmacological models.

In conclusion, PLK2 emerged as a novel biomarker and potential modulator of seizure recurrence in glioma-related epilepsy. Low PLK2 expression is associated with synaptic disruption, neuroinflammation, and shortened seizure-free survival, independent of established clinical and molecular predictors. These findings support the integration of PLK2 assessment into routine pathologic evaluation and highlight its potential as a therapeutic target to improve seizure control in glioma patients.

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Disclosure of conflict of interest

None.

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