Phosphorylation of NMDA 2B at S1303 in human glioma peritumoral tissue: implications for glioma epileptogenesis

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Object. Peritumoral seizures are an early symptom of a glioma. To gain a better understanding of the molecular mechanism underlying tumor-induced epileptogenesis, the authors studied modulation of the *N*-methyl-D-aspartate (NMDA) receptor in peritumoral tissue.

Methods. To study the possible etiology of peritumoral seizures, NMDA receptor expression, posttranslational modification, and function were analyzed in an orthotopic mouse model of human gliomas and primary patient glioma tissue in which the peritumoral border (tumor-brain interface) was preserved in a tissue block during surgery.

Results. The authors found that the NMDA receptor containing the 2B subunit (NR2B), a predominantly extrasynaptic receptor, is highly phosphorylated at S1013 in the neurons located in the periglioma area of the mouse brain. NR2B is also highly phosphorylated at S1013 in the neurons located in the peritumoral area from human brain tissue containing a glioma. The phosphorylation of the extrasynaptic NMDA receptor increases its permeability for Ca²⁺ influx and subsequently mediates neuronal overexcitation and seizure activity.

Conclusions. These data suggest that overexcitation of the extrasynaptic NMDA receptors in the peritumoral neurons may contribute to the development of peritumoral seizures and that the phosphorylated NR2B may be a therapeutic target for blocking primary brain tumor–induced peritumoral seizures.

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KEY WORDS • glioblastoma multiforme • peritumoral seizures • NMDA • NR2B

GLIOMAS are the most common form of primary brain tumors. Recurring seizures, often referred to as tumor-associated epilepsy, are a well-documented yet poorly understood comorbidity that affects approximately one-third of the individuals suffering from gliomas.¹⁴ Peritumoral seizures are an early symptom, and up to 80% of individuals with glioma experience at least 1 seizure during the course of their illness.^{27,38}

Conventional antiepileptic therapy is often not very successful at treating tumor-related seizures. Surgery is a palliative treatment even when a significant portion of the tumor can be removed.²³ However, the location of some gliomas may preclude their safe gross removal.³⁶ Tumor recurrence is often accompanied by the recurrence of the disabling seizure disorder.^{4,18} Thus, there is still significant need for an effective and noninvasive approach to treating glioma-associated epilepsy.

Abbreviations used in this paper: DAPI = 4',6-diamidino-2-phenylindole; EGFP = enhanced green fluorescent protein; FBS = fetal bovine serum; GBM = glioblastoma multiforme; GFAP = glial fibrillary acidic protein; Glu = glutamate; NeuN = neuron-specific nuclear protein; NMDA = *N*-methyl-D-aspartate; NMDAR = NMDA receptor; NR2B = NMDA receptor containing 2B subunit; PBS = phosphate-buffered saline; pNR2B = phosphorylated NR2B.

An understanding of the etiology of glioma-associated seizures may facilitate a novel therapeutic approach for their treatment. Alterations in the peritumoral microenvironment are believed to play a significant role in inducing epileptogenesis. The results of recent studies conducted both in patients with a glioma and in rodents implanted with a glioma have suggested that epileptiform activity originates within the peritumoral border, 1–2 mm away from the tumor mass, where invading tumor cells surround neurons.^{16,29,32} A recent clinical study measured the glutamate (Glu) concentrations of dialysates taken from the peritumoral cortex or from an uninvolved area of the brain. The results demonstrated peritumoral Glu concentrations higher than 100 μM, which is 100-fold higher than the levels in uninvolved brain.²⁴ Glutamate is considered the major mediator of excitatory signals in the mammalian central nervous system and is widely involved in normal brain function, including cognition, memory, and learning. Increased Glu levels result in hyperexcitability of the surrounding extratumoral neurons and, ultimately, seizures. Recently, a mouse model of glioma implicated the release of Glu by glioma cells as the key step in glioma-related seizures, suggesting that blockade of Glu-mediated overexcitation may be a therapeutic option

for treating tumor-associated seizures. Indeed, in a mouse model of glioma, the blockade of Glu release was shown to partially reduce seizure activity.² However, the process of how peritumoral neurons respond to Glu release from glioma cells is poorly understood. Inhibiting peritumoral neurons from being overexcited by Glu released from glioma cells may provide another pathway to block primary brain tumor–induced peritumoral seizures.

The *N*-methyl *D*-aspartate receptor (NMDAR) is a calcium-permeable subtype of the ionotropic Glu receptor in the mammalian brain.^{10,12,20} Pathological release of Glu may lead to overstimulation of the NMDA Glu receptor subtype. Most native NMDARs are tetrameric complexes consisting of two essential NR1 subunits and one or more regulatory NR2 subunits, most commonly NR2A and/or NR2B.¹⁹ The NR2A and NR2B subunits have differential roles. NR2A-containing NMDARs predominantly exist in the postsynaptic spines and conduct synaptic transmission, whereas NR2B-containing NMDARs are predominantly located extrasynaptically. When NR2B is activated, it allows the influx of Ca^{2+} into the cells and can trigger excitotoxic effects.²¹ To understand how peritumoral neurons respond to the pathological release of Glu from glioma cells, we assessed the phosphorylation of NR2B as an indicator for NR2B activation¹ in human glioma tissue and in a mouse model of human glioma.

Methods

Animals

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice (The Jackson Laboratory) were obtained from the In Vivo Therapeutics Core facility and group housed with a 12/12-hour light/dark cycle and were given access to food and water ad libitum. Mice were used in experiments at an age of 8–10 weeks. All procedures were performed under protocols approved by the Animal Care and Use Committee at Indiana University.

Primary Human Glioma Cell Lines

The primary human glioblastoma multiforme (GBM) cell line GBM10 was a kind gift from Dr. Jann Sarkaria (Mayo Clinic, Rochester, MN); it has been described elsewhere³ and validated in low-passage lines (GBM10: wild-type p53, CDKN2A/p16 deleted, wild-type PTEN, and MGMT positive). To preserve the molecular profiles, GBM10 cells were initially expanded as flank tumors, harvested, and maintained in 2.5% fetal bovine serum (FBS) for 4 days on Matrigel-coated plates to remove murine fibroblasts. Cells were expanded in DMEM/F12 (Gibco [11965]; 4.5 g/L *D*-glucose and *L*-glutamine) with 10% FBS (Advantage FBS [S11050], Atlanta Biologicals) for less than 2 weeks and used in the setup of intracranial models. Cells were routinely checked for mycoplasma before injection into mice.

Human Glioma Tissue Collection

We harvested tumor tissue during surgery from patients with pathologically diagnosed glioma. The border of each tumor was defined intraoperatively on the basis

of neuronavigation using MR imaging. After clear identification of the tumor border, microsurgical techniques were used to isolate a tissue block containing tumor, tumor border, and peritumoral brain. To avoid any injury to the tissue block, bipolar coagulation was not used. This “peritumoral tissue” area is usually removed to maximize tumor resection routinely unless the tumor is adjacent to highly functional cortices. Therefore, we did not change our standard resection techniques for this study. The orientation of the tissue block containing the tumor-brain interface was appropriately labeled to ensure correct identification of edge containing tumor or “peritumoral” tissue in the laboratory. Tissue specimens were immediately delivered to the laboratory in cold DMEM with 2.5% FBS on wet ice. Tissue collection was approved by the Indiana University Institutional Review Board.

Validation of a Humanized Orthotopic Glioma Model

For intracranial implantation of human GBM cells, a digitalized stereotaxic delivery system was used (5000 microinjection unit, David Kopf Instruments).^{13,30} For stereotaxic delivery of tumor cells, each mouse was placed under general anesthesia (intraperitoneal injection of 16 mg/kg xylazine and 150 mg/kg ketamine) and positioned in the stereotaxic device. A digitalized drill assembly was used to bore a hole 0.3 mm in depth and 0.8 mm in diameter into the cranium at a position 0.5 mm anterior and 1.2 mm lateral to the bregma anatomical landmark. Tumor cells (5×10^5 in 10 μl of phosphate-buffered saline [PBS]) were introduced slowly using a 10- μl Hamilton syringe at a depth of 3.5 mm at a rate of 2 $\mu\text{l}/\text{minute}$. Once injection was complete, the needle was kept in place for at least 5 minutes and then slowly removed, and the hole was sealed with bone wax. The incision was closed with a wound clip. Bioluminescence imaging of intracranial tumors that express a luciferase-enhanced green fluorescent protein (EGFP) fusion protein was performed to monitor and confirm longitudinal progression of the tumor, and details of the vector design will be published elsewhere (H. Wang, S. Cai, B. J. Bailey, et al., unpublished data). Survival analysis using predeath end-point criteria was also previously performed, and the median survival time is typically 35–50 days after the implantation of tumor cells in NOD/Scid mice (H. Wang, S. Cai, B. J. Bailey, et al., unpublished data).

Tissue Processing

The mice ($n = 3$) were killed according to the institutional Animal Care and Use Committee–approved protocol, and their brains were immediately removed and fixed overnight in 4% paraformaldehyde in PBS before embedding in paraffin. Serial 30- μm -thick coronal sections were cut using a cryostat (Leica CM 1950) and stored at -20°C . The sections were then processed for immunohistochemical analysis.

Immunohistochemistry

Free-floating sections were washed twice in PBS. The sections were incubated in blocking solution (0.1% Triton X-100, 1% bovine serum albumin, and 5% normal

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goat serum in PBS) for 1 hour at room temperature, followed by overnight incubation with primary antibody at 4°C. The sections were washed again 3 times with PBS and incubated at room temperature for 2 hours with the secondary antibody. After treatment for 2 minutes with 4',6-diamidino-2-phenylindole (DAPI), the sections were washed with PBS 3 times and mounted using Fluoromount G. Primary antibodies and their final concentrations were as follows: anti-phosphorylated NR2B (pNR2B) (1:400, rat; Accurate Chemical and Scientific), anti-glial fibrillary acidic protein (GFAP) (1:100, G3893; Sigma), anti-neuron-specific nuclear protein (NeuN) (1:100, MAB377; Millipore), and anti-Nestin (1:1000; rabbit; Covance). Secondary antibodies from Jackson ImmunoResearch Laboratories were applied in a dilution of 1:1000.

Microscopy

The sections were analyzed using an inverted microscopy system (Zeiss Axiovert 200 M) combined with an ApoTome (Zeiss) and interfaced with a digital camera (Zeiss AxioCam MRc5) controlled by a computer. Images were captured using the ApoTome's software (AxioVision version 4.8) and assembled and labeled in Photoshop 7.0 (Adobe Systems).

Results

Creating a Mouse Model of Human Glioma

To study how GBM cells affect the peritumoral neurons during tumorigenesis, a humanized orthotopic model was first optimized (Fig. 1) so that high-quality tumor samples could be obtained from the mice just before the predeath end point was reached. NOD/Scid mice were implanted with GBM10 cells at 3×10^5 ($n = 8-10$) or 5×10^5 ($n = 8-10$) GBM10 cells per mouse, and survival time was calculated by a Kaplan-Meier survival plot. The median survival times were 45 days for the mice implanted with 3×10^5 cells and 40 days for the mice implanted with 5×10^5 cells (Fig. 1A). At the predeath end point, H & E staining indicated that the intracranial GBM tumor encompassed a large region of the right cerebrum (Fig. 1B). Bioluminescence imaging was also used to verify tumor progression over time (Fig. 1C and D). In these studies, GBM10 cells that express a luciferase-EGFP fusion protein were implanted into the mice, and tumor progression was monitored for up to 40 days after the implantation. Intracranial tumor samples used in these studies were obtained from mice ($n = 3$) just before reaching the predeath end point.

Phosphorylation of NR2B at S1013 in the Neurons Located in the Periglioma Area in a Mouse Model of Human Glioma

We first distinguished the tumor tissue from peritumoral tissue and cortex on the basis of nuclear morphologies using DAPI staining. A large and extremely high-density cell mass was easily identified (Fig. 2A). There was a clear separation between the cell mass and peritumoral tissue (Fig. 2A). The morphologies of nuclei in the cell mass appeared obviously abnormal. They were large

and irregular with an extremely high density, showing characteristics of tumor cells (Fig. 2A). GFAP is highly expressed in the cells in the high-density tumor mass and glial cells at the peritumoral region (Fig. 2B), whereas Nestin is highly expressed by only the cells within the high-density tumor mass (Fig. 2C). These data indicate that the tumor and peritumoral tissues were easily and reliably identifiable, and Nestin, a progenitor cell marker, can be used as a marker to identify glioma cells.

To assess excitation of the peritumoral neurons stimulated by Glu released from glioma cells, we also assessed the phosphorylation of the extrasynaptic Glu receptor, NR2B (pNR2B) in mouse brains containing human GBM10 tumors. Mouse brain sections containing glioma were immunostained with antibody against pNR2B phosphorylated at S1303, and tissue was costained with DAPI to distinguish the glioma cell mass from peritumoral tissues. The smooth borderline between the tumor mass and peritumoral tissue was identified easily, suggesting a clear demarcation between the tumor tissue and nearby tissue in this mouse model of human glioma (Fig. 3D-F). The pNR2B was undetectable 1.5 mm away from the glioma cell mass (Fig. 3A-C). The pNR2B was highly detectable in the peritumoral cells (Fig. 3D-F) but not inside the cells of the glioma cell mass (Fig. 3G-I). Because phosphorylation is an indicator for the activation of NR2B, these results suggest that the extrasynaptic NR2B is highly phosphorylated at S1013 in the neurons located in the periglioma area in this mouse model of human glioma.

Phosphorylation of NR2B at S1013 in the Neurons Located in the Periglioma Area of Human Tissue

To verify whether extrasynaptic NMDARs in the peritumoral neurons are overexcited in human glioma tissue, we analyzed the primary patient GBM tissue in which the peritumoral border was preserved during surgery. The tumors ($n = 3$) were from either the frontal lobe ($n = 2$) or the temporal lobe ($n = 1$) and pathologically diagnosed as oligoastrocytoma (WHO Grade II [$n = 2$]) or GBM ($n = 1$). The tissue sections were immunostained with an antibody against pNR2B to assess NR2B phosphorylation, whereas an antibody against Nestin was used to detect glioma cells. A tissue mass with condensed cells expressing Nestin was easily observed. The morphologies of Nestin-positive cells were large and irregular (Fig. 4A and B). The borderline between the tumor and peritumoral tissue was not very clear and not smooth (Fig. 4A), which is in contrast to what we observed in the mouse model of human glioma (Fig. 2). The NR2B was phosphorylated in the cells both in the peritumoral area and at the surface of the glioma mass (Fig. 4). This result suggests that NR2B is activated in the cells at the tumor margins.

To further determine whether the NMDAR is overexcited in the neurons, glial cells, or both in the periglioma area of human tissue, we double-immunostained the primary patient GBM tissue with antibodies against pNR2B and 1 of the cell type-specific markers, NeuN or GFAP. NeuN is a widely used marker for neurons,^{17,28} and GFAP is a cellular marker for astroglial cells.³³ The results showed that pNR2B is highly expressed in neurons (Fig. 5A) and astroglial cells (Fig. 5B), indicating that the

Fig. 1. Validation of a humanized orthotopic GBM xenograft model. **A:** Kaplan-Meier survival plot of primary patient GBM10 cells implanted into the cerebrum at 3×10^5 or 5×10^5 cells per animal. **B:** Representative example of H & E-stained GBM10 tumor in the brain 35 days after implantation, original magnification $\times 50$. **C:** In another experiment, GBM10 cells were transduced with a lentiviral vector that expresses a luciferase-EGFP (LUCIE) fusion protein and 5×10^5 cells implanted into the cerebrum ($n = 11$ mice). GBM cell growth was monitored over time by using bioluminescence imaging. Photon emission over the region of interest is shown as photon emission/sec/mm² and determined using the Night Owl optical imaging system. Numbers in parentheses are the number of mice still available for imaging at each time point. **D:** Representative example of bioluminescence in the brains of mice implanted with GBM10 cells. Mouse 1 (Mo1) served as a background control (Bkgd) for bioluminescence, and Mice 2–4 (Mo2–Mo4) contained GBM tumors that express the LUCIE protein.

NMDAR is overexcited in both neurons and glial cells in the perigloma area of human tissue.

Discussion

Recurring seizures that are caused by the presence of a malignant primary brain tumor, such as GBM, are often referred to as tumor-associated epilepsy. Malignant tumors may induce seizures through a variety of mechanisms, including tissue damage, ischemia, hypoxia, pH changes, and other alterations of the cerebral microenvironment. These microenvironmental changes include isolation of cortical areas from the (inhibitory) inputs generated by other parts of the brain and stimulation of an imbalanced release of inhibitory and excitatory neurotransmitters (such as γ -aminobutyric acid [GABA] and Glu).³⁴ All of these changes may contribute to brain malfunction and epileptogenesis. However, the etiology of tumor-associated seizures and their relationship with tumor growth is still poorly understood.

A recent clinical study measured Glu concentrations of dialysates taken from the peritumoral cortex or uninvolved areas of the brain. The results showed peritumoral Glu concentrations higher than 100 μ M, which

is 100-fold higher than the levels in uninvolved brain.²⁴ Furthermore, a mouse model of glioma indicated that the release of Glu by glioma cells is the key step involved in glioma-related seizures.³⁷ Glutamate is almost exclusively (99.99%) located inside the cells. The Glu concentration inside the cells is around 1–10 mM, which is several thousand times higher than the concentration outside the cells. Maintenance of low extracellular concentrations of Glu is required for normal brain function. In disease conditions, Glu is released into the extracellular space, increasing the extracellular Glu concentration, suggesting that Glu-mediated NMDAR overexcitation in the peritumoral neurons may lead to epileptogenesis. When we created a mouse model of human glioma (Figs. 1 and 2) to study the excitation of NMDAR, we found that NR2B is highly phosphorylated in the peritumoral area (Fig. 3). This result is further confirmed in the human glioma tissue (Fig. 4). It is well known that phosphorylation of NR2B modulates its permeability to Ca^{2+} . Phosphorylated NR2B (pNR2B) increases permeability for calcium to influx; thus, pNR2B has been widely used as an indicator for the activation of NMDAR.^{5,21,31} These data suggest that the Glu released from glioma cells activates NR2B receptors in the neurons located at the peritumoral region.

Fig. 2. Immunostaining to distinguish tumor tissue from peritumoral tissue in a mouse model of human glioma using antibody against Nestin. **A:** DAPI staining was performed to exhibit nuclear morphologies. **B:** Immunostaining with antibody against GFAP to exhibit astrocytes in the glioma tissue and at the peritumoral area. **C:** Immunostaining with antibody against Nestin to exhibit glioma cells expressing Nestin. **D:** Merged image of A–C. The border between the tumor and peritumoral tissue is marked by a white line.

Fig. 3. Immunostaining to assess NR2B activation with antibody against pNR2B in a mouse model of human glioma. DAPI staining was performed to exhibit nuclear morphologies. **A–C:** pNR2B expression in the tissue 1.5 mm away from the tumor. **D–F:** pNR2B expression in the tissue at the peritumoral area. The border between the tumor and peritumoral tissue is marked by a white line. **G–I:** pNR2B expression in the tissue 1.5 mm inside the tumor.

FIG. 4. Double immunostaining to assess NR2B activation with antibodies against pNR2B and Nestin in human glioma. The *white line* (in A, C, E, and G) indicates the border between tumor and peritumoral tissue. **A:** Nestin immunoreactivity to detect the glioma cells and peritumoral tissue in human glioma. **B:** Enlargement of inset in A showing the Nestin-positive glioma cells. **C:** pNR2B immunoreactivity to detect the cells with NR2B activation and peritumoral tissue in the human glioma. **D:** Enlargement of inset in C showing the pNR2B-positive glioma cells. **E:** DAPI staining was performed to exhibit nuclear morphologies. **F:** Enlargement of inset in E showing the nuclear morphologies. **G:** Merged image of those shown in panels A, C, and E. **H:** Merged image of those shown in panels B, D, and F.

Fig. 5. The NMDAR is overexcited in neurons and glial cells in the perigloma area of human tissue. Double immunostaining was performed with antibodies against pNR2B and NeuN, or antibodies against pNR2B and GFAP in the perigloma area of human tissue. **A:** pNR2B (red) is highly expressed in a subpopulation of neurons (green) in the perigloma area of human tissue (white arrows). **B:** pNR2B (red) is highly expressed in a subpopulation of astroglial cells (green) in the perigloma area of human tissue (white arrows).

The overexcitation of these neurons may contribute to the generation of peritumoral seizures.

Our results also show that the cells with pNR2B are mainly located within 1.5–2 mm of the tumor mass. This observation correlated to those in recent studies conducted in humans with glioma and in glioma-implanted rodents. The results of these studies suggested that epileptiform activity originates within the peritumoral border, 1–2 mm away from the tumor mass.^{16,29,32}

In addition, the pattern of NR2B phosphorylation in the tissue in the mouse model of glioma is different from that in human patients with glioma. First, we observed an obvious difference in the glioma cell distribution within the peritumoral region between the implanted tumors in the mouse model and those of *de novo* tumors in patients. In the mouse model, there is a clear separation between the glioma and peritumoral tissue. It is easy to identify a smooth borderline between the tumor and peritumoral tissue (Figs. 1 and 2). In contrast, in the human glioma tissue, the glioma cells invade the peritumoral tissue. The edge of the tumor mass is not smooth, and there is a peritumoral zone that contains glia cells and surrounding neurons (Figs. 1 and 2). Second, in the mouse model, the NR2B phosphorylation was exclusively in the cells at the peritumoral region. In contrast, in tissue from humans with glioma, the NR2B phosphorylation was observed both in the peritumoral area and at the surface of the glioma mass. These observations reflect more complexity in cell composition within the peritumoral zone of glioma in humans than in the implanted tumors in the mouse model.

Glutamate-mediated neurotoxicity has been implicated in patients with stroke,^{15,35} traumatic brain injury,^{6–9,26,39} and neurodegenerative diseases.^{11,25} Excessive release of Glu from a glioma also mediates the death of peritumoral neurons and promotes glioma cell invasion, thus increasing the growth of malignant gliomas.³⁷ Additional study found that Glu hijacked NMDAR signaling to promote proliferation and invasion by activating

its downstream mitogen/extracellular signal-regulated kinase–mitogen-activated protein kinase (MEK–MAPK) and Ca^{2+} /calmodulin-dependent protein kinase (CaMK) in a mouse model of pancreatic neuroendocrine tumorigenesis.²² Treatment with NMDAR antagonist impaired cancer cell proliferation and invasion and slowed the growth of Glu-secreting tumors *in situ*.^{22,37} These results suggest that inhibiting Glu-mediated overexcitation may have doubly beneficial effects on blocking primary brain tumor-induced peritumoral seizures and reducing tumor growth and invasiveness.

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Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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References

- Bading H, Greenberg ME: Stimulation of protein tyrosine phosphorylation by NMDA receptor activation. **Science** **253**: 912–914, 1991
- Buckingham SC, Campbell SL, Haas BR, Montana V, Robel S, Ogunrinu T, et al: Glutamate release by primary brain tumors induces epileptic activity. **Nat Med** **17**:1269–1274, 2011
- Carlson BL, Pokorny JL, Schroeder MA, Sarkaria JN: Establishment, maintenance and in vitro and in vivo applications of primary human glioblastoma multiforme (GBM) xenograft models for translational biology studies and drug discovery. **Curr Protoc Pharmacol** **52**:1–14, 2011
- Chang EF, Zada G, Kim S, Lamborn KR, Quinones-Hinojosa A, Tyrrell JB, et al: Long-term recurrence and mortality after surgery and adjuvant radiotherapy for nonfunctional pituitary adenomas. **J Neurosurg** **108**:736–745, 2008
- Chen BS, Roche KW: Regulation of NMDA receptors by phosphorylation. **Neuropharmacology** **53**:362–368, 2007
- Choi DW: Excitotoxic cell death. **J Neurobiol** **23**:1261–1276, 1992
- Choi DW: Glutamate neurotoxicity and diseases of the nervous system. **Neuron** **1**:623–634, 1988
- Choi DW: Glutamate neurotoxicity in cortical cell culture is calcium dependent. **Neurosci Lett** **58**:293–297, 1985
- Choi DW: Ionic dependence of glutamate neurotoxicity. **J Neurosci** **7**:369–379, 1987
- Collingridge GL, Randall AD, Davies CH, Alford S: The synaptic activation of NMDA receptors and Ca²⁺ signalling in neurons. **Ciba Found Symp** **164**:162–175, 1992
- Doble A: The role of excitotoxicity in neurodegenerative disease: implications for therapy. **Pharmacol Ther** **81**:163–221, 1999
- Dodt HU, Frick A, Kampe K, Zieglgänsberger W: NMDA and AMPA receptors on neocortical neurons are differentially distributed. **Eur J Neurosci** **10**:3351–3357, 1998
- Giannini C, Sarkaria JN, Saito A, Uhm JH, Galanis E, Carlson BL, et al: Patient tumor EGFR and PDGFR gene amplifications retained in an invasive intracranial xenograft model of glioblastoma multiforme. **Neuro Oncol** **7**:164–176, 2005
- Hauser WA, Annegers JF, Kurland LT: Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. **Epilepsia** **34**:453–468, 1993
- Hazell AS: Excitotoxic mechanisms in stroke: an update of concepts and treatment strategies. **Neurochem Int** **50**:941–953, 2007
- Köhling R, Senner V, Paulus W, Speckmann EJ: Epileptiform activity preferentially arises outside tumor invasion zone in glioma xenotransplants. **Neurobiol Dis** **22**:64–75, 2006
- Korzhevskii DE, Gilerovich EG, Zin'kova NN, Grigor'ev IP, Otellin VA: Immunocytochemical detection of brain neurons using the selective marker NeuN. **Neurosci Behav Physiol** **36**:857–859, 2006
- Kurzweil D, Herrlinger U, Simon M: Seizures in patients with low-grade gliomas—incidence, pathogenesis, surgical management, and pharmacotherapy. **Adv Tech Stand Neurosurg** **35**:81–111, 2010
- Laube B, Kuhse J, Betz H: Evidence for a tetrameric structure of recombinant NMDA receptors. **J Neurosci** **18**:2954–2961, 1998
- Lester RA, Clements JD, Westbrook GL, Jahr CE: Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. **Nature** **346**:565–567, 1990
- Li B, Chen N, Luo T, Otsu Y, Murphy TH, Raymond LA: Differential regulation of synaptic and extra-synaptic NMDA receptors. **Nat Neurosci** **5**:833–834, 2002
- Li L, Hanahan D: Hijacking the neuronal NMDAR signaling circuit to promote tumor growth and invasion. **Cell** **153**:86–100, 2013
- Luyken C, Blümcke I, Fimmers R, Urbach H, Elger CE, Wiestler OD, et al: The spectrum of long-term epilepsy-associated tumors: long-term seizure and tumor outcome and neurosurgical aspects. **Epilepsia** **44**:822–830, 2003
- Marcus HJ, Carpenter KL, Price SJ, Hutchinson PJ: In vivo assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. **J Neurooncol** **97**:11–23, 2010
- Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL: Excitotoxicity: bridge to various triggers in neurodegenerative disorders. **Eur J Pharmacol** **698**:6–18, 2013
- Miller LP, Lyeth BG, Jenkins LW, Oleniak L, Panchision D, Hamm RJ, et al: Excitatory amino acid receptor subtype binding following traumatic brain injury. **Brain Res** **526**:103–107, 1990
- Moots PL, Maciunas RJ, Eisert DR, Parker RA, Laporte K, Abou-Khalil B: The course of seizure disorders in patients with malignant gliomas. **Arch Neurol** **52**:717–724, 1995
- Mullen RJ, Buck CR, Smith AM: NeuN, a neuronal specific nuclear protein in vertebrates. **Development** **116**:201–211, 1992
- Patt S, Steenbeck J, Hochstetter A, Kraft R, Huonker R, Haeisen J, et al: Source localization and possible causes of interictal epileptic activity in tumor-associated epilepsy. **Neurobiol Dis** **7**:260–269, 2000
- Sarkaria JN, Carlson BL, Schroeder MA, Grogan P, Brown PD, Giannini C, et al: Use of an orthotopic xenograft model for assessing the effect of epidermal growth factor receptor amplification on glioblastoma radiation response. **Clin Cancer Res** **12**:2264–2271, 2006
- Schumann J, Alexandrovich GA, Biegon A, Yaka R: Inhibition of NR2B phosphorylation restores alterations in NMDA receptor expression and improves functional recovery following traumatic brain injury in mice. **J Neurotrauma** **25**:945–957, 2008
- Senner V, Köhling R, Püttmann-Cyrus S, Straub H, Paulus W, Speckmann EJ: A new neurophysiological/neuropathological ex vivo model localizes the origin of glioma-associated epileptogenesis in the invasion area. **Acta Neuropathol** **107**:1–7, 2004
- Sergent-Tanguy S, Michel DC, Neveu I, Naveilhan P: Long-lasting coexpression of nestin and glial fibrillary acidic protein in primary cultures of astroglial cells with a major participation of nestin(+)GFAP(−) cells in cell proliferation. **J Neurosci Res** **83**:1515–1524, 2006
- Shamji MF, Fric-Shamji EC, Benoit BG: Brain tumors and epilepsy: pathophysiology of peritumoral changes. **Neurosurg Rev** **32**:275–286, 2009
- Silberstein M: Excitotoxicity and the future of stroke imaging. **Can Assoc Radiol J** **53**:307, 2002 (Letter)
- Simon M, Neuloh G, von Lehe M, Meyer B, Schramm J: Insular gliomas: the case for surgical management. Clinical article. **J Neurosurg** **110**:685–695, 2009
- Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M: Glutamate release promotes growth of malignant gliomas. **Nat Med** **7**:1010–1015, 2001
- Van Breemen MS, Wilms EB, Vecht CJ: Seizure control in brain tumors. **Handb Clin Neurol** **104**:381–389, 2012
- Yi JH, Hazell AS: Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. **Neurochem Int** **48**:394–403, 2006

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