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Short communication

Angiocentric glioma-associated seizures: The possible role of EATT2, pyruvate carboxylase and glutamine synthetase

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ABSTRACT

Purpose: Our purpose was to better understand the pathogenesis of seizures associated with angiocentric glioma. Angiocentric glioma is an indolent and rare low-grade glioma. Its typical clinical presentation is with epileptic seizures. The pathogenesis of tumor-associated seizures is poorly understood. Among the possible pathomechanisms, the increased neurotoxic concentrations of the glutamate has been proposed. Glutamate transporters, pyruvate carboxylase and glutamine synthetase are involved in maintaining the physiological concentration of glutamate in the inter synaptic spaces.
Methods: We evaluated the immunohistochemical expression of EAAT2 (the most important glutamate transporter), pyruvate carboxylase and glutamine synthetase in 17 angiocentric gliomas.
Results: EAAT2 was never expressed (0%) in the neoplastic cells in none of the cases studied. Pyruvate carboxylase was expressed in the cytoplasm of the neoplastic cells in 16/17 cases (94 %). Glutamine synthetase was expressed in the cytoplasm of the neoplastic cells in 15/17 cases (88 %).
Conclusion: The net result of this enzymatic expression, in particular considering the loss of EAAT2, could be an increased glutamate concentration in the synaptic clef, which might increase local network excitability initially involving intratumoral neurons. The observation that the angiocentric glioma-associated epilepsy might be at least in part related to EAAT2 deficiency opens up interesting therapeutic perspectives.

1. Introduction

Angiocentric glioma is a rare low-grade glioma [1]. The initial reports date back to 2005, when two independent research groups described 18 tumors exhibiting clinical-pathological similarities [2,3]. Angiocentric glioma was included in the 2007 World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) and classified as grade I [1].

It mainly affects young people and develops superficially in the cerebral hemispheres. On magnetic resonance imaging (MRI) it is

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hyperintense on FLAIR images, non-contrast-enhancing, and at times shows a band of hyperintensity on T1-weighed images. Calcifications are rare. The distinctive histopathological characteristics include perivascular arrangement of neoplastic bipolar cells (Figure 1a-b in Supplementary material) and features of ependymal differentiation, as evaluated by immunohistochemistry and electron microscopy [1,4].

An in-frame *MYB-QKI* gene fusion is typical of this tumor [5].

More than 90 % of patients have long-lasting drug-resistant epilepsy and the morbidity burden is often more related to epilepsy than to their neoplastic nature [6].

The pathogenesis of tumor-associated seizures is poorly understood. Many mechanisms have been proposed, including the increase in glutamate concentrations [7].

Glutamate is the main excitatory neurotransmitter. Elevated concentrations of extracellular glutamate may determine neuronal hyperexcitability and seizures and have been implicated in several other neurological disorders including strokes, autism, intellectual disability, amyotrophic lateral sclerosis and Alzheimer's disease [8]. Therefore, considering the potential neurotoxicity of glutamate, its eradication is of great importance.

In glutamatergic synapses, glutamate is released by pre-synaptic neurons into the synaptic cleft and binds to postsynaptic glutamate receptors. Excess of glutamate is removed by glutamate transporters, mainly present on the astrocytic processes. In the astrocytes glutamate is converted into glutamine by glutamine synthetase. Subsequently, glutamine is transported back to the neuronal presynaptic terminal, where it is reconverted into glutamate by glutaminase. Thus, glutamate-mediated neurotransmission is also based on recycle. However, during the recycling process a fraction of the neurotransmitter pool is lost in oxidative metabolism. This loss is replaced by *de novo* synthesis, which involves the action of astrocytic pyruvate carboxylase [9].

In order to better understand the pathogenesis of seizures associated with this rare tumor, we have studied the immunohistochemical expression of EAAT2 (the most important glutamate transporter), pyruvate carboxylase and glutamine synthetase in a cohort of 17 angiocentric gliomas.

2. Patients and methods

Tumor samples were obtained from 17 patients (age 2–50 years, mean 12 years; 14 males, 3 females) with a histological diagnosis of angiocentric glioma [1]. The commonest localizations were in the frontal, temporal, and parietal lobe. Data concerning symptomatology were known in 14 patients, of whom 12 (86 %) had epileptic seizures (Table 1 in Supplementary material)

Tissue specimens have been routinely formalin-fixed, paraffin-embedded and stained with hematoxylin-eosin for histopathological diagnosis.

Five μm sections of the most representative specimen of each case have been used for immunohistochemistry. Specimens in which peritumoral nervous tissue was present have been preferred (internal positive control). We have used rabbit polyclonal anti-human anti-SLC1A2 [EAAT2] (Sigma-Aldrich), rabbit polyclonal anti-human pyruvate carboxylase (Invitrogen), and mouse monoclonal anti-glutamine synthetase (Ventana Medical System) as primary antibodies.

Molecular analyses were performed in 8 previously reported cases (patients 1, 3, 5, 12–13 and 15–17, Table 1 in Supplementary material) [5,10] and showed the typical MYB genetic alterations. In the remaining cases, FISH analysis (SPEC MYB Dual Color Break Apart Probe, Zytovision) evidenced the presence of the 6q23.3 deletion-truncation breakpoint.

3. Results

We did not detect EAAT2 expression in the neoplastic cells in any of the cases studied (0%). Conversely, EAAT2 cytoplasmic immunostaining was present in intralesional and perilesional astrocytes (Figure 1c-d in Supplementary material).

Pyruvate carboxylase was diffusely expressed in the cytoplasm of neoplastic cells in 16/17 cases (94 %) (Figure 1e in Supplementary material). In these patients, a cytoplasmic immunostaining was present in intralesional and perilesional astrocytes.

Glutamine synthetase was expressed in the cytoplasm of neoplastic cells in 15/17 cases (88 %) (Figure 1f in Supplementary material). Furthermore, for glutamine synthetase cytoplasmic immunostaining was present in intralesional and perilesional astrocytes.

Peritumoral tissue was included in 14 slides immunostained with EAAT2, as well as in 15 slides immunostained with glutamine synthetase and pyruvate carboxylase.

4. Discussion

In this report, we document that angiocentric gliomas do not express EAAT2 whereas, analogously to non-neoplastic astrocytes, they express pyruvate carboxylase and glutamine synthetase.

EAAT2 is a predominantly glial glutamate transporter and is responsible for about 90 % of total forebrain glutamate uptake activity [8,11].

Considering that loss of EAAT2 causes glutamate accumulation, a consequent hyper-excitability and epilepsy is conceivable, despite the preserved expression of pyruvate carboxylase and glutamine synthetase. Several studies confirm this hypothesis.

Deletion of the *EAAT2* gene in mice causes nearly complete loss of glutamate uptake activity and seizures while transgenic mice over-expressing EAAT2 are less prone to seizures [11]. Recent studies suggest that in humans *de novo* mutations in *EAAT2* may determine early-onset of epilepsy with multiple seizure types and that dysplastic cortical tissue has a reduced EAAT2 expression [8,11]. It has also been demonstrated that high-grade gliomas associated with seizures have significantly higher glutamate levels and reduced expression of transporters (in particular EAAT2) [12]. Moreover, it has been referred that the loss of EAAT2 expression is more pronounced in high-grade gliomas than low-grade astrocytomas [12,13]. The mechanisms whereby neoplastic astrocytes silence their *EAAT2* expression remain to be defined. A possible epigenetic regulation of *EAAT2* transcription based on the methylation of the corresponding promoter and the expression of aberrant *EAAT2* mRNA has been postulated [12]. However, whatever the cause for absent or reduced *EAAT2* expression in gliomas is, it would seem that the consequent high glutamate concentration plays an important role in tumor-associated seizures. Consequently, an induced increased EAAT2 protein expression is considered a potential therapeutic approach for treating tumor-associated epilepsy [11].

No studies have specifically investigated the EAAT2, pyruvate carboxylase and glutamine synthetase expression in angiocentric glioma.

All angiocentric gliomas we have studied here lack EAAT2 expression, while the expression of pyruvate carboxylase and glutamine synthetase is mostly preserved. The net result of EAAT2 deficiency could be an increased glutamate concentration in the synaptic cleft, which might increase local network excitability initially involving intratumoral neurons (Figure 1b in Supplementary material).

The hypothesis that the angiocentric glioma-associated epilepsy may be related to EAAT2 deficiency might open interesting therapeutic perspectives, as several studies have emphasized the possibility to pharmacologically increase EAAT2 levels [11].

Although the possibility of a compensatory role of other glutamate transporters has yet to be investigated and further studies on larger series - also including other epilepsy-associated low-grade gliomas - are necessary, our results show for the first time that angiocentric glioma lost EAAT2 expression and suggest that epilepsy, the main clinical consequence of this tumor, might derive, at least in part, from a deficient expression of EAAT2. Moreover, considering the loss of EAAT2

expression has been reported as more pronounced in high-grade gliomas, further studies on different epileptogenic (or not) high- and low-grade gliomas will be useful to confirm the correlation of the EAAT2 loss to the epileptogenic potential of single entities among histological grades.

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Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.seizure.2021.02.014.

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