BRIEF REPORT

Stem-cell marker CD34, multidrug resistance proteins P-gp and BCRP in SEGA

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The resistance of human malignancy to multiple chemotherapeutic agents remains a major obstacle in cancer therapy due to in part to increased expression of ATP-binding cassette (ABC) transporters gene family, including "multidrug resistance 1", and "breast cancer resistant protein". These proteins are differentially expressed during normal hematopoiesis with the highest levels in primitive bone marrow CD34 stem cell population, and similarly, it was also suggested to occur transiently during neurulation. Subependymal Giant Cell Astrocytoma (SEGA) is a periventricular-low-grade tumor usually associated with tuberous sclerosis complex. We previously described that several multidrug-resistance proteins are overexpressed in brain cortical tubers associated with refractory epilepsy; however, they have not been investigated in SEGA. From a previous reported study of 15 brain specimens of SEGA, 6 randomized cases were selected for the immunostaining with specific monoclonal antibodies to multidrug resistance 1, breast cancer resistant protein and CD34 stem-cell marker. Heterogeneous distributions for these markers were detected with differential immunostaining pattern, showing high immunoreactivity in SEGA cells far of vessels, and low or negative expression in SEGA cells near the vessels. This particular expression pattern of both ABC-transporters, and CD34 antigen could identify different stem-cell subset in SEGA.

Keywords: CD34; P-gp; BCRP; SEGA; Hypoxia; Tuberous Sclerosis; Stem-Cell

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Introduction

Epilepsy can be associated with primary brain tumors (PBTs) due to PBTs cause seizures in 20–45% of patients[1]. The tumor itself may be the seizure focus, or the tumor may cause secondary several perilesional tissue alterations, thereby triggering seizure activity. Tumor-associated seizures that typically manifest as focal crisis with secondary generalization are commonly refractory to antiepileptic drug treatment[2].

Intractable seizures are generally related with early-

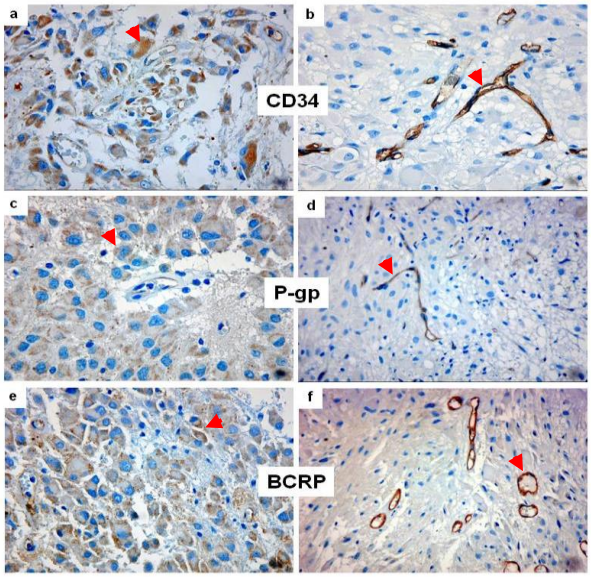

Figure 1.
a,c,e: Left panel: CD34, P-gp and BCRP expression in SEGA cells (magnification X20).
b,d,f: Right panel: CD34, P-gp and BCRP expression in BBB (➔) but not in the SEGA cells surrounding the vessels (magnification X10).

onset and lower-grade lesions and cause chronic seizures, and these features are commonly observed in low-grade tumors as oligodendrogliomas, or glioneuronal tumors such as gangliogliomas and dysembryoplastic neuroepithelial tumors, as well as in low-grade astrocytomas[3].

Tuberous sclerosis is a familial autosomal dominant disease and the most affected organs are skin, brain, kidney and heart. The major neuroradiologic lesions are tubers, subependymal nodules and subependymal giant cell astrocytoma (SEGA) and SEGA is a benign and slowly growing tumor corresponding to WHO grade I [4].

They are observed in 10% to 20% of patients with tuberous sclerosis complex (TSC) and are the major cause of morbidity in children and young adults with TSC[5]. Although increased intracranial pressure after misdiagnosed tumor growth, or intratumorous haemorrhage are their main neurological symptoms, also related with high risk of death[6] some reports describe SEGA presenting only with worsening of seizures[7]. The most frequent neurological signs of tuberous sclerosis are refractory epilepsy (RE) and mental retardation, which respectively affect about 80% and 60% of the patients whereas astrocytomas are present in 5% to 10% of the cases[8].

It was described that cortical tubers can act as seizure foci, and tumor cells themselves may create intrinsic epileptogenicity by several mechanisms that include glutamate secretion, inadequate homeostasis in the peritumoral tissue leading to alterations in the excitation-inhibition balance or membrane depolarization, resulting in higher seizure susceptibility. However, how pharmacoresistant seizures would relate to the presence of subependymal giant cell astrocytomas is not clear[9]. In previous study, we reported the abnormal overexpression of ATP-Binding cassette proteins (ABC-transporters: MDR1, MRP1 and BCRP) in cortical tubers from patients with TSC and RE[10,11] and more recently, the relationship between MDR-1 overexpression in hippocampus and epileptogenesis have been described[12].

The major cause of resistance of epilepsy to AEDs is overexpression of proteins belonging to mentioned ABC-transporters, in particular P-glycoprotein (P-gp) [13] and an overexpression of these proteins has been reported in brain tumor as glioma [14] ganglio-gliomas [15] that could lead to diminished drug transport into the brain parenchyma. Moreover, the release of glutamate upregulates P-gp expression [16].

As first described in cancer, ABC-transporter P-glycoprotein (P-gp) cause multidrug resistance by extruding their drug-substrates from the resistant cells and maintaining the intracellular level of these cytotoxic agents below cell-killing concentrations [17,18].

As above mentioned for RE, in many tumors multidrug resistance is caused by the activity of the several ABC-transporters, including MDR1/P-glycoprotein (ABCB1), the multidrug resistance-associated proteins MRP1 and MRP2 (ABCC1 and ABCC2), as well as the human ABC-half-transporter named breast cancer resistance protein BCRP (ABCG2)[19].

Interestingly, P-gp and BCRP not only are included as transient stem cell markers for normal hematopoietic side population stem cell (HSC) related with CD34(+) HSC[20-23], but also were related as markers of neural stem/progenitor cells [24,25]. Furthermore, isolated adult human hematopoietic CD34+ stem cells (HSCs) were differentiated into neurons and/or astrocytes according with differential culture conditions [26]. In spite several proteins have been studied as characteristic markers of brain tumor cells and a wide spectrum of new protein were recently described [27]. However, in SEGA, cellular expression of the stem-cell marker CD34 and the ABC-transporters P-gp and BCRP, have not investigated yet.

To investigate the immunostaining pattern expression

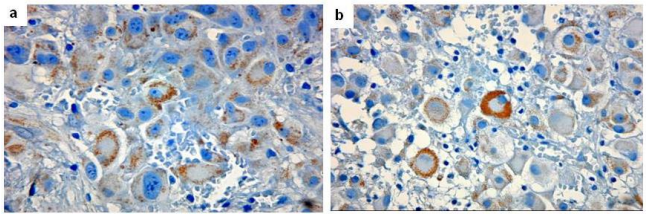

Figure 2.
a,b. Heterogeneous BCRP expression in SEGA cells (magnification X40).

of CD34 stem-cell marker and the ABC-transporters MDR-1 and BCRP, in brain specimens of SEGA.

Materials and methods

Selection of Brain tissue samples: from a total of 15 brain specimens of patients manifesting complete diagnostic criteria of SEGA surgically treated, 6 randomized cases were selected from the tissue collection of the Pathology Laboratory of the Garrahan Children's Hospital of Buenos Aires. Description of clinical presentation, hydrocephalus, neuroimaging by CT and MRI, surgery procedures, surgical effect on hydrocephalus, and tumor features including numbers of tumors, location, shape, and volume- size-growth ratio were previously reported in details [28].

Morphological analysis: Brain tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin and PAS methods for morphological analysis stained sections to confirm the diagnosis of SEGA.

Immunohistochemistry: The antibodies and dilutions were used as follows:

- BCRP monoclonal antibody (1:50; Kamiya Biomed Co-Seattle, USA)
- P-gp (MDR-1) polyclonal antibody (1:20; Santa Cruz, USA)
- CD34 monoclonal antibody (1:50; Clone: BIRMA-K3 Isotype: IgG1-kappa).

Thin sections of kidney and liver were used as immunostaining positive control specimens for MDR-1 and BCRP respectively; the specimens were collected from the same tissue archives (not shown).

Secondary polyclonal antibodies anti mouse (or anti-rabbit for MDR-1) were performed with a Streptavidin immunoperoxidase kit, according to the protocol recommended by the manufacturer (Biogenix, San Ramón, CA, USA). Brain normal tissue adjacent to both vascular malformation or brain tumors, were employed as normal controls. These samples were obtained from patients who had not received chemotherapy or any other chemical or radiological treatment.

Results

Clinical manifestation and seizures features as well as the tumors diagnosed as SEGA were previously described [28].

CD34, P-gp (MDR-1) and BCRP showed a particular distribution according with differential vascularization of the tumor´s area. SEGA-cells from poorly vascularized areas showed higher expression of these three markers compared with the cells from vascularized areas with low or negative immunoreactivity, where additionally, high expression of these 3 markers was observed in vascular endothelial cells (Figure 1a-f).

An evident heterogeneous intensity of the immune-reactivity was observed with BCRP in SEGA-cells (Figure 2a and 2b). All these particular immunostaing patterns were observed in all 6 cases studied. Positive and negative controls displayed the correct corresponding results (data not shown).

Discussion

Subependymal giant cell astrocytoma (SEGA) is a tumor that typically occurs in the lateral ventricle near the foramen of Monro and rarely in the third ventricle, and the association of this tumor with tuberous sclerosis complex (TSC) is well known [29]. SEGA is the most common intracranial tumor found in TS, it is a tumor thought to evolve from the enlargement of the hamartomatous subependymal nodule. Several studies have reported glial (astrocytic or rarely ependymal), neuronal or mixed glial-neuronal differentiation. However the histogenesis of this tumor is poorly understood [30,31].

Focal epileptic seizures are among the most common symptoms at disease onset in patients with different type of brain tumors and seizures can frequently predate other symptoms or diagnosis by many years [32].

Interestingly, seizures may be the only symptom for months or years in the non-progressive phase of the disease, and up to 80-90% of all patients with low-grade gliomas can experience seizures or epilepsy. In a recent study, approximately half of the patients with low-grade gliomas who presented with seizures were pharmacoresistant before surgery [33].

CD34 antigen, whose expression on primitive cells is

down-regulated as they differentiate into mature cells, has been considered to be the most critical marker for hematopoietic stem cells [34-36].

The MDR-1 gene encoded P-glycoprotein (P-gp) and the half-transporter known as the breast cancer resistant protein are expressed on CD34+ hematopoietic cells[37,38]. Furthermore, a highly enriched CD34 negative stem cell fraction termed *side population*, could also express P-gp and particularly BCRP as a marker of an earlier progenitor cell [39,40]. Similarly, in normal brain, CD34 occurs only transiently during neurulation [25], and recently CD34 immunoreactivity was also detected as a subpopulation of balloon cells confined to the white matter but not observed in neocortical layers from brain specimens of Taylor's focal cortical dysplasia [41].

In the central nervous system, the neural stem cells have been identified in different regions of fetal and adult brain [42,43]. These cells can self-renew and differentiate into all types of neural cells throughout life including neurons, astrocytes, and oligodendrocytes [44,45] and the expression of BCRP appears relatively specific for several stem cells, including neuronal stem cell [46,47].

Intriguingly, stem cells might demonstrate surprising plasticity, since purified hematopoietic stem cells were shown to give rise to liver tissues [48] or muscle cells [49] and several reports suggest bone marrow origin of a different type of neural cells, including mature neurons in brain [50-53].

Additionally, it has been shown that $CD34^+$ cells, contributes to vascular homeostasis, not only as a pool of endothelial progenitor cells, but as a source of growth-angiogenesis factors and identifying revasculatization or neovascularization at ischemic loci [54]. It was also demonstrated that under hypoxic conditions, a larger population of activated resident microglia express CD34 stem cell marker and undergo proliferation [55], and similarly in non-stressed conditions in vitro, expanded multipotent human neural progenitor cells also can express CD34 phenotype [56].

In tuberous sclerosis, the TSC2 gene loss upregulates the vascular endothelial growth factor expression through both mTOR-dependent and -independent pathways by accumulation of hypoxic inducible factor (HIF1□) and increased expression of HIF1□-responsive genes [57]. According with these observations, we could speculate that the high expression of these 3 markers in SEGA cells from non-vascularized areas of the tumor, suggest a link with the hypoxic condition, as a prone scenario for the annealing and growth of stem cells expressing these markers. In this regard, it is important to notice that brain expression of P-gp and BCRP genes are also related with hypoxic and/or inflammatory conditions [58-61].

Our data clearly indicate that P-gp and BCRP proteins are present in SEGA cells, which also display immunoreactivity toward the stem cell marker CD34.

The particular pattern of distribution of the markers, suggest that differential subsets of neoplastic cells could be present in SEGA, including the stem cell phenotype as demonastrated by CD34 antigen. The possibility that ABC-transporter expression indicates a stem-cell nature of SEGA cells still remains to be demonstrated, however, the expression of P-gp and BCRP, indicates that these cells could be refractory to pharmacological treatment.

Our group have described at the first time that several multidrug-resistance proteins are overexpressed in brain cortical tubers associated with RE [10,11,62]. Because all the cases studied were SEGA-associated with epilepsy, and patients selected were seizures free after surgical treatment. Now we additionally could also speculate that particularly P-gp overexpression could also related with the recently described role in the epileptogenesis [12].

The heterogeneous expression of the ABC-transporters (P-gp, BCRP) and CD34 stem-cell marker in SEGA suggests that subset of SEGA cells could be identified perhaps playing roles in tomourgenesis, pharmaco-resistance and epileptogenesis.

Conflict of interest

The authors declare that there is no conflict of interest.

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