Subependymal giant cell astrocytoma (SEGA): Is it an astrocytoma? Morphological, immunohistochemical and ultrastructural study.

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Original Article

Subependymal giant cell astrocytoma (SEGA): Is it an astrocytoma? Morphological, immunohistochemical and ultrastructural study

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Subependymal giant-cell astrocytoma (SEGA) is a rare intra-ventricular low-grade tumor which frequently occurs as a manifestation of tuberous sclerosis complex. The histogenesis of SEGA is controversial and its astrocytic nature has been doubted. First studies suggested the astrocytic nature of SEGA while several recent reports demonstrate its glio-neuronal nature. In spite of this, in the recently revised WHO classification of the CNS tumors, SEGA has been still included in the group of astrocytomas. We studied nine tuberous sclerosis complex-associated SEGAs. Patients were 1–18 years old. Eight patients (89%) had a solitary lesion located in the lateral ventricle close to of the head of the caudate nucleus, the remaining patient (11%) had two tumors, one located close to the head of the left caudate nucleus and the other in the central part of the right lateral ventricle. Histologically, tumors were composed of three types of cells: spindle, gemistocytic and ganglion-like. Four tumors (44%) had a prominent vascularization and three (33%) showed an angiocentric pattern. Calcifications were observed in six cases (66%). By immunohistochemistry, the majority of the tumors were GFAP- (9; 100%), neurofilament- (8, 89%), neuron-specific enolase- (9, 100%), and synaptophysin- (8; 89%) positive. Ultrastructural studies were performed on four cases. In all four there were glial cell processes filled with intermediate filaments. In one case dense core putative neurosecretory granules were appreciable. Our results emphasize the glio-neuronal nature of SEGA. We suggest moving it into the group of mixed glio-neuronal tumors under the denomination of subependymal giant cell tumor.

Key words: astrocytoma, immunohistochemistry, SEGA, subependymal giant cell astrocytoma, ultrastructure.

INTRODUCTION

Subependymal giant-cell astrocytomas (SEGA) are rare tumors associated with tuberous sclerosis complex. Tuberous sclerosis complex is a neurocutaneous syndrome which mainly involves the CNS where SEGAs, subependymal nodules, and cortical tubers may be present. In the majority of cases, SEGAs arise in the first two decades of life, from the head of the caudate nucleus near the foramen of Monro and grow inside the lateral ventricle. SEGAs are well circumscribed, often calcified, slowly growing, and low grade (World Health Organization [WHO] Grade I) tumors. Nevertheless, their outcome may be poor due to obstructive hydrocephalus or due to intratumoral hemorrhage.[1]

The histogenesis of SEGAs is controversial and their pure astrocytic nature has been doubted. Initial studies suggested the astrocytic character of SEGAs, while several recent reports demonstrate their mixed, glio-neuronal, nature.[2–5] Indeed, SEGAs are formed by three main kinds of cells, fibrillated spindle cells, swollen gemistocytic-like cells and giant pyramidal cells with a ganglioid appearance, which at the immunohistochemical and ultrastructural level, show both glial and neuronal features. In spite of this, in the recently revised WHO classification of CNS tumors,[1] SEGAs were still included in the group of astrocytic neoplasms. In this study we morphologically, immunohistoichemically, and ultrastructurally studied a series of

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nine tuberous sclerosis complex-associated SEGAs. Our purpose was to underline the mixed glio-neuronal nature of this tumor.

PATIENTS AND METHODS

Nine tuberous sclerosis complex-associated SEGA specimens were studied. They were obtained from nine consecutive patients surgically treated at the Neurosurgical Service of the Anna Meyer Pediatric Hospital of Florence, Italy, between 2004 and 2007. There were four male (44%) and five female (55%) patients. Ages ranged from 1 to 18 years at the time of the surgery (mean 6 years). Eight patients (89%) had a solitary lesion located in the lateral ventricle close to the head of the caudate nucleus, the remaining patient (11%) had two tumors, one located close to the head of the left caudate nucleus and the other located in the medial part of the right lateral ventricle. In all cases there were other CSN tuberous sclerosis-associated lesions: subependymal nodules in nine cases (100%) and cortical tubers in eight (89%). In three patients (33%) tuberous sclerosis occurred in a familial setting (father; father and grandmother; twins). In two patients SEGA was prenatally diagnosed. The most common symptom was seizures (7 cases; 78%). One patient (11%) had acute raised intracranial pressure. One patient (11%) was asymptomatic.

Histological, immunohistochemical, and ultrastructural studies were undertaken in the Department of Human Pathology and Oncology of the University of Florence. Surgical specimens were routinely fixed in 10% buffered neutral formalin and embedded in paraffin. Some 5-μm sections were stained with HE for morphological evaluation, and further 5-μm sections of the most representative sample were mounted on electrostatic slides and used for immunohistochemical studies. Immunohistochemical studies (standard streptavidin-biotin technique) were performed using the following primary antibodies: monoclonal antibody direct against GFAP (clone ZCG29), prediluted (Zymed Laboratories, San Francisco, CA, USA); neuron-specific enolase (NSE; clone MIG-N3, prediluted, BioGenex, San Ramon, CA, USA); synaptophysin (SP; clone Snp 88, prediluted, BioGenex) and multiclonal antibody against neurofilaments (NF; pan-clone-low, intermediate and high molecular weight subunits: DA2; FNP7; RMb020.11, 1:20 dilution, Zymed). Microwave antigen enhancement was utilized for all the used antibodies. Immunohistochemical staining was performed on NEXES automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA). Immunocoloration was evaluate separately in each type of neoplastic cell (spindle, gemistocytic, and ganglion-like cells) and was indicated as negative when it was present in no more than 50% of the cells and as positive if it was diffuse to more than 50% of the cells. Furthermore, we graded the immunocoloration as +/– or + on the basis of intensity of the staining (+/–: weak immunocoloration; +: strong immunocoloration).

Ultrastructural studies were performed on four cases. Tumor tissue fragments for electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.4) for 3 h, and post-fixed in 1% OsO_4 in 0.1 mol/L veronal acetate buffer (pH 7.4) for 1 h. The tissue samples were stained *en bloc* in 2% uranyl acetate in 50% ethanol, dehydrated in increasing concentrations of ethanol, cleared in propylene oxide, embedded in epoxy resin and cut using a diamond knife, with a Leica Ultracut R microtome. Ultrathin serial sections were mounted on formvar-coated Cu/Rh grids, stained with uranyl acetate and lead citrate, and observed with a Philips 410 LS transmission electron microscope.

RESULTS

Histologically, tumors were composed of three types of cells: spindle cells, gemistocytic-like cells and occasional ganglion-like cells (Fig. 1). A variable number of multinucleated cells were also detected. Four tumors (44%) had a prominent vascularization and three (33%) showed an angiocentric architectural pattern (Fig. 2). Calcifications were observed in six cases (66%).

By immunohistochemistry, the majority of the tumors were GFAP- (9; 100%), NF- (8, 89%), NSE- (9, 100%), and SP- (8; 89%) positive. In 5/9 GFAP-positive cases, spindle cells and the intercellular reticular matrix immunostained with anti-GFAP antibody (Fig. 3), while gemistocytic- and ganglion-like cells were negative; in two cases both spindle and gemistocytic cells were GFAP-positive; in the remain-

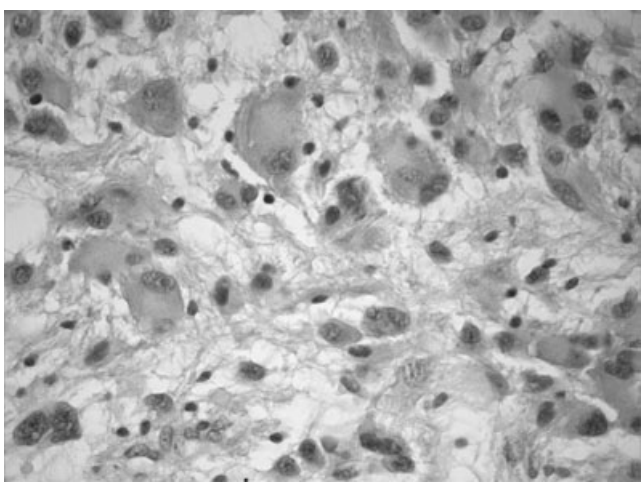

Fig. 1 Tumors were composed of three types of cells: spindle cells, gemistocytic-like cells and ganglion-like cells. HE staining. Original magnification × 200.

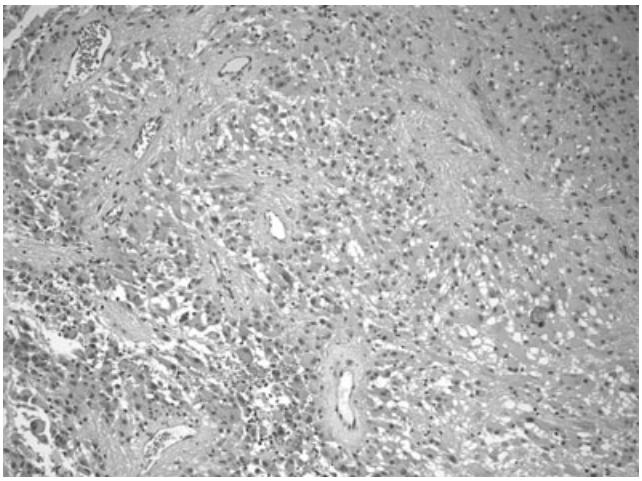

Fig. 2 Angiocentric architectural pattern. Original magnification × 100.

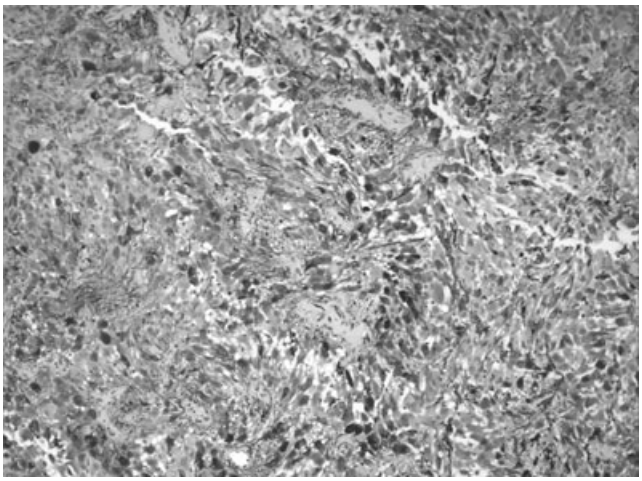

Fig. 4 Immunohisochemistry: diffuse GFAP immunostaining. Original magnification × 100.

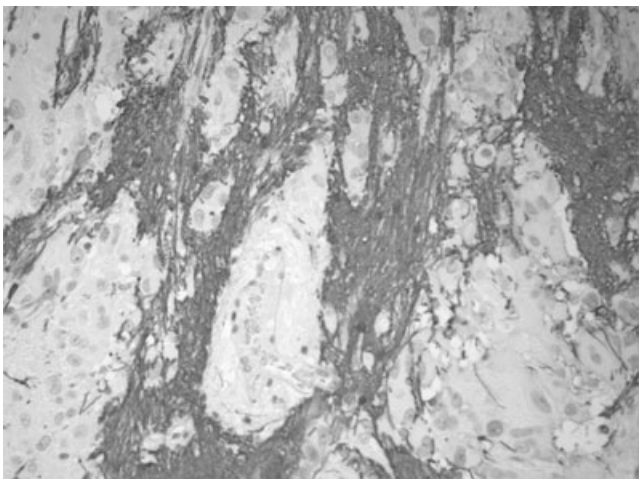

Fig. 3 Immunohisochemistry: GFAP immunostaining at the level of the spindle cells and of the intercellular reticular matrix. Original magnification × 200.

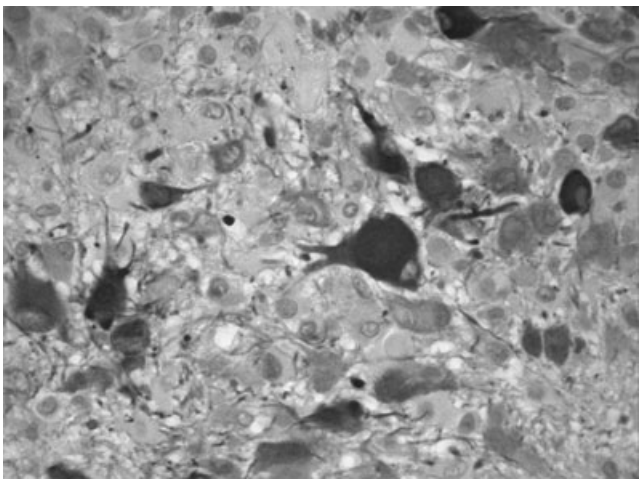

Fig. 5 Immunohisochemistry: neurofilament immunostaining at the level of ganglion-like cells. Original magnification × 400.

ing two GFAP-positive cases the immunostaining involved spindle, gemistocytic, and ganglion-like cells (Fig. 4). Antibody against NF stained only ganglion-like cells in six cases (Fig. 5), while it stained either ganglion-like and gemistocytic-like cells in two cases. NSE immunostaining was appreciable in all three types of cells in all tumors but one in which spindle cells were NSE-negative. SP immunostaining was almost always weak, focal and detected in gemistocytic- and ganglion-like cells in all eight SP-positive tumors (Fig. 6) (Table 1).

Ultrastructurally, all cases presented a background of glial cell processes filled with intermediate filaments, in which a variable number of giant and multinucleated cells were detected. The cytoplasm of these cells contained several mitochondria, rough endoplasmic reticulum profiles, free ribosomes, multiple Golgi profiles, several primary lysosomes and a well-developed cytoskeleton composed mainly of microtubules and less prominent bundles of intermediate filaments. In one case, we identified putative dense core neurosecretory granules in the cytoplasm of multinucleated giant cells. Deposits of calcium, which appeared as irregular electron-dense circular structures surrounding either amorphous matrix or cellular debris, were detected in one case (Figs 7,8).

DISCUSSION

The histogenesis of SEGA is still unclear. A pathogenetic link between subependymal nodules and SEGAs has been suggested. Several studies have been demonstrated, by sequential neuroimaging follow-up, that there is a

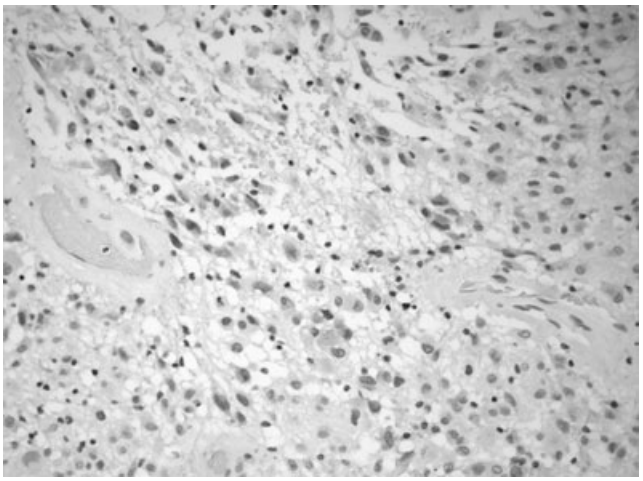

Fig. 6 Immunohistochemistry: synaptophysin immunostaining at the level of gemistocytic- and ganglion-like cells. Original magnification × 200.

continuum from subependymal nodules to SEGAs. Nevertheless, it is unclear if all or just a part of subependymal nodules have this potential. Moreover, it is difficult to distinguish either at the clinical or radiological level, the nodules with evolutive property from those without this property. Some authors have demonstrated that all subependymal nodules are clonal, have the capacity to proliferate, and behave as true neoplasms.[6–9]

It has been recently noted that there are also morphological, immunohistochemical and molecular similarities between SEGA and Type IIb focal cortical dysplasia.[10,11] Type IIb focal cortical dysplasia is morphologically characterized by the presence of large cells known as balloon cells. In the opinion of some authors, Type IIb focal cortical dysplasia could even be considered a focal form of tuberous sclerosis.[12] SEGA, cortical tubers, subependymal nodules, and Type IIb focal cortical dysplasia could originate from undifferentiated neuroepithelial cells which may remain undifferentiated, or differentiate into astrocytic, neuronal,

Table 1 Immunohistochemical and ultrastructural results

Case	Immunohistochemistry					Ultrastructure
		GFAP	NF	NSE	SP	
1	Spindle cells	+	–	+/–	–	–
	Gemistocytic-like cells	+	–	+/–	+/–	
	Ganglion-like cells	–	+	+/–	+/–	
2	Spindle cells	+	–	+	–	–
	Gemistocytic-like cells	+	+	+	+/–	
	Ganglion-like cells	+	+	+	+	
3	Spindle cells	+/–	–	+	–	–
	Gemistocytic-like cells	–	–	+	+/–	
	Ganglion-like cells	–	–	+	+/–	
4	Spindle cells	+	–	+	–	Large cells: microtubules, mitochondria, RER
	Gemistocytic-like cells	–	–	+	+/–	profiles, free ribosomes, primary lysosomes.
	Ganglion-like cells	–	+	+	+	Glial cell processes filled with intermediate filaments
5	Spindle cells	+	–	–	–	Large cells: microtubules, mitochondria, RER
	Gemistocytic-like cells	–	–	+/–	+/–	profiles, multiple Golgi complexes, primary and
	Ganglion-like cells	–	+	+/–	+/–	secondary lysosomes, calcium deposits around amorphous matrix or cellular debris.
						Glial cell processes filled with intermediate filaments
6	Spindle cells	+	–	+	–	–
	Gemistocytic-like cells	+	+	+	+/–	
	Ganglion-like cells	–	+	+	+/–	
7	Spindle cells	+	–	+/–	–	Large cells: microtubules, mitochondria, RER
	Gemistocytic-like cells	–	–	+/–	+/–	profiles, multiple Golgi complexes, primary
	Ganglion-like cells	–	+	+/–	+/–	lysosomes, pynocytotic vescicles
						Glial-cell processes filled with intermediate filaments
8	Spindle cells	+	–	+	–	Large cells: microtubules, intermediate filaments,
	Gemistocytic-like cells	–	–	+	+/–	mitochondria, RER profiles, primary lysosomes,
	Ganglion-like cells	–	+	+	+/–	dense core putative neurosecretory granules.
						Glial cell processes filled with intermediate filaments
9	Spindle cells	+	–	+/–	–	–
	Gemistocytic-like cells	+	–	+/–	–	
	Ganglion-like cells	+	+	+/–	–	

NF, neurofilament; NSE, neuron-specific enolase; SP, synaptophysin; RER, rough endoplastic reticulum.

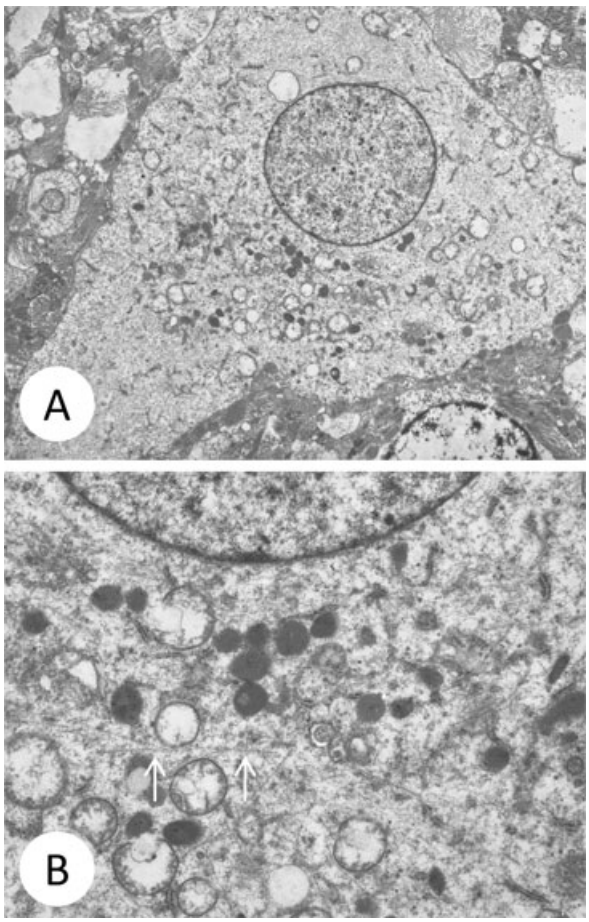

Fig. 7 A: Giant cell in a background of glial cell processes. The nucleus is round and the cytoplasm contains short rough endoplastic reticulum profiles, mitochondria and primary lysosomes. B: The cytoskeleton comprises microtubules (arrows) and intermediate filaments. Original magnification × 2100 (A); × 4400 (B).

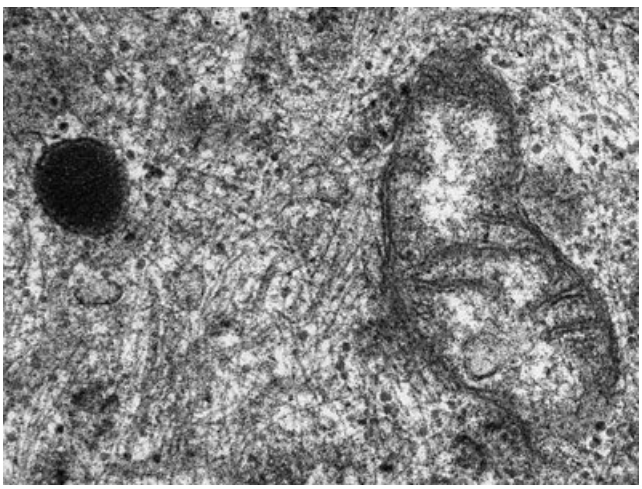

Fig. 8 Putative dense core neurosecretory granule measuring 240 nm. 10-nm intermediate filaments are visible in the background. Original magnification × 21 000.

or mixed glio-neuronal cells.[7,13] Analogously to SEGA, subependymal nodules and Type IIb focal cortical dysplasia express immunohistochemically both glial and neuronal markers.[3,7,14–16] Among neuronal markers, SP expression is generally reported as weak and focal in SEGAs.[4,17]

Subependymal giant-cell astrocytomas which we studied were homogeneous enough at a morphological level with only some differences concerning the architecture of the lesion and the amount of calcifications. At the immunohistochemical level, all studied cases revealed mixed glio-neuronal features. Indeed, all lesions were both neuronal- and glial marker-positive. However, there were variations in the immunoprofile among the different cell types and among the different tumors. At the ultrastructural level, SEGA giant cells which we studied had some features suggestive of neuronal differentiation, including the presence of microtubules, abundant rough endoplastic reticulum cysternae and free ribosomes, while bundles of intermediate filaments, indicative of glial differentiation, were seen only in cytoplasmic processes which likely originated from spindle astrocytic cells in the background. In addition, in one case we detected dense core granules that we interpreted as putative neurosecretory granules. In previous ultrastructural studies, other features strongly supportive of neuronal differentiation were the presence of a synapse between a tuber giant cell and an axonal termination.[18,19]

Further studies on larger series are certainly necessary to definitively understand the histogenesis of SEGA. Nevertheless, in consideration of our results and of the previous scientific reports, we would suggest moving this entity from the group of astrocytomas to the group of mixed glio-neuronal tumors under the denomination of subependymal giant cell tumor.

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