

RESEARCH ARTICLE

Enhanced expression of autophagy-related p62 without increased deposits of neurodegeneration-associated proteins in glioblastoma and surrounding tissue – An autopsy-based study

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Abstract

Neurodegenerative diseases are a major health burden. The underlying causes are not yet fully understood, but different mechanisms such as cell stress and chronic inflammation have been described as contributing factors. Neurodegenerative changes have been observed in the vicinity of brain tumors, typically around slowly growing benign lesions. Moreover, in-vitro data suggest a potential induction of pathological tau deposits also in glioblastoma, a highly malignant and proliferative brain cancer. The aim of this study was to evaluate neurodegeneration-associated protein deposition and autophagy as well as microglial activation within and surrounding glioblastoma. *Post-mortem* brain tissue of 22 patients with glioblastoma was evaluated immunohistochemically for phosphorylated tau, beta-amyloid, alpha-synuclein and phosphorylated TDP-43. Additionally, the autophagy marker p62 and the microglial marker HLA-DR were investigated. The data was compared to 22 control cases and ten cases with other space occupying brain lesions. An increase of p62-immunoreactivity was observed within and adjacent to the glioblastoma

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tumor tissue. Moreover, dense microglial infiltration in the tumor tissue and the immediate surrounding brain tissue was a constant feature. Deposition of neurodegeneration-associated proteins was found in the majority of cases (86.4%) but in distant sites. These findings suggested a preexisting neurodegenerative pathology, which followed a typical distributional pattern: ten cases with Alzheimer disease neuropathological changes, including two severe cases, eight cases with primary age-related tauopathy, six cases with aging-related tau astrogliaopathy and one case with progressive supranuclear palsy. Collectively, our data suggests enhanced autophagy in glioblastoma tumor cells and the surrounding brain. The variety and distribution of distant neurodegeneration-associated protein aggregates observed in the majority of cases, suggest a pre-existing rather than a tumor-induced neurodegenerative condition.

KEY WORDS

autophagy, glioblastoma, neurodegeneration, p62, Tau

1 | INTRODUCTION

Neurodegenerative diseases, with Alzheimer's disease as the most common representative, constitute a major global health issue and they gain even more importance due to demographic change and increases in life expectancy [1]. As a common feature, neurodegenerative diseases are characterized by progressive deposition of pathological proteins associated with cellular dysfunction and neuronal loss [2]. The exact mechanisms triggering pathological protein deposition are still not fully understood. Different influencing factors such as chronic cellular stress including chronic inflammation, oxidative stress and mitochondrial dysfunction have been proposed [3–5]. One of the most important risk factors of neurodegeneration is aging per se [6]. One hallmark of aging is deficient proteostasis [7], which plays a crucial role in the accumulation of neurodegeneration-associated proteins [2]. Neurodegeneration-associated protein deposits have been encountered in brain tumor patients, mostly in patients with slowly growing, low grade or benign tumor types such as meningioangiomas or gangliogliomas [8–12]. Neuropathologically, these deposits were characterized by tau-positive neurofibrillary tangles, neuropil threads as well as granulovacuolar degeneration [8–10]. This has been described mostly in adults, potentially proposing a synergistic effect of age on tumor development and the occurrence of neurodegeneration [8]. It was hypothesized that these changes could be partially due to chronic inflammatory infiltrates in the tumor and disturbance of its microenvironment [8]. In contrast, only a single study has explored the occurrence of neurodegenerative changes in a series of autopsy brains of patients with glioblastoma, a high-grade and proliferative active brain cancer. While the authors did describe Alzheimer pathology, their assessments were limited to few cortical regions and did not include further proteins linked to neurodegenerative diseases other than AD [13].

We therefore performed a comprehensive morphological characterization of different protein deposits linked to neurodegenerative diseases in glioblastoma, its vicinity and at distant sites. Using a local series of autopsy brains of individuals with glioblastoma, we aimed to identify particular patterns that would suggest a pathogenic link between glioblastoma and neurodegeneration.

2 | MATERIALS AND METHODS

2.1 | Post-mortem brain samples

Post-mortem brains of 22 adult patients with neuropathological diagnosis of glioblastoma between 2004 and 2019 were retrospectively identified from the Neuro-biobank of the Division of Neuropathology, Department of Neurology at the Medical University of Vienna. Reasons for performing an autopsy included a rapid disease evolution, lack of previous biopsy for diagnostic confirmation or rarity of co-existing diseases. Patient characteristics were retrospectively collected from the clinical files, where available (Table 2). In those patients who underwent previous surgery, biopsy or resection specimens were reexamined.

Additionally, an age-matched control group of 22 patients who died of non-neurological causes (cardiac causes, systemic tumor without neurological affection, infectious diseases without direct neurological affection, suicide) and an age-matched group consisting of ten patients with other space occupying lesions of the brain (metastasis, lymphoma, abscess) were analyzed.

2.2 | Immunohistochemical stainings

Formalin-fixed, paraffin-embedded tissue blocks from the tumor region, distant regions with areas

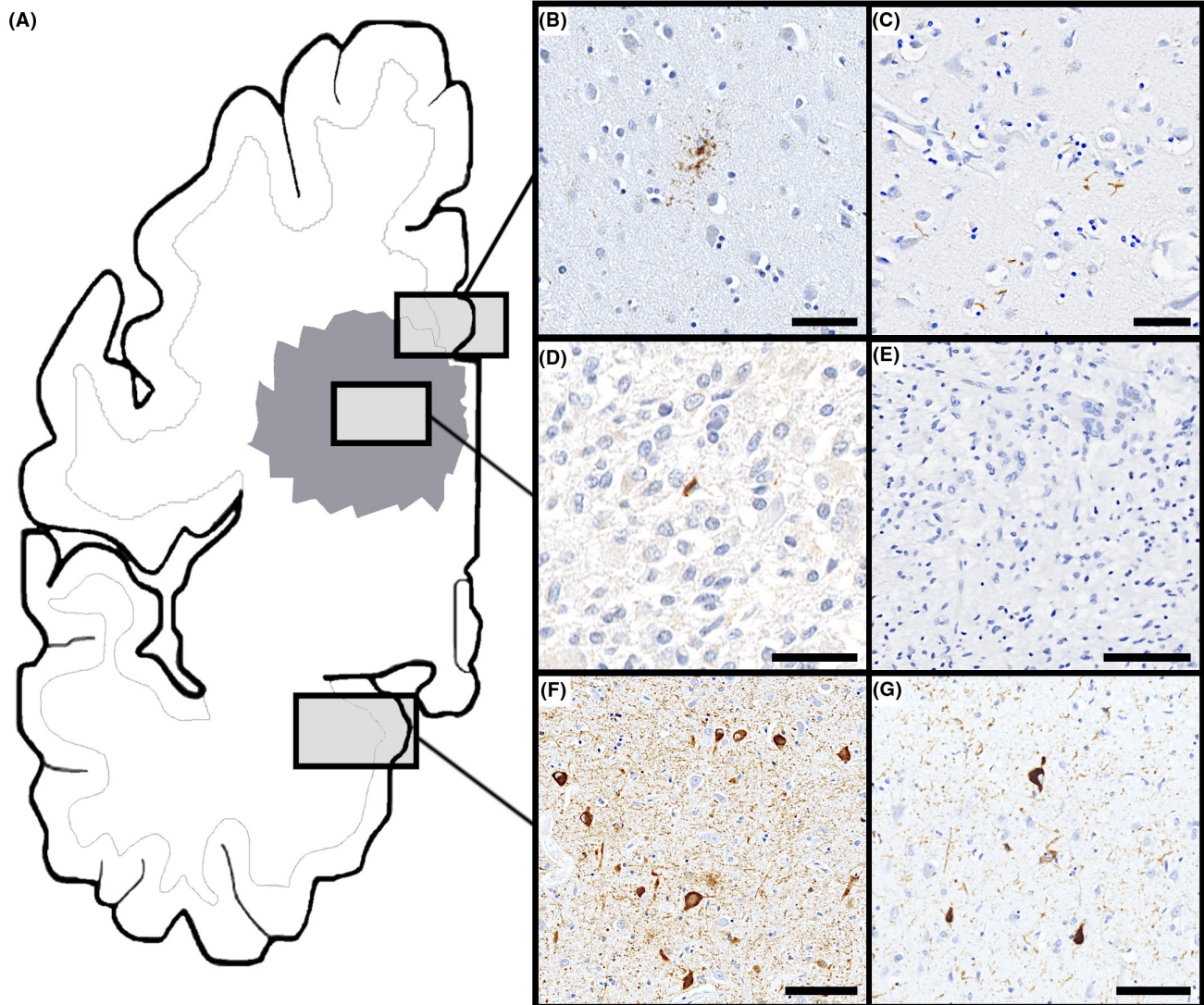


FIGURE 1 Neurodegeneration-associated proteins found within the tumor, the immediate surroundings and in areas prone to neurodegenerative pathology depicting two exemplary cases. (A) Figure of a coronary section through a brain with a tumor (depicted in grey) and strategy of evaluation. (B, D, F) PSP case showing tufted astrocytes in the immediate tumor surroundings, isolated tau-positive remnants in the tumor tissue and more pronounced pathology in the medial temporal lobe, distant from the tumor (AT8); (C, E, G) Isolated neuropil threads in the cortex adjacent to the tumor in a case with ADNC, no immunoreactivity in the tumor itself, tangles, pretangles and neuropil threads in the medial temporal lobe (AT8); Scale bars: B, C, D: 50 μ m; E, F, G: 100 μ m

sensitive to neurodegeneration (frontal cortex, basal ganglia, hippocampus, amygdala, pons, medulla oblongata; [Figure 1](#)) were stained with hematoxylin and eosin (H&E; [Figure 3A](#)) and by immunohistochemistry applying a panel of primary antibodies ([Table 1](#)). Immunoreaction was visualized by the DAKO Envision System kit (DAKO, Glostrup, Denmark) using the Dako-Autostainer 48 Link platform, diaminobenzidine was used as chromogen.

2.3 | Neuropathological examination

Morphological assessment was performed on H&E-stained sections. Brain regions were further analyzed by

immunohistochemistry for the identification of different neurodegeneration-associated proteins, the autophagy marker p62 and for the inflammatory marker HLA-DR to provide information on the tumor microenvironment and inflammation.

Densities of immunohistochemical reactions were evaluated semiquantitatively as previously described [[14](#)]. A magnification of 100x was used for the following antibodies:

Tau: none (-); isolated (i) 1–2 immunoreactive structures; mild (+) 3–6 immunoreactive structures; moderate (++) 7–10 immunoreactive cells; severe (+++) >10 immunoreactive cells. Neurofibrillary pathology was staged according to Braak&Braak [[15,16](#)]. Alzheimer disease neuropathologic changes were staged according

TABLE 1 Primary antibodies used for immunohistochemical characterization

Antibody name	Clone	Company	Dilution
Anti-p62	Clone 3/p62 lck ligand	BD Transduction Laboratories, Franklin Lakes, NJ, USA	1:500
Anti-phosphorylated tau	Clone AT8, pS202/pT205	Thermo Scientific, Rockford, IL, USA	1:200
Anti-βA4-amyloid	Clone 6F/3D	DAKO, Glostrup, Denmark	1:100
Anti-alpha-Synuclein	Clone 5G4	Roboscreen, Leipzig, Germany	1:4000
Anti-phosphorylated TDP-43	Clone 11-9, pS409/410	Cosmo Bio, Tokyo, Japan	1:20,000
Anti-HLA-DR	Clone CR3/43	DAKO, Glostrup, Denmark	1:400
Anti-Ubiquitin	Clone Ubi-1	Millipore, Temecula, CA, USA	1:50,000
Anti-CD68	Clone KP1	DAKO, Glostrup, Denmark	1:5000

to the National institute on aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease. [17]

BA4: none (-); isolated (i) 1–5 plaques; mild (+) 6–15 plaques; moderate (++) 15–30 plaques; severe (+++) >30 plaques. Neuritic plaques were staged according to CERAD criteria [18]. Amyloid phases were assessed according to Thal [19]. Cerebral amyloid angiopathy (CAA) was described as absent (-) or present (+) and further described according to Thal [20].

alpha-Synuclein: none (-); isolated (i) 1–2 dots/neurites, mild (+) 3–6 immunoreactive structures; moderate (++) 7–10 immunoreactive structures; severe (+++) >10 immunoreactive structures. Lewy bodies were staged according to McKeith and Braak [21–23].

phospho-TDP-43: none (-); isolated (i), 1–2 neuropil dots or single threads, mild (+), 3–6 immunoreactive structures; moderate (++) , 7–10 immunoreactive structures; and severe (+++) , >10 immunoreactive structures.

p62: none (-), isolated (i), 1–4 immunoreactive structures, mild (+), 5–10 immunoreactive structures; moderate (++) , 11–15 immunoreactive structures; severe (+++) , >15 immunoreactive structures.

HLA-DR was evaluated using a magnification of 400x: none (-); isolated (i), 1–20 immunoreactive structures, mild (+), 20–50 immunoreactive structures; moderate (++) , 51–100 immunoreactive structures; severe (+++) , >100 immunoreactive structures.

CD68 was evaluated using a magnification of 400x as follows: none (-); isolated (i), 1–20 immunoreactive structures, mild (+), 20–50 immunoreactive structures; moderate (++) , 51–100 immunoreactive structures; severe (+++) , >100 immunoreactive structures.

3 | RESULTS

3.1 | Patients' characteristics

Post-mortem brain samples from 22 adult patients with glioblastoma were collected. Mean *post-mortem* delay was 24.1 hours (range 5–62, SD 15.8), mean formalin fixation time was 30.6 days (range 2–134, SD 30.1). Six

patients were female. One case was a secondary glioblastoma. Mean age at death was 63.9 years (range 37–88, SD 14.3) and mean disease duration was 5.3 months (0.5–35, SD 7.7, median 2.5 months). One case (case 11) had a clinical diagnosis of Parkinson's disease and two cases (case 5, case 18) had an initial suspicion of a prion disease due to rapidly progressive dementia and confusion, respectively. One patient (case 19) had preexisting cognitive dysfunction due to Trisomy 21. In the other cases no preexisting neurological conditions were noted. 13 patients underwent previous surgery (biopsy: five, resection: eight). Nine patients received chemotherapy, twelve did not, and data on therapy was lacking in one case. Nine patients underwent radiotherapy, twelve did not, and data on radiotherapy was lacking in one case. Clinical and demographic characteristics of the GBM group are summarized in Table 2.

Mean age of the non-neurological control group was 63.6 years and consisted of seven females and 15 males. Mean age of the control group with other space occupying lesions was 60.8 years; there were three females and seven males. Further patients' characteristics of these two groups are summarized in Table S1.

3.2 | Neuropathological examination

3.2.1 | Neurodegeneration-associated proteins

The tumor masses rarely exhibited neurodegeneration-associated protein deposits (Figure 2A–C). One case revealed isolated within-tumor tau deposition. Beta-amyloid was found inside the tumor in two cases, one showing isolated and the other a low density of deposits. No intra-tumoral alpha-synuclein or phospho-TDP-43 was identified. No cases with protein deposits confined to the tumor tissue without affection of the surrounding brain tissue were found and generally the amount of protein deposits was higher in the surrounding brain tissue.

In the immediate surroundings (Figure 2D–F) of the tumor severe tau deposition was found in one case (4.5%) with neuropil threads, neurofibrillary tangles

TABLE 2 Patients' clinical and demographic characteristics

No.	Sex	Age (m)	Duration (m)	Diagnosis	Location	Surgery	CTx	RTx	Symptoms at onset	Dementia	Comorbid disease	Effective cause of death	PMD (h)	FFT (d)
1	M	61	1	Primary GBM	Frontal	No	No	No	Somnolence	No dementia	COPD, arterial hypertension, alcoholism	Pneumonia	10	21
2	M	78	2.5	Primary GBM	Parietal	No	Yes	No	Dysarthria, hemiparesis	No dementia	Cardiomyopathy, coronary heart disease, AV block, arterial hypertension, PAOD, diabetes	Pneumonia, cardiomyopathy	22	2
3	M	77	2.5	Primary GBM	Fronto-basal	No	No	No	Impaired vision, aphasia, hemiparesis	No dementia	Arterial hypertension, diabetes, chronic kidney disease	Cardiorespiratory failure	25	21
4	M	73	2.5	Primary GBM	Frontal	No	No	No	Epileptic seizure	No dementia	Arterial hypertension, diabetes, chronic kidney disease	Intracerebral bleeding	41	2
5	F	61	13	Primary GBM	Basal ganglia	No	No	No	Epileptic seizure	Dementia	Atrial fibrillation, arterial hypertension	Cardiorespiratory failure	48	21
6	M	56	6	Primary GBM	Fronto-basal	Resection	Yes	Yes	Organic psychosyndrome	No dementia	Arterial hypertension, chronic kidney disease	Cardiac arrest	6	8
7	M	45	13	Primary GBM	Amygdala	Resection	Yes	Yes	Epileptic seizure	No dementia	Axial spondyloarthritis	Cardiac arrest	22	59
8	F	59	2	Primary GBM	Parietal	Biopsy	Yes	Yes	Epileptic seizure	No dementia	Diabetes, COPD, arterial hypertension, pulmonary adenocarcinoma, pulmonary emphysema, atherosclerosis	Cardiorespiratory failure	25	53
9	M	70	3.5	Primary GBM	Temporal	Resection	Yes	Yes	Recurrent falls	No dementia	COPD, arterial hypertension, PAOD	Pneumonia, heart failure	11	58
10	M	81	NA	Primary GBM	Central	No	NA	NA	NA	NA	Arterial hypertension, coronary heart disease, obesity	Cardiorespiratory failure	27	134
11	M	77	4.5	Primary GBM	Frontal	No	No	No	Epileptic seizure	No dementia	Parkinsonism, pulmonary emphysema, coronary heart disease	Cardiac arrest	62	10
12	M	43	1	Primary GBM	Basal ganglia	resection	Yes	Yes	NA	No dementia	Multiple sclerosis, atherosclerosis	Pneumonia	24	6
13	F	56	1	Primary GBM	Temporal	No	No	No	Aphasia	No dementia	Atherosclerosis, steatosis hepatitis	Multi organ failure	18	13
14	M	38	35	secondary GBM	Medulla oblongata	resection	No	Yes	Double vision	No dementia	None	Unknown	12	28
15	F	68	0.5	Primary GBM	Parietal	Resection	No	No	Dysarthria, hemiparesis	No dementia	Hemithyroidectomy	Cardiac arrest	9	27
16	F	83	7	Primary GBM	Centro-parietal	Biopsy	No	No	Dizziness, headache	No dementia	Diabetes mellitus, arterial hypertension, coronary heart disease, PAOD	Cardiac arrest	21	14
17	M	64	3	Primary GBM	Frontal	Biopsy	Yes	Yes	Fatigue, apathy	No dementia	Diabetes mellitus, coronary heart disease	SEPSIS	61	28
18	M	88	3	Primary GBM	Parietal	No	No	No	Confusion	No dementia	Cardiac insufficiency	Cardiorespiratory failure	24	21
19	M	61	1.5	Primary GBM	Fronto-parietal	Biopsy	No	No	Hemiparesis	Dementia	Trisomy 21, chronic kidney disease, cardiac vitium	Multi organ failure	5	23

(Continues)

TABLE 2 (Continued)

No.	Sex	Age (m)	Duration (m)	Diagnosis	Location	Surgery	CTx	RTx	Symptoms at onset	Dementia	Comorbid disease	Effective cause of death	PMD (h)	FFT (d)
20	F	37	1.5	Primary GBM	Frontal	Resection	Yes	Yes	Organic psychosyndrome	No dementia	Arteriosclerosis, ovarian cyst	Brain edema, brain herniation	17	67
21	M	61	2	Primary GBM	Insula	Biopsy	No	No	Dizziness, aphasia, gait disturbance	No dementia	Chronic bronchitis, pulmonary emphysema	Cardiorespiratory failure	20	48
22	M	69	5	Primary GBM	Parieto-occipital	Resection	Yes	Yes	Impaired vision, confusion	No dementia	Arterial hypertension, condition after stroke right brain hemisphere	Cardiac arrest	21	8

Abbreviations: Age, age at death; AV block, atrioventricular block; COPD, chronic obstructive pulmonary disease; CTx, chemotherapy; d, days; F, female; FFT, formalin fixation time; GBM, glioblastoma; h, hours; M, male; m, months; NA, not available; No., case number; PAOD, peripheral artery occlusive disease; PMD, *post-mortem* delay; RTx, radiotherapy.

(NFT) and pretangles. One case (4.5%) exhibited a moderate amount of tau pathology with NFT, tufted astrocytes and coiled bodies, two cases (9.1%) showed a mild amount of tau pathology with few threads and single pretangles, six cases (27.3%) showed isolated tau deposits with single neuropil threads and twelve cases (54.5%) did not exhibit tau deposits.

Peritumorally, one case (4.5%) showed severe beta-amyloid-pathology, two cases (9.1%) exhibited moderate pathology, four cases (18.2%) showed mild beta-amyloid deposits, two cases (9.1%) showed isolated beta-amyloid deposits and 13 cases (59.1%) did not harbor any beta-amyloid. No alpha-synuclein or phospho-TDP-43 deposits were found peritumorally.

Those cases showing neurodegeneration-associated protein deposits within the tumor and in the surrounding brain tissue were associated with more widespread neurodegenerative conditions following typical spatial distribution. Ten cases (45.5%) showed Alzheimer disease neuropathologic changes (ADNC, [Figure 2F–H](#)), eight cases (36.4%) had primary age-related tauopathy (PART), six cases (27.3%) exhibited ageing-related tau astrogliopathy (ARTAG, [Figure 2I,J](#)), five cases (22.7%) had cerebral amyloid angiopathy (CAA, [Figure 2C,H](#)) and one case (4.5%) showed tufted astrocytes (TA), neurofibrillary tangles (NFT) and coiled bodies (CB), typical of progressive supranuclear palsy (PSP, [Figure 1B,D,F](#) and [2E,K,L](#)). In summary, 19 cases (86.4%) showed any neurodegeneration-associated protein deposits. One case (4.5%) was totally devoid of neurodegeneration-associated proteins. In two cases (9.1%), the available tissue was restricted to the tumor and its immediate surroundings, not allowing further assessment of neurodegenerative changes. Most cases exhibited a low amount of neurofibrillary change with a Braak stage I in seven cases (31.8%) and a Braak stage II in five cases (22.7%). No tau-positive astrocytic plaques, argyrophilic grains or atypical neuronal or glial pathology were observed. No alpha-synuclein deposits were found. One case exhibited isolated phospho-TDP-43-positive threads in the amygdala only. For detailed staging see [Table 3](#). In the previous surgical biopsy or resection samples tau and beta-amyloid deposits were found in three cases. These findings were comparable to the changes observed in the post-mortem tissue of the same patients.

In the non-neurological control group, ten patients (45.5%) had ADNC, seven patients (31.8%) had PART, seven patients (31.8%) had ARTAG, three patients (13.6%) exhibited CAA, one patient (4.5%) showed changes consistent with argyrophilic grain disease (AGD), there was one patient (4.5%) with incidental PSP, two patients (9.1%) showed alpha-synuclein-deposits and one patient (4.5%) had isolated phospho-TDP-43-positive threads in the frontal cortex. Only one case was totally devoid of neurodegeneration-associated proteins. Also in this group, most cases exhibited a low amount of neurofibrillary tau pathology with a Braak stage I in six cases

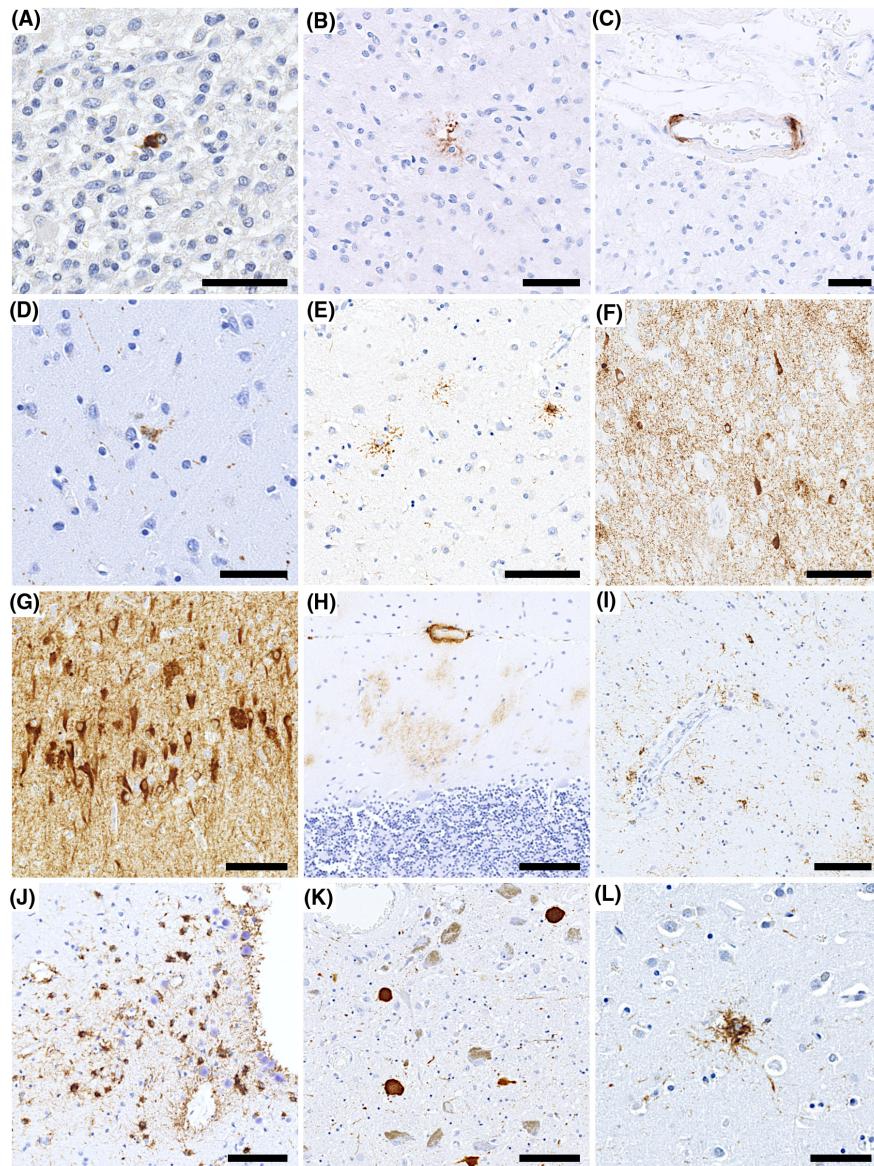


FIGURE 2 Different neurodegenerative changes found in the cohort. (A–C) Pathological deposits found inside the tumor; (A) Isolated cytoplasmic deposits resembling a coiled body found inside of the glioblastoma in the single case with PSP (AT8); (B) Remnants of a beta-amyloid-plaque found inside the tumor in a case with ADNC (β A4); (C) Beta-amyloid in the vessel wall in the tumor region (β A4); (D–F) Pathological protein deposits found in the immediate surroundings of the tumor; (D) Pretangle and neuropil threads in an Alzheimer's disease case (AT8); (E) Tufted Astrocytes in the PSP case (AT8); (F) Abundant pretangles, tangles and neuropil threads in an Alzheimer's disease case (AT8); (G–L) Pathologies found distant from the tumor; (G) Abundant tangles, pretangles and neuropil threads in the CA1-sector of the hippocampus in a case with ADNC (AT8); (H) Diffuse beta-amyloid deposits and cerebral amyloid angiopathy in the cerebellar cortex in a case with severe ADNC (β A4); (I) Perivascular ARTAG with thorn shaped astrocytes (AT8); (J) Subpial ARTAG with abundant thorn shaped astrocytes (AT8); (K) Globose tangles in the locus coeruleus of the patient with PSP (AT8); (L) Tufted astrocyte in the basal ganglia of the patient with PSP (AT8); Scale bars: A, B, C, D, L: 50 μ m; E, F, G, H, I, J, K: 100 μ m

(27.3%), and a Braak stage II in three cases (13.6%). For a detailed staging see Table S2.

In the third study group with other space occupying lesions, three patients (30%) had ADNC, five patients (50%) had PART, three patients (30%) had ARTAG, one patient (10%) had AGD, and one patient (10%) showed alpha-synuclein-deposits in the brainstem. The space occupying lesions themselves rarely contained neurodegeneration-associated protein deposits. For a detailed staging see Table S2.

3.3 | Microglial infiltration

Microglial reaction inside the glioblastoma as evaluated by anti-HLA-DR-immunohistochemistry was severe in ten cases (45.5%) (Figure 3B,C), moderate in eight (36.4%), mild in one (4.5%), and showed only isolated activated cells in two cases (9.1%); no significant microglial reaction was observed in one case (4.5%). Peritumorally, one case (4.5%) showed a moderate microglial reaction, 18 (81.8%) had mild reaction, isolated activated microglial cells were

TABLE 3 Detailed mapping and semiquantitative assessment of immunohistochemical stainings of neurodegeneration-associated proteins, HLA-DR, CD68 and p62 and staging of neurodegenerative pathologies

No.	Tau								BetaA4-amyloid					Phosho-TDP-43									
	Tu	PeTu	Fr	BG	HC + EC	Amy	Pons	Bx Tu	Bx PeTu	Tu	PeTu	Fr	BG	HC + EC	Bx Tu	Bx PeTu	Tu	PeTu	Fr	HC + EC	Amy	MO	
	1	0	i	i	i	i		0			0	i	i	0	1			0	0	0	0		
2	0	i	1	i	2	2	1			0	1	2	2	1			0	0	0	0	0	0	0
3	0	0	1	i	3	3	1			0	i	i	0	i			0	0	0	0	0	0	0
4	0	0	0	0	1		0			0	0	0	0	0			0	0	0	0			0
5	0	3	3	3	3		2			0	3	3	3	3			0	0	0	0			
6	0	i	i	0	i		i	0	1	0	1	1	0	i	0	1	0	0	0	0			0
7	0	i	1	0	i	0	0	0	0	0	0	0	0		0	0	0	0	0		0	0	
8	0	0	0	i	1	i	i	0		0	0	0	0	0	0		0	0	0	0	0	0	0
9	0	0	i	0	i		i	0		0	0	0	0	0	0		0	0	0	0			0
10	0	0	0	i	3	1	i			0	0	0	0	0			0	0	0	0	i		0
11	i	2	2	2	3	2	2			0	0	0	0	0			0	0	0	0	0		0
12	0	0	0	0	i		0			0	0	0	0	0			0	0	0	0			0
13	0	0	i	1	1					1	1	2	2	1			0	0	0	0			
14	0	0	0	0	0		0	0		0	0	0	0	0	0		0	0	0	0			0
15	0	0	0	0	1		1			0	0	0	0	0			0	0	0	0			0
16	0	i	2	i	3	3	2	0	i	i	1	2	1	i	0	1	0	0	0	0	0	0	0
17	0	0	0	0	1	i	1	0		0	0	0	0	0			0	0	0	0	0	0	0
18	0	0	0	1	1		1			0	0	1	0	0			0	0	0	0			0
19	0	1	1	1	3	3	1	0		0	2	2	1	1	0		0	0	0		0		0
20	0	0					0			0	0						0	0					0
21	0	1					0			0	0						0	0					
22	0	i	i	1	2		i	0	1	0	2	2	1	1	1	2	0	0	0	0			0

Abbreviations: ADNC, Alzheimer disease neuropathological change; Amy, amygdala; ARTAG, aging-related tau astrogliaopathy; BG, basal ganglia; Bx, biopsy/resection specimen; CAA, cerebral amyloid angiopathy; Fr, frontal; HC + EC, hippocampus and entorhinal cortex; MO, medulla oblongata; No, case number; PART, primary age-related tauopathy; PeTu, peritumoral brain tissue; PSP, progressive supranuclear palsy; Tu, tumor tissue.

The different shades of colors represent different severity of the pathology with lighter colors indicating less severe pathology and darker colors indicating more severe pathology.

found in two cases (9.1%) and no microglial reaction in one case (4.5%). In the surgical specimen intensity and distribution of the microglial reaction was similar to that observed in the post-mortem brain tissue. Additional staining for CD68 showed similar intensities and distribution patterns but was in general less pronounced than HLA-DR. For a detailed staging see [Table 3](#).

In the other non-GBM space occupying lesions enhanced HLA-DR expression was also observed within the lesion and in the immediate surroundings, particularly in lymphoma; overall, these changes were, however, not more prominent than in glioblastoma.

3.4 | Autophagic marker p62

p62 was enhanced within the glioblastoma in 21 cases (95.5%). One case (4.5%) exhibited no p62-immunoreactivity. Different patterns, as depicted in

[Figure 3](#) could be identified. On the one hand, a faint cytoplasmic and nuclear immunoreactivity was seen in tumor cells ([Figure 3D](#)), especially surrounding vascular proliferations and perinecrotic areas ([Figure 3E](#)). Some tumor cells exhibited dense intranuclear p62-positive inclusions ([Figure 3F](#)). On the other hand, there were cells with a granular cytoplasmic / lysosomal pattern of p62 positivity in cells resembling macrophages ([Figure 3G](#)). In the adjacent cortex in some cases there was a nuclear positivity, which was highly resembling the p62-immunoreaction in metabolic gliosis [24] ([Figure 3I](#)), and some cases showed fibrillar cytoplasmic inclusions, representing NFT ([Figure 3H](#)). Generally, the density of p62 inside the tumor was high in three cases (13.6%), moderate in six (27.3%), mild in seven (31.8%) and isolated in five cases (22.7%).

In the immediate tumor surroundings, 15 cases (68.2%) showed p62-positivity, seven (31.8%) showed no p62 accumulation adjacent to the tumor. Four cases

TABLE 3 (Continued)

		Alpha-synuclein				p62				HLA-DR				CD68				Overall neurodegenerative pathology
Bx Tu	Bx PeTu	Tu	PeTu	Amy	MO	Bx Tu	Bx PeTu	Tu	PeTu	Bx Tu	Bx PeTu	Tu	PeTu	Bx Tu	Bx PeTu			
		0	0					2	1			3	1			i	i	
		0	0	0	0			i	0			3	1			1	i	ADNC A2B1C1, ARTAG
		0	0	0	0			2	0			2	1			1	i	ADNC A1B2C1
		0	0		0			i	i			2	i			1	i	PART BII, ARTAG
		0	0					2	1			2	1			1	1	ADNC A3B3C3, CAA Thal 2
0	0	0	0		0	0	0	1	i	2	i	2	1	2	1	1	i	ADNC A1B1C0
0	0	0	0	0	0	0	0	2	i	2	1	2	1	2	1	i	i	PART BI
0	0	0	0	0	0			1	0	1		3	1	1		2	i	PART BI
0	0	0	0	0	0			2	i	2		3	1	2		2	i	PART BI
		0	0	0	0			0	0			i	1			i	i	PART BIII, ARTAG
		0	0	0	0			1	i			3	1			1	i	PSP
		0	0		0			3	i			3	1			1	i	PART BI
		0	0					1	i			1	1			i	i	ADNC A2B1C2, CAA Thal 2
0	0	0	0		0	0		i	i	1		0	0	1		i	i	None
		0	0		0			i	0			2	2			2	i	PART BII
		0	0	0	0	0	0	i	0	1	i	i	1	1	1	i	i	ADNC A2B3C2, CAA Thal 2
		0	0	0	0			1	i	i		3	1	2		2	i	PART BI, ARTAG
		0	0		0			1	i			3	1			2	i	ADNC A1B1C1, CAA Thal 2, ARTAG
0	0	0	0	0	0			3	1	1		3	i	3		2	i	ADNC A3B3C3, CAA Thal 2
		0	0					3	1			2	1			1	i	Not assessable
		0	0					2	i			3	1			2	i	Not assessable
0	0	0	0		0	0	0	1	0	2	1	2	1	2	1	2	i	ADNC A2B2C1

(18.2%) showed a mild degree of p62-positivity and eleven (50.0%) showed only isolated p62-positive cells. In the biopsy/resection specimens p62-immunoreactivity was similar to that observed in the post-mortem brain tissue and showed similar patterns. For detailed staging see Table 3.

In the other non-GBM space occupying lesions moderate p62 immunoreactivity was observed in two metastases (adenocarcinoma of the lung and esophagus), while only isolated positive cells were identified in a breast carcinoma metastasis. Immunoreaction in the four lymphomas as well as in the three abscesses was mostly mild and was also less pronounced than in glioblastoma. For detailed staging see Table S2.

3.5 | Correlation with cognitive dysfunction and age groups

Only one patient had a clinically overt dementia and one patient presented cognitive impairment due to Trisomy

21. However, we found ADNC in roughly half of the patients (n = 10; 45.5%). When we took patients' age into consideration, we found that AD-type pathology was present in all age-groups except in the group below 50 years (n = 4). Among three patients aged 50–60 years, two had mild ADNC (one Braak I, Thal phase 2, and one Braak II, Thal phase 3 with CAA). In the group aged 61–70 years (n = 8), four patients exhibited ADNC and in the age group >70 (n = 7) four patients exhibited ADNC. Six cases (27.3%) had a Braak&Braak NFT stage of at least III [15,16]. The two patients with cognitive dysfunction (presenile dementia) had advanced Alzheimer's pathology with Braak&Braak NFT stages of V and VI, respectively. One of them (female, 62 years) had a history of rapidly progressive dementia over the course of two years prior to diagnosis of the brain tumor. In this patient no genetic testing was conducted. The second case (male, 62 years) had Trisomy 21, which is associated with a high risk of presenile Alzheimer's disease due to the overexpression of amyloid precursor protein (APP) on chromosome 21 [25].

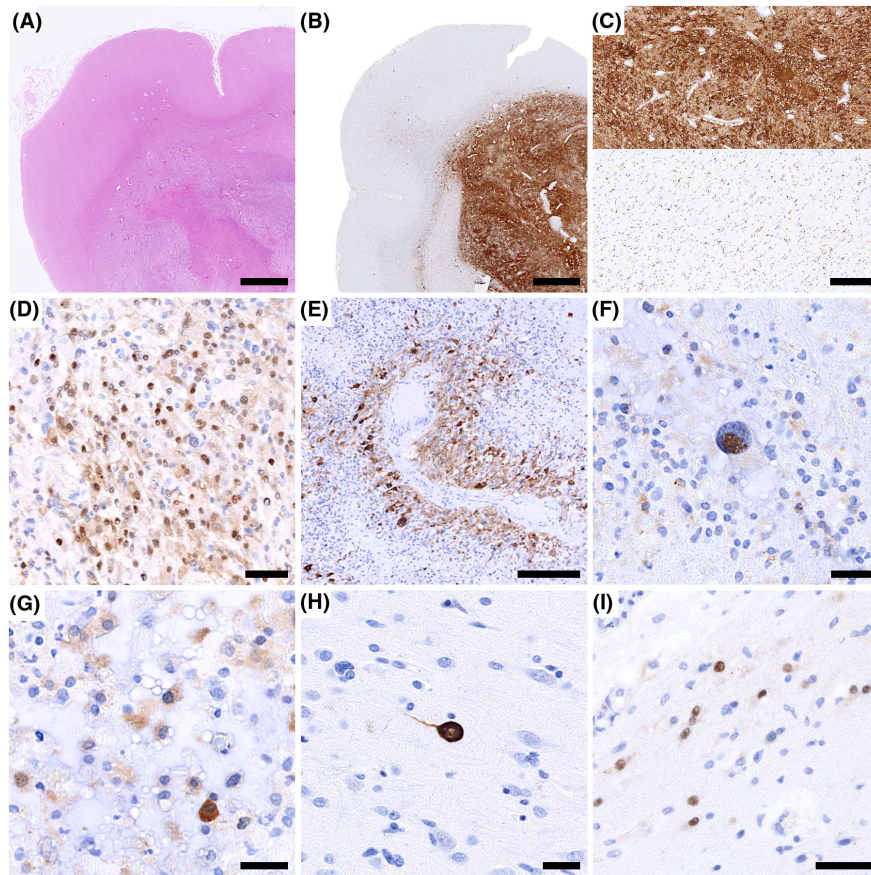


FIGURE 3 Microglial reaction and p62-immunoreactivity. (A) Glioblastoma located mainly in the white matter, showing characteristic vascular proliferations and necrosis (H&E); (B) HLA-DR staining with a severe reaction in the tumor tissue (HLA-DR); (C) Upper inset: Severe microglial reaction inside the tumor tissue, lower inset: Mild microglial reaction in the adjacent cortex (HLA-DR); (D) Diffuse nuclear and cytoplasmic p62 immunopositivity inside tumor cells (p62); (E) Enhanced p62-positivity around vascular proliferations (p62); (F) Intranuclear inclusions in a tumor cell (p62); (G) Diffuse granular cytoplasmic (lysosomal) pattern in small intratumoral cells resembling macrophages (p62); (H) Fibrillary cytoplasmic aggregate in a neuron of the adjacent cortex, corresponding to a neurofibrillary tangle (p62); (I) Diffuse nuclear astroglial positivity reminiscent of metabolic gliosis (p62). Scale bars: A, B: 3mm; C: 250 μ m; E: 200 μ m; D, I: 50 μ m; F, G, H: 25 μ m

4 | DISCUSSION

In this study, we systematically assessed the presence and distribution of neurodegeneration-associated protein deposits in a large *post-mortem* cohort of patients with glioblastoma in the tumor itself, in the immediately surrounding brain tissue and at distant sites that are prone to neurodegenerative changes (Figure 1).

We observed that the accumulation of neurodegeneration-associated proteins was generally rare in the tumor itself. In the immediate tumor surroundings, however, we found different levels of tau deposition in roughly half of the patients. These deposits were morphologically and spatially associated with a range of known neurodegenerative conditions such as ADNC, PART, ARTAG and PSP. Moreover, a relatively high percentage of neurodegenerative pathology was identified in tumor-distant brain areas, in an amount and distribution that would be expected in persons at that age.

Considering an extensive set of brain regions (also distant from the glioblastoma) that are prone to

neurodegenerative changes, the most frequent pathological protein involved was hyperphosphorylated tau with at least isolated neuropil threads somewhere in the brain in almost all patients. This is of interest, since tau protein has been previously linked to glioblastoma [26,27], while similar links have not (yet) been described for other proteins linked to neurodegenerative diseases. Comparing the pathology with an aged-matched control group we found no differences in the amount and distribution of pathologies between both groups. This is in line with previous reports on age-related neurodegenerative changes [28,29]. We also did not find a significant difference between the overall neurodegenerative changes observed in the glioblastoma group and the group of patients with other space occupying lesions. Tau deposits were increasingly identified in glioblastoma patients over 50 years, with higher pathology load in the oldest patients. Previous reports have identified early deposits in subcortical nuclei already below the age of 30 years [28,30]. It is known that AD pathology can be found in patients without clinically apparent dementia



and the so-called cognitive resilience is a topic of great scientific interest [31–33]. Indeed, although cognitive impairment was noted in <10% of cases, we found ADNC in almost half of the cohort. Tau deposition was however mild in most cases [34], and only about one third had a Braak&Braak neurofibrillary stage >III. [15,16]. The most severe AD pathology was identified in a woman with presenile dementia, and in one patient with trisomy 21, a condition that might also be associated with brain tumors [35]. Another case showed histological features of progressive supranuclear palsy (PSP) [36]. In this disease, tau pathology typically affects glial cells, which are usually the cells affected in glioblastoma. However, no such pathology was found in any other glioblastoma case. Due to the typical neuropathological distribution and typical clinical symptoms of PSP, it is likely that this patient had two different concurrent pathologies rather than a PSP-pathology induced by the tumor. The association of glioblastoma and PSP seems to be rare and, to our knowledge, has not been described before. Apart from these three unusual concurrences no unusual distribution pattern or extent of pathology was found.

Glioblastoma is an astrocytic tumor and the role of astrocytes, particularly in brain's homeostasis, is of growing interest in different neurodegenerative conditions. One recently characterized astroglial neurodegenerative pathology, which is commonly found in elderly people is ARTAG (Aging-related Tau Astroglipathy) [37]. This pathology shares characteristics with chronic traumatic encephalopathy (CTE), another tauopathy with astroglial and neuronal pathology [38]. Recently, two patients with arachnoid cysts and a history of repetitive concussions were described to have extensive ARTAG and CTE-pathology. The authors suggest that chronic mechanical stress could represent another trigger of astrocytic tau pathology [39]. In our study about one third of glioblastoma patients exhibited mild ARTAG, but the frequency and distribution did not differ from both control groups.

Neither tau nor beta-amyloid pathology seemed to be restricted to or enhanced in the tumor itself or its immediate surroundings when compared to more distant regions, which does not support a causative link between glioblastoma and neurodegenerative changes as suggested for slowly growing, low grade tumors [8]. However, median disease duration in our cohort was only 2.5 months and only one patient with secondary glioblastoma survived 35 months. This single patient did, however, also not show more extensive neurodegenerative pathology.

In contrast, we found a surprisingly high accumulation of p62 within the tumor tissue and in the immediate surroundings. p62 or sequestosome-1 (SQSTM1) is a protein, which binds ubiquitinated misfolded proteins to introduce them into the autophagosome allowing their degradation. It represents a link between the autophagy pathway and the ubiquitin-proteasome pathway [40].

p62-immunohistochemistry can be used as autophagy-marker and can be found in different pathological inclusions in neurodegenerative diseases [41]. Autophagy has been a topic of increasing interest in the context of neurodegenerative diseases [42]. Astroglial p62-immunoreactivity has been recently described to be up-regulated in metabolic/hepatic gliosis/encephalopathy [24]. We observed a similar pattern in several cases that showed a nuclear astroglial upregulation of p62 in the gray matter directly adjacent to the tumor, most likely indicating some metabolic derangement of glial cells. We additionally found a nuclear and faint cytoplasmic staining of some tumor cells, especially in perivascular and perinecrotic areas, a pattern which has been previously described in glioblastoma [43,44]. Similar findings were observed in brain carcinoma metastasis but not in cerebral lymphoma or brain abscess, although the number of studied cases was too low to draw firm conclusions. Chronic treatment effects of chemotherapy and radiotherapy on the CNS need to be also considered as possible cellular stressors. Temozolomide, the mostly used chemotherapy in glioblastoma, has been found to induce autophagy in glioblastoma [45]. In our study however, p62-densities did not differ between chemotherapy-treated and un-treated patients, although this could be attributed to small sample size and biased inclusion of clinically undiagnosed cases.

Regarding microenvironmental inflammatory changes as potential influencing factor on neurodegenerative processes we also observed distinct HLA-DR immunoreactivity in most cases, as described previously [46]. Except for the long surviving secondary glioblastoma patient, all primary glioblastoma cases showed prominent microglia infiltration of the tumor and a milder microglial reaction in the adjacent brain tissue. Similar changes were observed in the non-glioblastoma space occupying lesions. These findings could suggest that a neuroinflammation-associated cell stress, independently of the cause, could potentially lead to neurodegeneration in the lesion surroundings, as particular states of microglial activation may play a detrimental role in neurodegenerative processes [47–49].

Our study has several limitations. The retrospective and descriptive nature of this postmortem study does not allow the drawing of firm conclusions on mechanistic aspects. Moreover, even if the study cohort is relatively large, the interpretation of results is hampered by the non-homogenous type (primary and secondary glioblastomas) and location of the tumors (supratentorial and infratentorial location) as well as variable treatment regimes (elderly patients receiving no treatment [50]), and different disease durations. Of particular interest would be the analysis of a homogenous patient population with long disease duration after effective treatments, to assess whether neurodegenerative pathologies are indeed more frequently observed in long-term survivors. Some epidemiological studies on the most prevalent

neurodegenerative disease, such as Alzheimer's disease, have reported an inverse relationship between cancer in general and Alzheimer's disease [51], while another study suggested a positive correlation between Alzheimer's disease and glioblastoma [52]. Parkinson's disease has been related to a higher incidence of some peripheral tumors, including melanoma but also brain tumors [53–55].

With these limitations in mind, our *post-mortem* study shows that abnormal neurodegeneration-associated protein aggregates in glioblastoma patients are more likely related to silent, age-related conditions. However, both conditions glioblastoma and neurodegeneration may reflect a defective aging. Whether these two, apparently different pathways influence each other in one or the other direction is a matter of debate and needs further analyses. With increasing personalized treatments it is, however, important to be aware that cellular aging begins early in the brain, long before being clinically evident. Particular treatments, an altered autophagy pathway and a prominent microglial response within and/or around a brain tumor may be detrimental for the brain tissue and accelerate predisposing conditions.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Drafting and conceptualization of the manuscript: Sigrid Klotz, Adelheid Wöhrer, Gabor G. Kovacs, Ellen Gelpi. Methodology: Sigrid Klotz, Gerda Ricken, Adelheid Wöhrer, Gabor G. Kovacs, Ellen Gelpi. Data collection: Sigrid Klotz, Matthias Preusser, Karin Dieckmann, Georg Widhalm, Karl Rössler, Peter Fischer, Ognian Kalev, Adelheid Wöhrer, Gabor G. Kovacs, Ellen Gelpi. Formal analysis: Sigrid Klotz, Adelheid Wöhrer, Gabor G. Kovacs, Ellen Gelpi. Writing, reviewing and editing of the manuscript: Sigrid Klotz, Gerda Ricken, Matthias Preusser, Karin Dieckmann, Georg Widhalm, Karl Rössler, Peter Fischer, Ognian Kalev, Adelheid Wöhrer, Gabor G. Kovacs, Ellen Gelpi. Visualization: Sigrid Klotz, Gabor G. Kovacs, Ellen Gelpi. Supervision: Adelheid Wöhrer, Gabor G. Kovacs, Ellen Gelpi.

ETHICS APPROVAL

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Vienna (EK 1636/2019).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study is available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

TABLE S1 Clinical and demographic characteristics of control groups

TABLE S2 Detailed mapping and semiquantitative assessment of immunohistochemical stainings of neurodegeneration-associated proteins, HLA-DR, CD68 and p62 and staging of neurodegenerative pathologies

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