Clinicopathological Significance of Autophagy-related Proteins and its Association With Genetic Alterations in Gliomas

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Abstract. Aim: To investigate clinicopathological significance of autophagy and its association with genetic alterations in gliomas. Materials and Methods: The expression of three autophagy-related proteins, light chain-3 (LC3), beclin 1, and p62 was immunohistochemically analyzed in 32 low-grade gliomas and 65 high-grade gliomas. Results: LC3, beclin 1, and p62 expression was positive in 70/94 (74%), 51/94 (54%) and 55/96 (57%) gliomas, respectively. High expression of LC3, beclin 1 and p62 was significantly more frequent in high-grade gliomas than in low-grade. Positive expression of LC3, beclin 1 and p62 were significantly positively correlated with overall survival, methylation of *O*⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter, mutations of isocitrate dehydrogenase 1 (*IDH1*) and telomerase reverse transcriptase (*TERT*) promoter, and 1p/19q co-deletion. Kaplan–Meier analyses revealed that LC3, p62 and autophagy status (positivity for at least two of the three proteins) were significantly associated with poorer survival. Conclusion: Autophagy might be associated with the progression of glioma, particularly high-grade, and thus might be a useful prognostic factor in patients with glioma.

Glioma accounts for nearly 80% of all brain tumors and arises from glial cells of the brain cells (1). Based on their aggressiveness, they can be classified as low-grade gliomas (LGG) or high-grade gliomas (HGG) (2). LGG can progress into malignant tumor of World Health Organization (WHO)

grade III or IV, with an incidence of 23-72% and conferring a median survival time of 2.7-5.4 years (3-5). HGG, mainly glioblastoma (GBM), classified as grade IV by the WHO, is one of the most lethal forms of malignant gliomas, is highly infiltrative, and progresses rapidly (2, 6, 7). Despite multimodal therapies including standard surgical resection followed by radiation and chemotherapy, HGG continues to confer a poor prognosis, with a median survival time of 9-12 months (1, 7). Therefore, uncovering the mechanism of tumorigenesis of glioma is crucial for discovering novel treatments to improve the prognosis of patients (8, 9). Furthermore, genetic mutations such as of *O*⁶-methylguanine-DNA methyltransferase (*MGMT*), isocitrate dehydrogenase 1 (*IDH1*), tumor protein 53 (*TP53*), telomerase reverse transcriptase (*TERT*) promoter, and 1p/19q chromosomal co-deletion are essential molecular gene mutations for the final pathological diagnosis of gliomas (10).

Over the years, the role of autophagy in the pathogenesis of various cancer types has been widely recognized and considered as a vital target in cancer therapy (8, 11, 12). Recent studies have shown autophagy to play dual functions in cancer: it can suppress the development of cancer in early stages by eliminating damaged proteins and organelles, or facilitate tumor growth in low oxygen and nutrient-deprived conditions (9, 13, 14). Autophagy is a 'self-eating' catabolic process occurring in all eukaryotic cells in response to starvation in which cellular organelles (cargo), proteins and cytoplasm are engulfed, digested and recycled to sustain cellular metabolism (15, 16). These recycled intracellular constituents also serve as an alternative source of energy during stress conditions (16). In most cells, autophagy usually occurs at low levels, but can be up-regulated in stress conditions such as starvation, hypoxia, infection or nutrient deprivation (11, 17). There are three types of autophagy based on how the cellular constituents are delivered to the lysosome: Microautophagy, chaperone-mediated autophagy

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(CMA) and macroautophagy (8, 18). Microautophagy is a non-selective process which involves the direct engulfment of the cytoplasm at the lysosomal surface by invagination, protrusion or septation of the lysosomal limiting membrane (16, 19). CMA is a selective process which delivers soluble proteins recognized by molecular chaperones to the lysosome (8). In contrast, in macroautophagy (hereafter referred to as autophagy), portions of cellular organelles, proteins and cytoplasm are engulfed, digested and recycled to sustain cellular metabolism *via* a double-membrane vesicle termed an autophagosome (19). Subsequently, autophagosomes bind with lysosomes to degrade and recycle their content (20).

The process of autophagy is regulated by a series of autophagy-related genes (ATGs) (21). For the formation of autophagosome and cargo recruitment, two ubiquitin-like conjugation systems, namely ATG12–ATG5–ATG16 and ATG8 conjugation system are crucial (22). Microtubule-associated protein 1 light chain 3 (LC3) belongs to the ATG8 protein family and is a structural component of autophagosomes (7, 23). Various studies have shown autophagy to be highly selective [reviewed in (18)]. This selectivity is ascribed to autophagy receptors that are able to interact with the autophagy machinery and on the other hand recognize a ligand-bound cargo (24). One of the most studied autophagy receptors is p62, which is involved in autophagic degradation (20). Beclin 1 is a tumor-suppressor gene and is an autophagosome initiation protein (7, 25): Studies have revealed LC3, beclin 1 and p62 to be essential markers for autophagy (25-28).

There are studies that have reported high expression of different autophagy-related proteins in various cancer cells (29-31). For this reason, using a single autophagy-related protein marker alone to determine the autophagy status might be unreliable. Therefore, we included the study of three autophagy-related proteins, LC3, beclin 1 and p62 to determine the autophagy status and evaluate their expression in different forms of glioma.

In the present study, the expression of the autophagy-related proteins, LC3, beclin 1, and p62, in glioma specimens were evaluated, and the correlation between clinicopathological features and autophagy in glioma was investigated. Additionally, the correlation between genetic alterations in glioma and autophagy-related proteins was also investigated. The correlation between autophagy-related proteins with clinicopathological features along with genetic alterations of gliomas, are rare in the literature. To the best of our knowledge, this is the first study to investigate genetic features and autophagy-related proteins, LC3, beclin 1, and p62, in gliomas.

Materials and Methods

Clinical materials. A total of 97 patients who had undergone surgery for different grades of diffuse glioma from 2001 to 2017 at the Department of Neurosurgery, Osaka City University, were included in the study. Exclusion criteria included samples from biopsy,

insufficient tumor sample or unavailable follow-up data. All specimens were histopathologically classified and revised genetically according to the World Health Organization (WHO 2016) of tumors of the central nervous system (1) by an expert neuropathologist. None of the patients received preoperative radiation or chemotherapy. All glioma tissues were obtained at the time of surgery. The tumors were further sub-grouped as LGG (n=32) and HGG (n=65), where the LGG group consisted of grade II and the HGG consisted of grade III and IV (Table I). Overall survival (OS) was defined as the time from diagnosis to death of the patient. This study was carried out in accordance with the principles of the Helsinki Declaration and was approved by the Osaka City University Ethics Committee (approval number: 3084 and 2047) and all collaborative institutes. Written informed consent was obtained from all patients.

Immunohistochemical techniques. Immunohistochemical determination of LC3, beclin 1 and p62 were examined as per the manufacturer's instructions. Immunohistochemical staining was performed on 4- μ m sections of formalin-fixed paraffin-embedded tissue using a Bond Polymer Refine Detection system (catalogue #DS 9800; Leica Biosystems Newcastle Ltd, Newcastle upon Tyne, UK). Immunohistochemistry was performed using the following antibodies: anti-LC3 (ab48394, 1:1200; Abcam, Cambridge, UK), anti-beclin 1 (NB500-249, 1:100; Novus, Littleton, CO, USA) and anti-p62 (SF2, 1:400; MBL, Nagoya, Japan) on Leica BOND-MAX (Leica).

Assessment of immunohistochemical staining. Immunostaining was interpreted by two independent investigators who were blinded to the clinicopathological features of the patients. The cutoff values were decided by the cumulative mean value of the total score of all samples: Immunoreactivity of more than 30% of cancer cells was regarded as positivity for beclin 1 and p62, whereas immunoreactivity of more than 25% of cancer cells was regarded as positivity for LC3. Each sample was scored individually using the cumulative mean score of five 200 $\times$ magnification fields. Autophagy status was considered positive when the sample was positive for at least two out of the three autophagy proteins.

Genetic analysis. Genetic analyses were conducted at two laboratories: Osaka National Hospital (ONH), Osaka, Japan and the National Cancer Center Research Institute (NCC), Tokyo, Japan. For all cases, tumor DNA was extracted from frozen tumor tissue using a DNeasy Blood & Tissue kit (Qiagen, Tokyo, Japan). The methylation status of the *MGMT* promoter was analyzed and assessed by quantitative polymerase chain reaction (qPCR) at ONH or by pyrosequencing after bisulfite modification of genomic DNA at NCC. Hotspot mutations of *IDH1/2* (codon 132 of *IDH1* and codon 172 of *IDH2*), *TERT* promoter (termed C228 and C250) and *TP53* gene were analyzed by Sanger's sequencing and pyrosequencing at either lab. The copy number status of 1p and 19q were analyzed using multiplex ligation-dependent probe amplification in a unified workflow at either laboratory. Detailed information on genetic analysis, including PCR and sequencing of each gene, can be found in previous publication (32):

Statistical analysis. The chi-square test was used to compare the immunohistochemical findings with the clinicopathological features. The overall survival curves were estimated using the Kaplan–Meier method and log-rank test was used to compare the cumulative survival durations among each patient groups. Additionally, the Cox proportional hazards model was used to compute univariate and

Table I. Summary of clinicopathological and molecular characteristics of 97 glioma cases.

Clinicopathological feature	Value
Gender	
Female	43
Male	54
Age, years	
Median (range)	46 (13-82)
Histopathological type (WHO grade) ^a	
II	32
III	22
IV	43
Histopathological group	
LGG	32
HGG	65
Status at 5 years	
Dead	24
Alive	73
Recurrence	
No recurrence	56
Recurrence	41
2016 WHO classification, n	
Grade II	
Diffuse astrocytoma, mutant <i>IDH</i>	19
Diffuse astrocytoma, wild-type <i>IDH</i>	5
Oligodendroglioma, mutant <i>IDH</i> and 1p/19q co-deletion	6
Oligodendroglioma, NOS	2
Grade III	
Anaplastic astrocytoma, mutant <i>IDH</i>	6
Anaplastic astrocytoma, wild-type <i>IDH</i>	10
Anaplastic oligodendroglioma, mutant <i>IDH</i> and 1p/19q co-deletion	4
Anaplastic oligodendroglioma, NOS	2
Grade IV	
Glioblastoma, wild-type <i>IDH</i>	37
Glioblastoma, mutant <i>IDH</i>	6
<i>MGMT</i> promoter, n (%)	
Methylated	61 (62.8%)
Unmethylated	36 (37.1%)
<i>IDH1</i> , n (%)	
Wild-type	56 (57.7%)
Mutant	41 (42.2%)
<i>TP53</i> , n (%)	
Mutation	34/76 (44.7%)
<i>TERT</i> promoter, n (%)	
Mutation	38/90 (42.2%)
1p/19q co-deletion	
Yes	11/52 (21.1%)

^aHistopathological type was classified genetically according to the 2016 World Health Organization of Tumors of the Central Nervous System (1). LGG: Low-grade glioma; HGG: high-grade glioma; *IDH*: isocitrate dehydrogenase; NOS: not otherwise specified; *MGMT*: *O*⁶-methylguanine-DNA methyltransferase; *TP53*: tumor protein 53; *TERT*: telomerase reverse transcriptase.

multivariate hazards ratios for the study parameters. For all of the tests, a *p*-value <0.05 was considered as being statistically significant. The SPSS software program (SPSS Japan, version 22, Tokyo, Japan) was used for the analyses.

Results

Correlation between clinicopathological features and autophagy.

The expression of autophagy markers in resected specimens of LGG and HGG was evaluated by immunohistochemistry. A summary of the clinicopathological characteristics of the glioma cases are shown in Table I. Strong expression of LC3, beclin 1, and p62 were found in both the nucleus and cytoplasm of HGG compared with LGG cells (Figure 1). Among the 97 glioma cases, LC3, beclin 1, and p62 expression were positive in 70/94 (74%), 51/94 (54%) and 55/96 (57%) cases respectively.

The relationship between expression of autophagy proteins and clinicopathological variables are shown in Table II. LC3, beclin 1 and p62 expression was significantly associated with HGG (*p*=0.004, *p*≤0.001 and *p*=0.006 respectively); LC3 and p62 expression also significantly correlated with poorer overall survival (*p*=0.005 and *p*=0.020, respectively). Autophagy status was defined as positive when two out of the three autophagy-associated proteins were expressed in each sample. Expression of LC3, beclin 1 and p62 and positive autophagy status was higher in samples from patients who received radiation post operatively (*p*=0.029, *p*=0.002, *p*=0.001 and *p*=0.006 respectively). Interestingly, positivity for LC3 (n=52, 74.2%), beclin 1 (n=42, 82.3%), p62 (n=43, 78.1%) and autophagy (n=50, 79.3%) was more prominent in HGG than in LGG. In most of the HGG cases, extensive staining of autophagy proteins was seen in both the nucleus as well as in the cytoplasm of the tumor cells. Out of the 97 cases, autophagy status was positive in 63 cases (64.9%). Autophagy-positive status was also significantly associated with HGG (*p*≤0.001), poorer overall survival (*p*=0.023) and postoperative radiation (*p*=0.006).

Correlation between autophagy markers and genetic alterations in gliomas.

The relationship between the autophagy protein expression and genetic alterations are shown in Table II. There was strong association between LC3 expression and methylation of *MGMT* promoter (*p*=0.021) and mutation of *TERT* (*p*=0.010) promoter; beclin 1 expression with *IDH1* mutation (*p*=0.003) and 1p/19q co-deletion (*p*=0.045); p62 expression with *MGMT* promoter methylation (*p*=0.034), *IDH1* mutation (*p*=0.022) and 1p/19q co-deletion (*p*=0.038). Statistically, there was a significant correlation between autophagy-positive status: and: *MGMT* methylation (*p*=0.002), *IDH1* mutation (*p*=0.004) and 1p/19q co-deletion (*p*=0.034). However, there was no correlation between autophagy status and *TP53* and *TERT* mutation (*p*=0.254 and *p*=0.170).

Correlation among autophagy markers. There was significant positive correlation among the autophagy proteins LC3, beclin 1 and p62 (*p*-value=0.005, 0.080 and 0.001 respectively) (Table III).

Figure 1. Representative immunohistochemistry staining of autophagy-related proteins light chain 3 (LC3), beclin 1 and p62, in low-grade (upper panels) and high-grade (lower panels) gliomas at low (A; $\times 200$) and high (B; $\times 400$) magnification. The expression level of autophagy-related proteins LC3, beclin 1, and p62 was found to be weak in low-grade glioma (LGG), compared to high-grade glioma (HGG).

Survival. Regional recurrences occurred in 41 out of 97 patients (42.2%).

Evaluation of the association of autophagy proteins with overall survival was estimated using Kaplan–Meier survival analysis (Figure 2). The prognosis of patients with LC3-positive, p62-positive and autophagy-positive status was

significantly poorer ($p=0.007$, $p=0.012$ and $p=0.013$ respectively) than that of patients negative for the respective marker. The survival prognosis of beclin 1-positive cases was non-significant ($p=0.074$). Univariate analyses revealed that overall survival was significantly poorer in those with LC3-positive ($p=0.027$), p62-positive ($p=0.018$), autophagy-

Table II. Correlation of autophagy-related protein expression light chain 3 (LC3), beclin 1 and p62 with clinicopathological factors.

Factor	LC3			Beclin 1			p62			Autophagy		
	Positive n=70	Negative n=24	p-Value	Positive n=51	Negative n=43	p-Value	Positive n=55	Negative n=41	p-Value	Positive n=63	Negative n=30	p-Value
Age												
<50 Years	42 (77.7%)	12 (22.2%)	0.393	26 (49.0%)	27 (50.9%)	0.250	29 (52.7%)	26 (47.2%)	0.295	37 (69.8%)	16 (30.1%)	0.623
≥50 Years	28 (70.0%)	12 (30.0%)		25 (60.9%)	16 (39.0%)		26 (63.4%)	15 (36.5%)		26 (65.0%)	14 (35.0%)	
Gender												
Female	29 (69.0%)	13 (30.9%)	0.279	22 (52.3%)	20 (47.6%)	0.743	26 (60.4%)	17 (39.5%)	0.571	26 (61.9%)	16 (38.0%)	0.274
Male	41 (78.8%)	11 (21.1%)		29 (55.7%)	23 (44.2%)		29 (54.7%)	24 (45.2%)		37 (72.5%)	14 (27.4%)	
Histological type												
LGG (grade II)	18 (56.2%)	14 (43.7%)	0.004	9 (28.1%)	23 (71.8%)	<0.001	12 (37.5%)	20 (62.5%)	0.006	13 (40.6%)	19 (59.3%)	<0.001
HGG (grade III & IV)	52 (83.8%)	10 (16.1%)		42 (67.7%)	20 (32.2%)		43 (67.1%)	21 (32.8%)		50 (81.9%)	11 (18.0%)	
Recurrence												
No	43 (76.7%)	13 (23.2%)	0.532	32 (58.1%)	23 (41.8%)	0.364	32 (58.1%)	23 (41.8%)	0.838	39 (70.9%)	16 (29.0%)	0.432
Yes	27 (71.0%)	11 (28.9%)		19 (48.7%)	20 (51.2%)		23 (56.0%)	18 (43.9%)		24 (63.1%)	14 (36.8%)	
Status at 5 years												
Dead:	23 (95.8%)	1 (4.1%)	0.005	15 (65.2%)	8 (34.7%)	0.225	18 (78.2%)	5 (21.7%)	0.020	20 (86.9%)	3 (13.0%)	0.023
Alive	47 (67.1%)	23 (32.8%)		36 (50.7%)	35 (49.2%)		37 (50.6%)	36 (49.3%)		43 (61.4%)	27 (38.5%)	
MGMT promoter												
Unmethylated	30 (88.2%)	4 (11.7%)	0.021	21 (61.7%)	13 (38.2%)	0.271	25 (71.4%)	10 (28.5%)	0.034	29 (87.8%)	4 (12.1%)	0.002
Methylated	40 (66.6%)	20 (33.3%)		30 (50.0%)	30 (50.0%)		30 (49.1%)	31 (50.8%)		34 (56.6%)	26 (43.3%)	
IDH1												
Wild-type	43 (78.1%)	12 (21.8%)	0.327	37 (67.2%)	18 (32.7%)	0.003	37 (67.2%)	18 (32.7%)	0.022	43 (79.6%)	11 (20.3%)	0.004
Mutated	27 (69.2%)	12 (30.7%)		14 (43.5%)	25 (64.1%)		18 (43.9%)	23 (56.0%)		20 (51.2%)	19 (48.7%)	
TP53												
Wild-type	29 (69.0%)	13 (30.9%)	0.207	20 (48.7%)	21 (51.2%)	0.261	21 (51.2%)	20 (48.7%)	0.088	26 (63.4%)	15 (36.5%)	0.254
Mutated	27 (81.8%)	6 (18.1%)		21 (61.7%)	13 (38.2%)		24 (70.5%)	10 (29.4%)		25 (75.7%)	8 (24.2%)	
TERT promoter												
Wild-type	42 (84.0%)	8 (16.0%)	0.010	31 (62.0%)	19 (38.0%)	0.214	28 (54.9%)	23 (45.0%)	0.596	36 (73.4%)	13 (26.5%)	0.170
Mutated	22 (59.4%)	15 (40.5%)		18 (48.6%)	19 (53.8%)		23 (60.5%)	15 (39.4%)		22 (59.4%)	15 (40.5%)	
Co-deletion of 1p/19q												
No co-deletion	32 (78%)	9 (21.9%)	0.591	26 (65.0%)	14 (35.0%)	0.045	25 (62.5%)	15 (37.5%)	0.038	30 (75.0%)	10 (25.0%)	0.034
Co-deletion	7 (70.0%)	3 (30.0%)		3 (30.0%)	7 (70.0%)		3 (27.2%)	8 (72.7%)		4 (40.0%)	6 (60.0%)	
Radiation												
Not received	21 (60.0%)	14 (40.0%)	0.029	12 (34.2%)	23 (65.7%)	0.002	13 (35.1%)	24 (64.8%)	0.001	17 (48.5%)	18 (51.4%)	0.006
Received	43 (81.1%)	10 (18.8%)		36 (67.9%)	17 (32.0%)		37 (69.8%)	16 (30.1%)		40 (76.9%)	12 (23.0%)	

Pearson's Chi-square test was applied for the statistical analysis. LGG: Low-grade glioma; HGG: high-grade glioma; IDH: isocitrate dehydrogenase; NOS: not otherwise specified; MGMT: O⁶-methylguanine-DNA methyltransferase; TP53: tumor protein 53; TERT: telomerase reverse transcriptase.

Table III. Association among autophagy-related proteins light chain 3 (LC3), beclin 1 and p62.

Factor	Beclin 1		<i>p</i> -Value	p62		<i>p</i> -Value
	Negative (n=43)	Positive (n=50)		Negative (n=38)	Positive (n=55)	
LC3						
Negative (n=24)	17 (70.8%)	7 (29.1%)	0.005	17 (70.8%)	7 (29.1%)	0.001
Positive (n=69)	26 (37.6%)	43 (62.3%)		21 (30.4%)	48 (69.5%)	
Beclin 1						
Negative (n=43)				(n=39)	(n=55)	0.080
Positive (n=51)				22 (51.1%)	21 (48.8%)	
				17 (33.3%)	34 (66.6%)	

Pearson's Chi-square test was applied for the statistical analysis.

Table IV. Univariate and multivariate analyses with respect to overall survival of patients with glioma.

Variable		Univariate analysis			Multivariate analysis		
		Hazard ratio	95% CI	<i>p</i> -Value	Hazard ratio	95% CI	<i>p</i> -Value
LC3	Positive vs. negative	9.6	1.3-71.6	0.027	9.731	0.6-138	0.093
Beclin 1	Positive vs. negative	2.1	0.9-5.0	0.081			
p62	Positive vs. negative	3.3	1.2-9.0	0.018	1.417	0.3-6.2	0.644
Autophagy	Positive vs. negative	4.1	1.2-13.9	0.022	0.258	0.02-2.3	0.229
Age	<50 vs. ≥50 Years	2.1	0.9-4.8	0.060			
Gender	Female vs. male	0.6	0.2-1.5	0.344			
Tumor recurrence	Positive vs. negative	0.7	0.3-1.7	0.552			
Histological type	High-grade vs. low-grade	18.9	2.5-141.2	0.004	1.833	0.09-34	0.687
<i>MGMT</i>	Methylated vs. unmethylated	0.3	0.1-0.6	0.004	0.612	0.2-1.6	0.327
<i>IDH1</i>	Mutated vs. wild-type	0.1	0.05-0.04	0.001	0.302	0.05-1.5	0.156
<i>TP53</i>	Mutated vs. wild-type	0.8	0.3-2.0	0.758			
<i>TERT</i> promoter	Mutated vs. wild-type	1.2	0.5-2.8	0.604			
1p/19q co-deleted	Co-deletion vs. no co-deletion	0.1	0.02-1.1	0.069			
Radiation	Received vs. not received	26.8	3.5-200.7	0.001	8.606	0.6-115	0.104

Cox proportional hazard model was applied for the statistical analysis. LC3: Light chain 3; *IDH*: isocitrate dehydrogenase; *MGMT*: *O*⁶-methylguanine-DNA methyltransferase; *TP53*: tumor protein 53; *TERT*: telomerase reverse transcriptase.

positive status ($p=0.022$), HGG ($p=0.004$) and those who received radiation therapy ($p=0.001$), whereas cases with *MGMT* methylation ($p=0.004$) and *IDH1* mutated type ($p=0.001$) had favorable prognosis. However, multivariate analysis revealed that the above parameters were not independent prognostic factors for overall survival (Table IV).

Discussion

In the current study, expression of autophagy-related proteins in various types of gliomas and their relationship with clinicopathological parameters and clinical outcomes were evaluated. Remarkably, the expression of all three autophagy-related proteins LC3, beclin 1 and p62, along with the

autophagy status was found to be closely associated with tumor histology and was relatively more frequent in HGG than in LGG. Positivity for LC3, p62 and autophagy was found to be significantly associated with poor prognosis, whereas the expression of beclin 1 was not significantly correlated in determining the overall prognosis as shown in the Kaplan–Meier survival analysis. Additionally, close association between the autophagy-related proteins LC3 and p62 was also seen, suggesting that these proteins may interact with each other and play a significant role in the regulation of autophagy activation. These findings could also be due to the active role of LC3 and p62 during autophagy as they are the structural component of autophagosomes (16, 29, 30). Beclin 1 is an autophagosome initiation protein and thus this could

Figure 2. Kaplan–Meier survival curves for the 97 glioma cases according to expression of light chain 3 (LC3), beclin 1 and p62, and autophagy status. Positivity for the expression of LC3 and p62, and autophagy was associated with significantly poorer overall survival. The expression of beclin 1, however, was not significantly correlated with patient survival. The overall survival of patients with autophagy-positive tumors was significantly poorer than that for those with autophagy-negative tumors.

be the reason for the insignificant correlation of beclin 1 with the overall prognosis in our series. High levels of LC3 and p62 have also been reported to be significantly associated with prognosis in patients with HGG, breast cancer and oral cell carcinoma (31, 32). Studies have shown beclin 1 expression to be associated with longer survival in patients with colon and esophageal cancer and its low expression has been associated with poor prognosis in hepatocellular carcinoma (32–34). Cytoplasmic protein expression of beclin 1 was found to be reduced in HGG *versus* LGG, suggesting a loss of gene function (35). One of the possible mechanisms for this is that beclin 1 expression reduces the frequency of additional mutation and limits chromosomal instability (9). Positivity for LC3, beclin 1, p62 and autophagy was also found to be closely

associated with radiation therapy but whether radiation induces autophagy or protects cells is still debatable (36).

With the introduction of 2016 WHO classification of tumors of the central nervous system, the molecular identification of gliomas, such as for *MGMT* methylation, *IDH1*, *TP53* and *TERT* promoter mutation, and 1p/19q co-deletion, is now a pivotal procedure incorporated into the diagnosis and treatment strategy of gliomas. These molecular markers also assist in predicting the sensitivity of the tumor to chemotherapy and radiation therapy (37, 38). Recently, these molecular markers were shown to be useful in predicting the prognosis and treatment response in gliomas (10, 32, 39). However, studies on autophagy and the molecular markers of glioma are limited.

In our study, we evaluated these molecular markers and investigated their association with autophagy. Individually, there was significant association of LC3 with *MGMT* methylation and *TERT* promoter mutation; beclin 1 with *IDH1* mutation and 1p/19q co-deletion; and p62 with *MGMT* methylation, *IDH1* mutation and 1p/19q co-deletion. Autophagy status was also significantly associated with *MGMT* methylation, *IDH1* mutation and 1p/19q co-deletion, suggesting that there might be a close association between autophagy and *MGMT* methylation, *IDH1* mutation and 1p/19q co-deletion. One possible hypothesis linking autophagy mechanisms with *MGMT* methylation: could be that the presence of methylated *MGMT* promoter reduces the proficiency to repair DNA damage, ultimately resulting in cancer cell death (apoptosis); autophagy has a unique role in cell death pathways and although autophagy and apoptosis are controlled by multiple signals, they are also thought to cross-regulate each other (39). This could be one possible linkage, however, the crosstalk between autophagy and apoptosis is still controversial and complex (40). Our results also showed a close association between autophagy and 1p/19q co-deletion and *IDH1* mutation. Combined loss of chromosomal arms 1p and 19q is associated with improved survival as damaged DNA is unable to be repaired thus eventually leading to cancer cell death or apoptosis. A study showed that mutant *IDH1* can have profound effect on autophagy but whether *IDH1* mutation develops spontaneously or depends on other co-existing genetic alterations is yet to be investigated (41): However, there is paucity of data regarding the association between autophagy and genetic mutations of gliomas in literature and further studies need to be carried out to fully determine its association. Autophagy activity was closely associated with *MGMT* methylation, *IDH1* mutation and 1p/19q co-deletion, making this the first report of such a finding.

The poor response of brain tumors to multi-modality treatment has urged the need to target autophagy as an alternative death pathway. Recently, studies have shown chloroquine, a widely used antimalarial agent, to actively inhibit autophagy (42-44). Thus, autophagy-interfering agents in conjunction with radiation and chemotherapy may prove to be a promising novel treatment strategy for gliomas. Further studies incorporating autophagy markers and molecular alterations are also warranted to determine their putative prognostic or diagnostic importance for gliomas.

In conclusion, autophagy may be associated with the progression of glioma, especially in HGG type. LC3 and p62 significantly correlated with poorer prognosis, suggesting that LC3 and p62 might be considered as useful prognostic factors of gliomas.

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Conflicts of Interest

No financial or other interest exists that might be construed as a conflict of interest in regard to this study.

Authors' Contributions

ST and MY designed the study; ST performed the research; TK provided sample collection; TK, TU, YT and KO provided clinical support; ST, MY, YK and MO contributed to staining interpretation; ST wrote the manuscript, and MY critically revised the manuscript and interpretation of the data. All Authors reviewed, edited and approved the final version of the manuscript.

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