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ORIGINAL ARTICLE

Molecular characteristics and clinical outcomes of elderly patients with *IDH*-wildtype glioblastomas: comparative study of older and younger cases in Kansai Network cohort

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Abstract
Aging is a known negative prognostic factor in glioblastomas (GBM). Whether particular genetic backgrounds are a factor in poor outcomes of elderly patients with GBM warrants investigation. We aim to elucidate any differences between older and younger adult patients with *IDH*-wildtype GBM regarding both molecular characteristics and clinical outcomes. We collected adult cases diagnosed with *IDH*-wildtype GBM from the Kansai Network. Clinical and pathological characteristics were analyzed retrospectively and compared between older (≥ 70 years) and younger (≤ 50 years) cases. Included were 92 older vs. 33 younger cases. The older group included more patients with preoperative Karnofsky performance status score < 70 and had a shorter survival time than the younger group. *MGMT* promoter was methylated more frequently in the older group. *TERT* promoter mutation was more common in the older group. There were significant differences in DNA copy-number alteration profiles between age groups in *PTEN* deletion and *CDK4* amplification/gain. In the older group, no molecular markers were identified, but surgical resection was an independent prognostic factor. Age-specific survival difference was significant in the *MGMT* methylated and *TERT* wildtype subgroup. Elderly patients have several potential factors in poor prognosis of glioblastomas. Varying molecular profiles may explain differing rates of survival between generations.

Keywords Elderly · *IDH*-wildtype glioblastoma · Molecular marker · Overall survival

Introduction

Glioblastoma (GBM), IDH-wildtype is one of the most common primary tumors in the central nervous system (CNS) according to 2016 World Health Organization Classification of Tumors of the CNS (2016 CNS WHO) [1]. Incidences of GBM increase with age. In Japan, the median age at diagnosis of GBM is 63.0 years from a range between 0 and 94 years of age [2]. Meanwhile, the population of elderly people is increasing, so GBM in the elderly is becoming more common [3]. In geriatric patients in particular, there are, therefore, major concerns regarding the pathological and clinical nature of GBM.

Advanced age at diagnosis of GBM is known to be significantly associated with shorter rate of survival [3], but the reasons for it being such a negative prognostic factor remain unclear. Compared with younger populations, older patients tend to have poorer outcomes and several potential clinical factors affect their poor prognosis. Physicians may be apprehensive to offer aggressive treatments because of concerns relating to tolerance due to advanced age-related fragility, to co-morbidities, or because of underlying propensity for complications. Meanwhile, resection followed by radiation and temozolomide (RT + TMZ) can lengthen the duration of survival, even in elderly patients with GBM [3]. Treatment-associated adverse events appear to be more common in the elderly, and the previous studies have suggested that there are age-specific molecular signatures and differences in the

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biology of GBMs [4, 5]. There could be genetic factors for poor prognosis in elderly patients with GBM.

Mutations in *IDH*, *TP53*, *TERT* promoter, and codeletion of chromosome arms 1p and 19q (1p/19q codeletion) have been highlighted as clinically relevant prognostic markers of diffuse gliomas [6–10]. We recently reported that a combination of *EGFR*, *CDKN2A,* and *PTEN* copy-number alteration (CNA) status had a prognostic impact in patients with GBM [11]. Some molecular markers have been reported to indicate the potential benefits of specific therapeutic intervention. Particularly in elderly patients, *MGMT* promoter methylation status has been reported to be important information for deciding adjuvant treatment regimen [12, 13]. These molecular biomarkers in association with age-specific distribution have not been investigated in detail. Age-specific prevalence and the impact of previously established biomarkers are considered to be the main areas of investigation for GBMs.

Age is a powerful predictor of survival in adult patients with GBM, yet the molecular basis for the difference in clinical outcome is mostly unknown [4]. We recently collected clinical and pathological information about adult patients with *IDH*-wildtype GBM from Kansai Molecular Diagnosis Network for CNS Tumors (Kansai Network), as previously reported [11]. In the current study, we investigate molecular characteristics as well as the clinical outcomes and the differences between older (≥ 70 years) and younger (≤ 50 years) patients in a Japanese cohort.

Methods

Ethics

This study was carried out in accordance with the principles of the Declaration of Helsinki. Approval was obtained from the Wakayama Medical University Institutional Review Board (No. 98), Osaka National Hospital Institutional Review Board (No. 713), and from all collaborative institutes. Written informed consent was obtained from all patients.

Determining "older" and "younger" groups

For comparative study, we defined "older" as ≥ 70 years and "younger" as ≤ 50 years. For two-sample tests, samples from 'middle' ages (51–69 years), which are between the older and younger groups, were not included. As described in the previous reports, if there is an age-specific biology, then cases within the "middle" ages may represent a mix of the "older" and "younger" biology [4]. To define the age boundaries for the "older" and "younger" groups, we examined the survival curve for different age groups (Fig. 1) and number of samples in each age group (Table 1). From this, we assigned patients ≥ 70 years old to the "older" group and patients ≤ 50 years old to the "younger" group. The "older" and "younger" patients constitute 43.4% and 15.6% of all cases in Kansai Network cohort (Table 1).

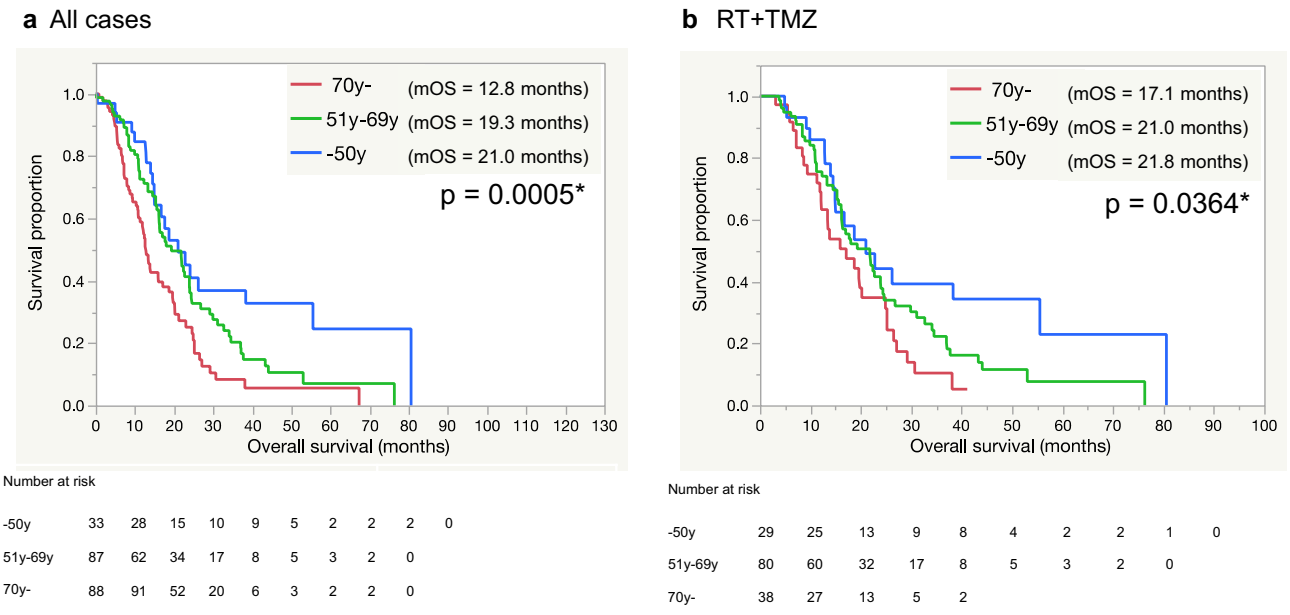

Fig. 1 Kaplan–Meier survival curves of patients with *IDH*-wildtype GBM in Kansai Network cohort. **a** All cases. **b** Patients treated with RT + TMZ. Median overall survival (mOS) of the older group (≥ 70 years) was shorter than in the middle-aged (51–69 years) and younger (≤ 50 years) groups

Table 1 Clinical characteristics of patients with *IDH*-wildtype GBM in Kansai Network

		Kansai Network ($n=212$)			
		≥ 70 year	69–51 year	50 year ≥	*p* value
Number		92 (43.4%)	87 (41.0%)	33 (15.6%)	
Clinical characteristics					
Age (years)	Median (range)	76 (70–93)	62 (51–69)	42 (18–50)	
Gender					0.4078
Male		53 (57.6%)	42 (48.3%)	19 (57.6%)	
Female		39 (42.4%)	45 (51.7%)	14 (42.4%)	
Preoperative KPS score					< 0.0001*
	80–100	27/91 (29.7%)	46 (53.5%)	26 (78.8%)	
	0–70	64/91 (70.3%)	40 (46.5%)	7 (21.2%)	
	N/A	1	1	-	
Extent of resection					0.2066
	≥ 90%	40 (43.5%)	49 (56.3%)	15 (45.5%)	
	< 90%	52 (56.5%)	38 (43.7%)	18 (54.5%)	
Concomitant RT + TMZ					< 0.0001*
	+	39/89 (43.8%)	80/85 (94.1%)	29 (87.9%)	
	–	50/89 (56.2%)	5/85 (5.9%)	4 (12.1%)	
	N/A	3	2	-	

*Chi-square test

N/A not available

Patient population of Kansai Network cohort

We recently collected patients with *IDH*-wildtype GBM [11]. The cases were all treated at affiliated institutions or hospitals participating in the Kansai Network between December 2006 and November 2017. Established in the Kansai area of western Japan, Kansai Network collects tumor samples and clinical information from affiliated institutions or hospitals and analyzes the molecular status of tumors for diagnosis and research [3]. From this data bank, we focused on *IDH* wild-type GBM cases. Diagnosis of GBM was initially confirmed by histopathological examination at each institution or hospital. After central review of histopathological diagnoses and molecular analyses including absence of *IDH1/2* and *H3F3A* mutation and 1p/19q codeletion, 212 cases were enrolled from seven institutions as the Kansai Network cohort.

Histopathological examination of Kansai Network cohort

All cases underwent central pathology review by a senior board-certified neuropathologist (Y.K.). Integrated diagnoses of GBM, IDH-wildtype, and WHO grade IV were made based on the 2016 CNS WHO classification [1].

Clinical information of Kansai Network cohort

Clinical information was collected from medical records including patient demographics, preoperative Karnofsky performance status (KPS) scores, extent of surgical resection (EOR), adjuvant radiation and chemotherapy (RCT) regimens, and survival time. EOR was classified as ≥ 90% and < 90% according to the assessment by the surgeon. Patients underwent RCT consisting of RT plus concomitant and adjuvant TMZ, RT alone, or TMZ monotherapy, or they received no treatment. Adjuvant RCT regimen was determined by attending physicians with consideration of the patient's condition.

Genetic analysis of Kansai Network cohort

Tumor genomic DNA was extracted with NucleoSpin Tissue kit (Macherey–Nagel, Inc., Bethlehem, PA) or DNeasy Blood and Tissue Kit (Qiagen, Tokyo, Japan), according to the manufacturer's protocol. Details of genetic analysis, including PCR and sequencing for each gene status, were

previously reported [14]. Mutational statuses of *IDH1/2*, *TERT* promoter, and *TP53* were determined using the Sanger technique. Details of Sanger sequencing have been previously reported [11]. Methylation status of the *MGMT* promoter was analyzed by quantitative methylation-specific PCR (qMSP) after bisulfite modification of genomic DNA [11, 15]. Based on an outcome-based study to determine an optimal cut-off to judge *MGMT* promoter methylation in a series of newly diagnosed GBM, we used a cut-off of ≥ 1% for *MGMT* methylation.

To assess CNAs, we performed Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA KIT P105 (version D2), in accordance with the manufacturer's protocol (MRC Holland, Amsterdam, The Netherlands) [16]. The P105 kit is designed to detect CNAs typical in gliomas, and includes probes against *PDGFRA*, *EGFR*, *CDKN2A*, *PTEN*, *TP53*, *CDK4*, and *MDM2* genes. Based on a previous report, the CNA category was classified by the following thresholds: homozygous deletion ($x \leq 0.4$), hemizygous deletion ($0.4 < x \leq 0.7$), gain ($0.7 \leq x < 1.3$), and amplification ($x \geq 1.3$) [17]. For convenience, homozygous and/or hemizygous deletion were collectively referred to as 'del', while amplification and/or gain was referred to as 'amp/gain'.

Statistical analysis

Statistical analysis was performed using an SAS package and JMP Pro version 14 (SAS Institute, Cary, NC, USA). Categorized data were compared between subgroups using Chi-square test. Overall survival curves were estimated by Kaplan–Meier method and compared with log-rank test. Univariate and multivariate analyses of risk factors were performed using Cox proportional hazards model. p value < 0.05 was considered statistically significant.

Results

Differences in clinical outcomes between older and younger patients

Table 1 shows clinical characteristics of older (≥ 70 years), middle-aged (51–69 years), and younger (≤ 50 years) patients from Kansai Network cohort. There were 92 (43.4%) older cases in the cohort ($n = 212$). In the older group, 64 of 91 cases (69.6%) had preoperative KPS score of < 70. Younger patients tended to have high KPS scores (80–100) (78.8%). Regarding EOR, the difference was not significant among groups ($p = 0.2066$). After resection, adjuvant RT + TMZ was less likely to be performed in the older group (56.2%). Figure 1a shows age-specific overall survival curves of all patients in the cohort. Median overall survival (mOS) of the older group was 12.8 months and significantly shorter than in the middle-aged or younger groups (19.3 or 21.0 months). Estimated survival curve of middle-aged group was close to that of younger group.

For survival analysis considering homogenous treatment background, cases in which patients underwent temozolomide-based chemoradiation were selected from the cohort. As a result, included in the analysis were 148 cases in the cohort. Figure 1b shows age-specific overall survival curves of patients treated with adjuvant RT + TMZ. There were

Table 2 Clinical characteristics of patients with *IDH*-wildtype GBM treated with RT + TMZ in Kansai Network cohort

		Kansai Network ($n = 148$)			
		≥ 70 year	69–51 year	50 year ≥	p value
Number		39 (26.4%)	80 (54.0%)	29 (19.6%)	
Clinical characteristics					
Age (years)	Median (range)	71 (82–70)	62.5 (69–51)	43 (50–18)	
Gender					0.2574
	Male	25 (64.1%)	39 (48.8%)	17 (58.6%)	
	Female	14 (35.9%)	41 (51.2%)	12 (41.4%)	
Preoperative KPS score					0.0030*
	80–100	15 (38.5%)	43 (53.8%)	24 (82.8%)	
	0–70	24 (61.5%)	37 (46.2%)	5 (17.2%)	
	N/A				
Extent of resection					0.0278*
	≥ 90%	14 (35.9%)	48 (60.0%)	12 (41.4%)	
	< 90%	25 (64.1%)	32 (40.0%)	17 (58.6%)	

*Chi-square test

N/A not available

significant differences between groups. Median OS time of the older group was 17.1 months and longer than those in Fig. 1a. Table 2 shows clinical characteristics of patients treated with RT + TMZ in the cohort. There were 39 (26.4%) elderly cases. In the elderly group, 24 cases (61.5%) had preoperative KPS score < 70. Regarding EOR, elderly patients were likely to receive < 90% resection (64.1%). Univariate and multivariate analyses identified EOR as an independent prognostic factor for elderly cases in the cohort (Online Resource 1). According to KPS or EOR, survival difference between the older and younger cases was not significant (Online Resource 2).

Differences in molecular characteristics between older and younger patients

Next, we compared molecular characteristics between the older and younger groups, in which there was a significant difference in overall survival (Fig. 1). Table 3 shows molecular characteristics and the frequency of each genetic status. *TERT* promoter mutations were observed in 51 patients of the older group (55.4%) and 13 patients of the younger group (39.3%), but the difference did not reach statistical significance ($p = 0.1680$). *MGMT* promoter methylation in the cohort was present in 50 tumors in the older group (54.3%) and 14 tumors in the younger group (40.0%), but there was no statistical difference between them ($p = 0.2389$). In the cohort, *TP53* mutation was detected in the older group less frequently than in the younger group.

The molecular disproportions of CNA were dissected in light of age-specific differences (Table 3). Major CNA differences between the older and younger groups included *PTEN* del and *CDK4* amp/gain, and there was a significant difference in frequency. These CNAs were more frequent in the older cases than in the younger cases. Notably, although the age-specific difference does not reach a statistical significance, the triple overlapping CNAs of *EGFR*, *CDKN2A*, and *PTEN* (termed triple CNA) were more frequent in the older group than in the younger group [11]. Co-amplification of *MDM2* and *CDK4* was also observed more frequently in the older group.

Differences of clinical outcomes between older and younger patients according to molecular biomarkers

According to each molecular status, survival differences between the older and younger cases were examined in patients treated with adjuvant RT + TMZ in the cohort. Figures 2 and 3 show mOS and Kaplan–Meier survival curves of the older and younger groups that were treated with RT + TMZ. Regarding *MGMT* promoter methylation status, in the older cases, no significant difference was found in OS time between methylated and unmethylated subgroups (18.7 months vs. 17.1 months) ($p = 0.3885$) (Fig. 2a, b). Survival difference between the older and younger cases was significant in the methylated subgroup ($p = 0.0136$) (Fig. 2a), while the older cases showed comparable OS with the younger cases in the unmethylated subgroup ($p = 0.1742$) (Fig. 2b).

Regarding *TERT* mutation status, mutated subgroup had a shorter OS time (13.8 months) than wildtype subgroup (19.6 months) in the older group, but the difference did not reach statistical significance ($p = 0.2934$) (Fig. 2c, d). Age-specific survival difference was significant in wildtype subgroup ($p = 0.0270$) (Fig. 2c), but not in mutated subgroup ($p = 0.6609$) (Fig. 2d).

The older cases with triple CNA (13.6 months) showed a poorer prognosis, but survival difference from non-triple CNA (19.6 months) did not reach statistical significance ($p = 0.1734$) (Fig. 3). In cases with triple CNA, age-specific survival difference was not significant ($p = 0.8861$) (Fig. 3a). On the other hand, there was significant difference between the older and younger groups with non-triple CNA ($p = 0.0454$) (Fig. 3b). Regarding *PTEN* or *CDK4* CNA status, survival difference between the older and younger cases was not significant (Online Resource 2).

As shown in Online Resource 3, there was no significant survival difference in the younger group according to molecular markers including *MGMT*, *TERT,* and triple CNA.

Discussion

To elucidate molecular characteristics and clinical outcomes of GBM in elderly patients, we examined the difference between older (≥ 70 years) and younger (≤ 50 years) patients in Kansai Network cohort. The older group had several clinical characteristics. Preoperative KPS scores below 70 were more common and adjuvant RT + TMZ were performed less commonly than in the younger groups. Although resection followed by RT + TMZ lengthened duration of survival even in the older group, mOS of the older group was significantly shorter than that of the younger group. Age-specific differences in molecular characteristics were also suggested. *TERT* promoter mutation in the older group was more frequent than that in the younger group. *MGMT* promoter methylation was more common in the older group than in the younger group. Significantly different CNA profiles between the older and younger groups were *PTEN* del and *CDK4* amp/gain.

Kaplan–Meier survival curves clearly showed age-dependent difference of OS; elderly patients had the shortest survival and, even in the non-elderly cases (< 70 years), those of higher age had poorer prognosis. The result supports the findings of the previous report that age is one of

Table 3 Molecular characteristics of patients with *IDH*-wildtype GBM in Kansai Network cohort

		Kansai Network ($n=212$)			
		≥ 70 year	69–51 year	50 year ≥	*p* value
Number		92 (43.4%)	87 (41.0%)	33 (15.6%)	
Genetic status					
TERT promoter					0.1680
	Wild	41 (44.6%)	26 (29.9%)	20 (60.6%)	
	Mut	51 (55.4%)	61 (70.1%)	13 (39.3%)	
	N/A				
MGMT promoter					0.2398
	Met	50 (54.3%)	34 (39.1%)	14 (42.4%)	
	Unmet	42 (45.7%)	53 (60.9%)	19 (57.6%)	
	N/A				
TP53					0.1443
	Wild	58 (63.0%)	30 (34.5%)	16 (48.5%)	
	Mut	34 (37.0%)	57 (65.5%)	17 (51.5%)	
	N/A				
Copy Number Alteration (CNA)					
EGFR					0.9482
	Amp	17 (18.5%)	30 (34.5%)	7 (21.2%)	
	Gain	31 (33.7%)	24 (27.6%)	10 (30.3%)	
	Non	44 (47.8%)	33 (37.9%)	16 (48.5%)	
PDGFRA					0.0678
	Amp/Gain	22 (23.9%)	18 (20.7%)	3 (9.1%)	
	Non	70 (76.1%)	69 (79.3%)	30 (90.9%)	
PTEN					0.0404*
	Del	43 (46.7%)	43 (49.4%)	8 (24.2%)	
	Non	49 (53.3%)	44 (50.8%)	25 (75.8%)	
CDKN2A					0.1780
	Homo	34 (37.0%)	32 (36.8%)	13 (39.4%)	
	Hemi	23 (25.0%)	27 (31.0%)	3 (9.1%)	
	Non	35 (38.0%)	28 (32.2%)	17 (51.5%)	
MDM2					0.1990
	Amp/gain	12 (13.0%)	8 (9.2%)	1 (3.0%)	
	Non	80 (87.0%)	79 (90.8%)	32 (97.0%)	
TP53					0.9974
	Wild	53 (57.6%)	54 (62.1%)	19 (57.6%)	
	Mut/Del	39 (42.4%)	33 (37.9%)	14 (42.4%)	
CDK4					0.0301*
	Amp/gain	17 (18.5%)	12 (13.8%)	1 (3.0%)	
	Non	75 (81.5%)	75 (86.2%)	32 (97.0%)	
Triple CNA (*EGFR*/*PTEN*/*CDKN2A*)					0.1950
	Triple	23 (25.0%)	24 (27.6%)	4 (12.1%)	
	Non-triple	69 (75.0%)	63 (72.4%)	29 (87.9%)	
CDK4/*MDM2* coamp					0.251
	Coamp	11 (12.0%)	4 (4.6%)	1 (3.0%)	
	Non-coamp	81 (88.0%)	83 (95.4%)	32 (97.0%)	

*Chi-square test

Amp Amplification, *Del* homozygous and/or hemizygous deletion, *Hemi* hemizygous deletion, *Homo* homozygous deletion, *Met* methylated, *Mut* mutated, *Mut/Del* Mut or Del, *N/A* not available

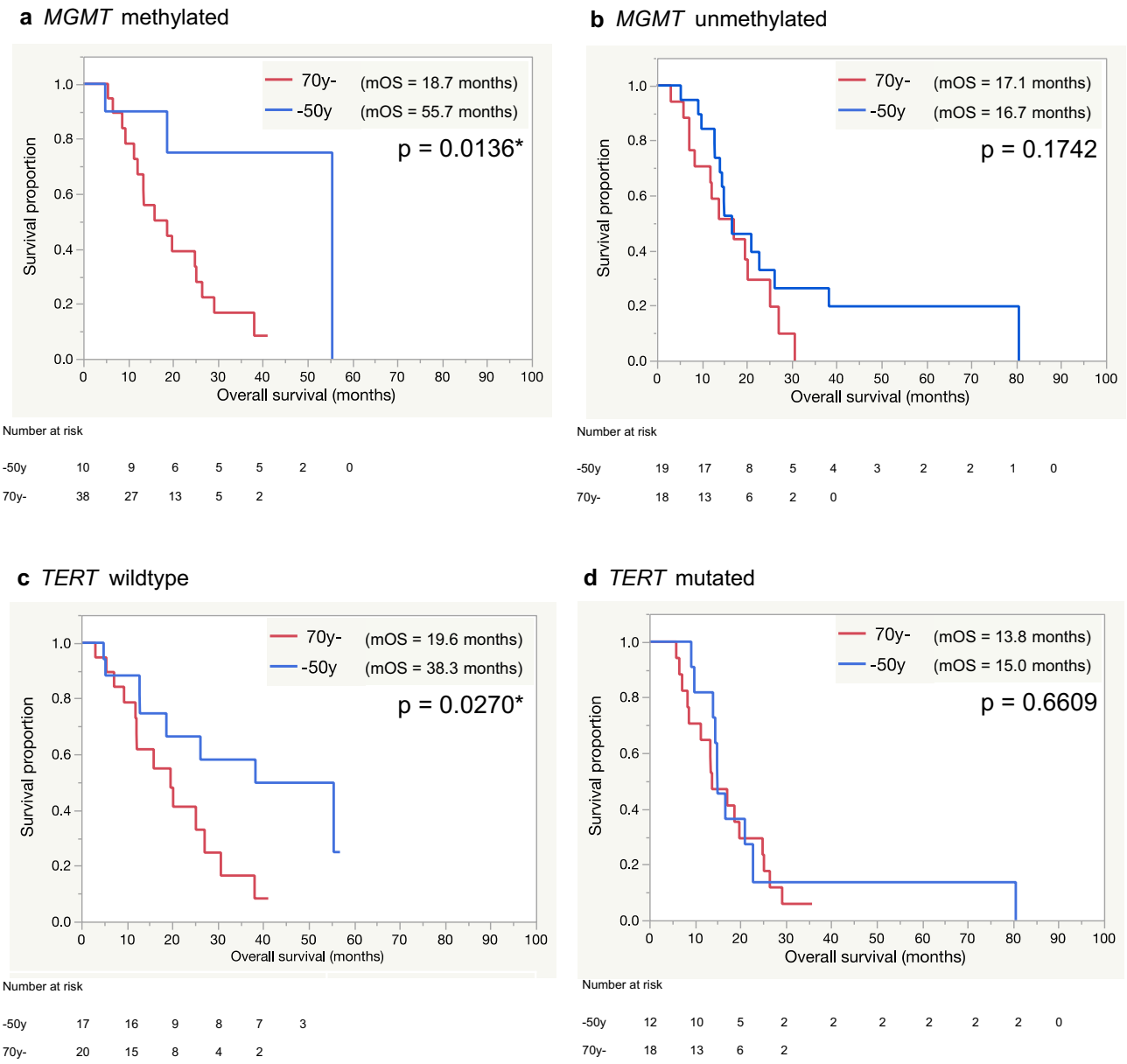

Fig. 2 Kaplan–Meier survival curves of patients treated with RT + TMZ according to *MGMT* promoter methylation status (**a**, **b**) and *TERT* promoter mutation status (**c**, **d**). Survival difference between the older and younger cases was significant in *MGMT* methylated subgroup, while the older cases showed similarly long overall survival to the younger cases in *MGMT* unmethylated subgroup. Age-specific survival difference was significant in *TERT* wildtype subgroup, not in *TERT-mutated* subgroup

the most important prognostic factors [3, 18]. It should be considered that preoperative KPS scores < 70 were seen more frequently in the older group. Performance status is independently associated with survival, which has also been discussed repeatedly in the literature [3, 18]. Although the optimal treatment remains controversial in the elderly, and resection followed by RT + TMZ was conducted in the minority of our study cohort, adjuvant RT + TMZ was associated with a longer rate of survival of the elderly [19]. In general, treatment outcomes were mostly consistent with those in the previous reports [3, 18].

In this study, univariate and multivariate analyses identified EOR as an independent prognostic factor in the elderly group (Online Resource 1). This result suggests that considering potential risk factors, even in the elderly patients, maximal and safe resection is warranted. Heterogeneous treatments and the small population of the cohort are possible reasons why factors such as preoperative KPS scores

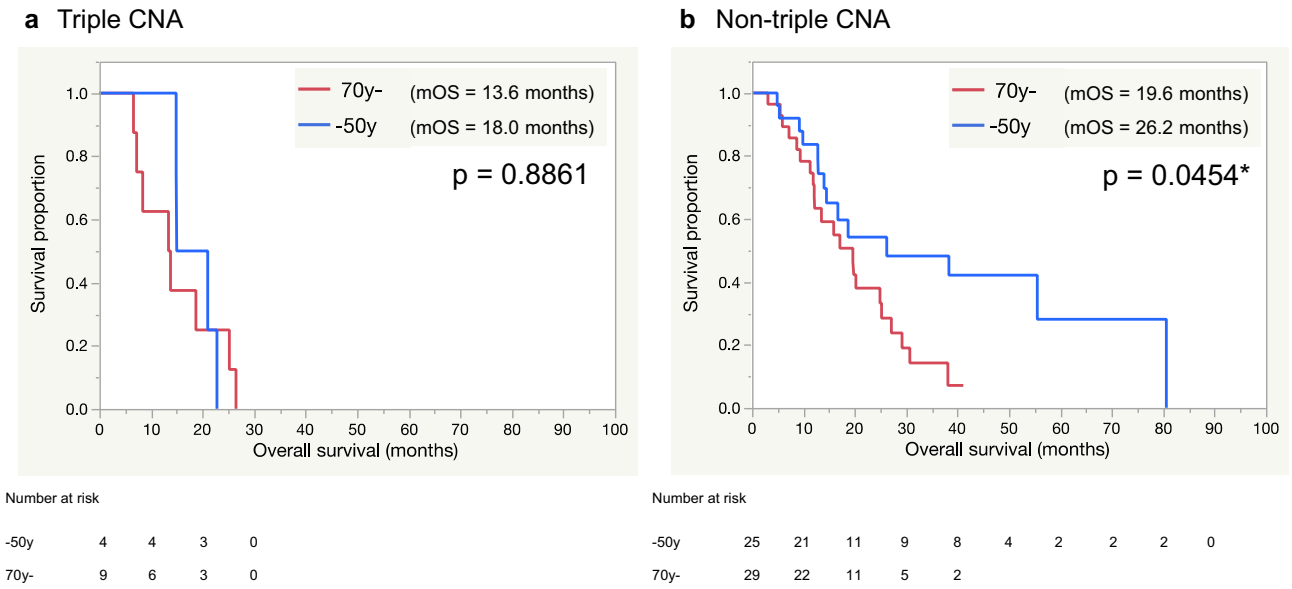

Fig. 3 Kaplan–Meier survival curves of patients treated with RT + TMZ according to CNA status. Age-specific survival difference was not significant in cases with triple CNA (**a**). There was a significant difference between the older and younger groups with non-triple CNA (**b**)

and molecular markers were not associated with survival in this cohort.

In a previous study of Japanese adult GBM, *TERT* promoter mutation rate was reported to be around 60% [9]. The frequency in the older group of our Kansai Network cohort (55.4%) was almost equivalent to that in the literature, although less frequent in the younger group (39.4%) (Table 3). Age-specific difference in frequency was suggested in our study, but should be further examined in a larger cohort.

In our previous publication, we proposed molecular classification of diffuse glioma based on *IDH* and *TERT* mutation, which was divided into four molecular groups, each showing distinct patient characteristics, histology, or clinical outcome [9]. In our study, which focused on *IDH-wildtype* GBM, *TERT*-mutated patients expected shorter survival in both older and younger groups (Fig. 2). It is notable, however, that survival difference between the groups was apparent in *TERT* wildtype patients, but not in mutated patients.

MGMT methylation status is generally regarded as a prognostic/predictive marker. In the elderly patients treated with RT + TMZ, however, there was no significant difference between methylated and unmethylated cases (Fig. 2a, b). Additionally, difference in survival between older and younger groups was notably apparent in *MGMT* methylated patients, but not in unmethylated patients.

Previously, a significant interaction between *TERT* and *MGMT* was demonstrated as prognostic markers, and it was suggested that a combination of *TERT* and *MGMT* would refine clinically relevant classification of diffuse gliomas [3, 9]. In four groups with the *TERT*/*MGMT* classification, patients with GBM with *TERT* wildtype and *MGMT* methylated had the best prognosis. In our study, age-specific survival difference was prominent in *TERT* wildtype or *MGMT* methylated cases. The difference was hypothesized to be the biggest in the *TERT* wildtype and *MGMT* methylated patients, but it did not reach statistical significance (data not shown).

We recently investigated the prognostic impact of CNAs in IDH-wild-type GBM [11]. Triple CNA, such as *EGFR*, *CDKN2A*, and *PTEN,* was identified as a negative prognostic factor. In the current study, triple CNA was observed more frequently in the elderly group than in the younger group. Difference in frequency, however, did not reach statistical significance.

In the literature, co-amplification of *CDK4* and *MDM2* in addition to gain of the whole chromosome 1, not 19, has also been discussed as a negative prognostic factor in GBM [20]. In the current study, the frequency was relatively low, and age-specific difference was not apparent in our cohort.

Regarding prognostic significance of molecular markers, *MGMT* and triple CNA were identified as independent prognostic factors for the cases overall in the cohort, as reported previously [11]. However, these molecular markers were not prognostic in the age-specific analyses of the cohort (Online Resource 1). This could be in part because of the small population of each group, especially the younger cases. Age-specific effect on survival of molecular markers is a matter of interest and remains unsolved. Further investigation is ongoing with a larger cohort of older and younger adult cases.

Several studies have focused on a potential age-specific difference in the biology of GBMs. Bozdag et al. [4] investigated age-specific signatures of GBM at the genomic, genetic, and epigenetic levels. They found age-specific hypermethylation in polycomb group protein target genes and upregulation of angiogenesis-related genes in older GBMs [4]. By analyses of methylation patterns and other integrated data, other researchers have suggested that age correlates with distinct GBM clusters [21, 22]. On the basis of gene expression classification, Lee et al. suggested that the prognostic effect of age may reflect less favorable subtypes occurring in older patients [23]. Our study revealed that different molecular profiles exist between generations, although we were unable to determine molecular markers that could explain the varying rate of survival in our study cohort. Further investigation in a larger population would contribute to better understanding of poor prognoses of GBM in the elderly.

There are several limitations concerning this study; it uses a retrospective cohort design and unlike a complete survey, selection bias could exist, and the selection could affect the molecular and survival findings. The limited number of patients could explain the absence of statistical power to detect differences between groups. Molecular characteristics might be different between Kansai Network and Western cohorts, although the reason was unclear. Other than racial difference, the different techniques utilized in the previous reports might be a consideration.

In conclusion, we report molecular characteristics and clinical outcomes of elderly patients with *IDH-wildtype* GBM in Kansai Network cohort. Elderly patients have several potential factors in poor prognosis, such as low KPS and non-aggressive treatment strategy. Different molecular profiles might explain the survival inconsistency between generations. The prognostic impact of several molecular biomarkers should be investigated in the next step and further investigation in a larger population to better understand the pathogenesis of GBM in the elderly is required. Elucidating a molecular basis that would explain age-specific differences could generate novel therapeutic strategies for elderly patients with GBM.

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