Distinct *TERT* Promoter C228T and C250T Mutation in a Patient with Oligodendroglioma: A Case Report

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Abstract

A majority of oligodendroglial tumors harbor *telomerase reverse transcriptase (TERT)* promoter and *isocitrate dehydrogenase 1/2 (IDH1/2)* mutations and 1p/19q codeletion. Generally, *TERT* promoter mutations of C250T and C228T are mutually exclusive. We present a case with oligodendroglioma harboring both C250T and C228T mutations in *TERT* promoter. A 38-year-old man presented with grand mal seizure underwent resection surgery for the left frontal lobe tumor. He was pathologically diagnosed with oligodendroglioma and was carefully observed. At aged 48 years, he underwent resection surgery due to tumor regrowth, with the pathological diagnosis of anaplastic oligodendroglioma. Genetic analysis of the initial tumor specimen revealed *IDH1* R132H mutation and both C250T and C228T mutations in *TERT* promoter. Using mutation-specific primers, two mutations were considered to distribute in different alleles. In the tumor specimen obtained during the second surgery, *IDH1* R132H mutation was detected to be similar with the initial specimen; however, only C228T mutation was detected in *TERT* promoter. A 1p/19q codeletion was detected in both the initial and recurrent specimens. According to the sequencing data of tumor during the initial and second surgeries, although *TERT* promoter mutation has been considered as an early genetic event in the tumorigenesis of oligodendroglial tumors, C250T and C228T mutations in *TERT* promoter were considered to be subclonally distributed in the same tumor specimen. (207 words)

Keywords: TERT, oligodendroglioma, oligodendroglial tumor, C228T, C250T

Introduction

Oligodendroglioma and anaplastic oligodendroglioma are currently diagnosed based on the presence of *isocitrate dehydrogenase 1/2* (*IDH1/2*) mutation and whole-arm deletion of chromosome 1p and 19q (1p/19q-codeletion) in addition to pathological findings[1, 2]. Majority of oligodendroglial tumors harbor mutation in the promoter region of *telomerase reverse transcriptase* (*TERT*) as well as *IDH1/2* mutation and 1p/19q-codeletion[3-7]. *TERT* promoter mutation is currently

excluded from the World Health Organization (WHO) classification, but has been considered as an early genetic event in the tumorigenesis of oligodendroglial tumors[8]. In addition to *IDH* mutation, 1p/19q-codeletion, and *TERT* promoter mutation, mutations in *capicua transcriptional repressor* (*CIC*) and *far upstream element binding protein 1* (*FUBP1*) have been also known as coexisting mutations for the tumorigenesis of oligodendroglial tumors[7, 9].

TERT promoter mutation is commonly observed in adult glioblastomas (GBMs) and oligodendroglial tumors[3-5]. Two distinct hotspot mutations with positions 146 and 124 bp upstream of the transcription start site (C250T and C228T, respectively) have been reported[3, 10]. Both C250T and C228T of *TERT* promoter mutation predicted to generate E-twenty-six (ETS)-binding site and result in *TERT* transcriptional upregulation[5, 11].

Among previous studies on *TERT* promoter mutation in gliomas, most patients with mutation possessed either C250T or C228T with mutually exclusive fashion[4, 5]. Few reports have indicated that patients with gliomas have both C250T and C228T or subclonal distribution of *TERT* promoter mutation in oligodendroglial tumors. Herein, we present the case of oligodendroglioma harboring *TERT* promoter mutation with both C250T and C228T and discuss its biological mechanism.

Case Presentation

A 38-year-old male patient presented with grand mal seizure. His head magnetic resonance imaging (MRI) revealed a left frontal lobe tumor originating from the superior frontal gyrus. The tumor presented high intensity on T2-weighted imaging (WI) and was not enhanced on gadolinium-enhanced T1-WI (Fig. 1A, B). Therefore, subtotal tumor resection was performed (Fig. 1C), and the pathological diagnosis was oligodendroglioma (WHO grade 2). He was discharged from the hospital without neurological deficit and carefully followed up without adjuvant treatment. However, MRI presented gradual growth of the residual tumor, and thereby, additional tumor resection was performed by awake-craniotomy at aged 48 years (Fig. 1D-E). Therefore, he was pathologically diagnosed with anaplastic oligodendroglioma (WHO grade 3) and underwent local

irradiation with 54 gray combined with concomitant maintenance chemotherapy with temozolomide.

Pathological examination

Hematoxylin and eosin (HE) staining of the initial tumor specimen diffusely presented growing tumor cells with perinuclear halo (Fig. 2A, B). On immunohistochemistry, tumor cells were negative for glial fibrillary acidic protein and positive for Olig2. The Ki-67 labeling index was approximately 2%–3%. The initial tumor specimen was diagnosed as oligodendroglioma.

HE staining of the tumor specimen during the second surgery partly presented tumor cells with perinuclear halo that was similar to the initial specimen; however, the density was higher (Fig. 2C, D). In the dense area with tumor cells, mitoses were 1 per 10 high-power fields. The Ki-67 labeling index was approximately 25%. The tumor was positive for anti-IDH1 R132H antibody. Fluorescent in-situ hybridization revealed 1p/19q-codeletion. Finally, he was pathologically diagnosed with anaplastic oligodendroglioma, with *IDH*-mutant and 1p/19q-codeleted.

Genetic analysis

For the DNA extraction from the frozen tumor sample, polymerase chain reaction (PCR) and Sanger sequencing of the *IDH1/2* and *TERT* promoter were performed as previously described[12]. Oligonucleotide primers used in this study are summarized in Table 1. Tumor specimen obtained during the first surgery presented *IDH1* R132H mutation and both C228T and C250T mutation in the *TERT* promoter region (Fig. 3A). Using the knowledge that PCR is hindered by a single nucleotide mismatch in the 3’ end of primer[13], we designated 6 wild-type- or mutation-specific forward primers in which the 3’ end was set in C250 or C228 and wild-type (C) or mutated (T) allele was set in the respective site (Fig. 3B). After performing a nested PCR using these forward primers, reverse primers, and PCR products according to screening *TERT* promoter primers, Sanger sequencing was performed using the reverse primer for the nested PCR. Sequencing data of the

initial tumor revealing the presence of alleles with C250T and C228-wild, C250-wild and C228T, or C250-wild and C228-wild were detected using mutation-specific primers (Fig. 3C). In contrast to the initial specimen, only *TERT* promoter C250T was detected in the tumor specimen obtained during regrowth (Fig. 3D). Multiplex ligation-dependent probe amplification and fluorescent in-situ hybridization revealed a 1p/19q codeletion in both the initial and recurrent specimens. The sequencing analysis of each exon of *CIC* and *FUBP1* did not reveal any pathogenic mutations in their coding regions and splice sites (Supplementary Table 1).

Discussion and Conclusions

According to the sequencing data of the initial surgical specimen that harbor both C250T and C228T mutations in *TERT* promoter, three possible situations were considered: 1) both mutations exist in a single allele, 2) each mutation exists in different alleles in the same tumor cell, or 3) each mutation exists in different cells in the same tumor specimen. Results of sequencing using mutation-specific primers indicated the presence of two distinct alleles that harbor either *TERT* promoter C250T or C228T mutation, suggesting that these mutations were biallelic or subclonal distribution. Considering that C228T was not detected in the tumor specimen during the second surgery, these two mutations in the initial tumor specimen were strongly considered to be subclonally distributed, and clones with C250T dominantly occupied the recurrent tumor during the clonal evolution. To certify the subclonality of *TERT* promoter mutation in this tumor specimen, a single-cell DNA sequencing would be required[14]. Besides, the spatial heterogeneity of *TERT* promoter mutation was considered as another potential pathogenesis in this case. For such cases, genetic analysis with multiregional sampling would provide further information about the spatial heterogeneity of mutation[7]. However, because of the limited amount of preserved specimen with the initial and recurrent tumors, it could not be performed, which is a limitation of this study.

In *IDH*-wild-type GBMs, *TERT* promoter mutation has been considered to occur during the early tumorigenesis[15, 16] and commonly observed in both primary and recurrent tumors[17]. However, a recent study has indicated the subclonality of *TERT* promoter mutation in one-third of *IDH*-wild-type GBMs[18]. To date, patients with GBM that harbor both C250T and C228T mutations in *TERT* promoter or that presented mutational status changes of *TERT* promoter in recurrence have not been reported. Kim et al. has reported a case of anaplastic oligodendroglioma without *IDH* mutation in which *TERT* promoter mutation was detected during the initial specimen but was not detected during the recurrent tumor[19].

In oligodendroglial tumors or diffuse gliomas with oligodendroglioma components, several patients presenting with the spatial and/or temporal heterogeneity of *TERT* promoter mutations with common *IDH* mutation have previously been reported[19-24] (Table 2). Aihara et al. have reported a patient with oligodendroglioma that harbors *TERT* promoter C228T and C250T mutations in different areas of the same patient, respectively[20]. Patients with oligodendroglioma or oligoastrocytoma partially harboring *TERT* promoter mutation have been reported[20-23]. Two patients with oligodendroglioma with *TERT* promoter mutation that lost mutation in recurrent tumors despite of preserved *IDH* mutation have also been reported[24]. In these two patients, one patient presented loss of *TERT* promoter mutation after chemoradiotherapy, whereas another patient has not received chemoradiotherapy. This patient also presented loss of *TERT* promoter C228T mutation after the regrowth without chemoradiotherapy, suggesting that *TERT* promoter mutation is a subclonal genetic event and would present clonal changes spontaneously without pharmacologic and radiological burden. The mechanism of such clonal replacement was unclear. Although *TERT* expression is higher in cases with *TERT* promoter C250T and C228T mutations regardless of *IDH* status, there were no statistical differences of *TERT* expression between groups[5]. However, a previous report indicated that the activation of noncanonical NF-κB signaling cooperatively promotes the tumor growth of GBMs with *TERT* C250T[11]. We speculate that some kinds of intracellular signaling, such as noncanonical NF-κB, dominantly promoted the proliferation of

TERT C250T cells and resulted in clonal replacement as shown between the initial tumor and recurrence in our case.

This case of oligodendroglioma harboring both C250T and C228T mutation in *TERT* promoter suggests the subclonal distribution in a same tumor specimen. Because there was no pathogenic mutation detected in *CIC* and *FUBP1*, whether *TERT* promoter mutation occurs before or after these mutations in the tumorigenesis of oligodendroglial tumors is still unclear. Further study would be necessary to identify the subclonality of *TERT* promoter mutation and mechanism of clonal evolution in oligodendroglial tumors.

Abbreviations

CIC, capicua transcriptional repressor; *FUBP1, far upstream element binding protein 1*; GBM, glioblastoma; HE, hematoxylin and eosin; *IDH1/2, isocitrate dehydrogenase 1/2*; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; *TERT, telomerase reverse transcriptase*; WHO, World Health Organization; WI, weighted imaging

Declarations

Ethics approval and consent to participate: Approval from institutional review board in Hokkaido University Hospital (015-0154) was obtained prior to this study and the consent was obtained from the patient.

Consent for publication: Written informed consent was obtained from the patient for the publication of this study and accompanying images.

Availability of data and material: All data of this study are included in this published article.

Competing interests: The authors declare that they have no conflict of interest.

Funding: Not applicable,

Authors' contributions: YI contributed to study designation, data collection, data analysis, and draft writing. OM, HM, and TM contributed to data collection. HO, ST, and SY contributed to data

collection and revising the draft. All authors approved the final manuscript.

Acknowledgments: Not applicable

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Figure legends

FIG. 1. Head MRI of the patient

A. T2WI of MRI before the initial surgery presenting the left frontal lobe tumor originating from the superior frontal gyrus.

B. Gd-T1WI before the initial surgery presenting no apparent enhanced lesion in the tumor.

C. T2WI after the initial surgery presenting a partially resected tumor.

D. T2WI obtained 10 years after the initial surgery presenting regrowth of the residual tumor.

E. Gd-T1WI before the second surgery presenting no apparent enhanced lesion.

FIG. 2. Pathological examination

A and B. HE staining of the initial tumor specimen (A: scale bar = 100μm, B: scale bar = 20μm) presenting diffusely proliferating tumor cells with perinuclear halo.

C and D. HE staining of the second tumor specimen (C: scale bar = 100μm, D: scale bar = 20μm) presenting tumor cells with perinuclear halo similar but denser than the initial specimen.

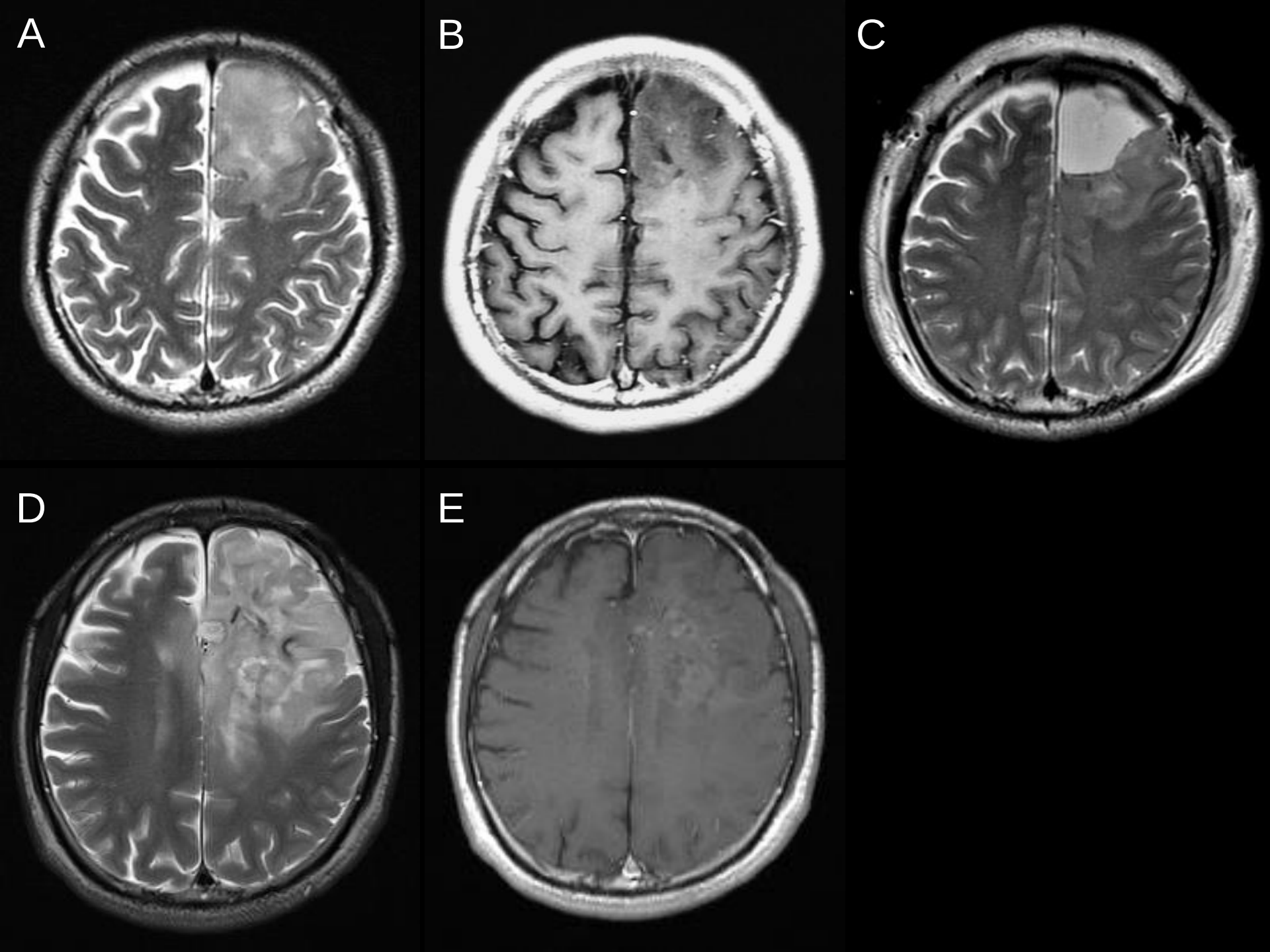
FIG. 3. Genetic analysis of the tumor specimen on the initial and second surgeries.

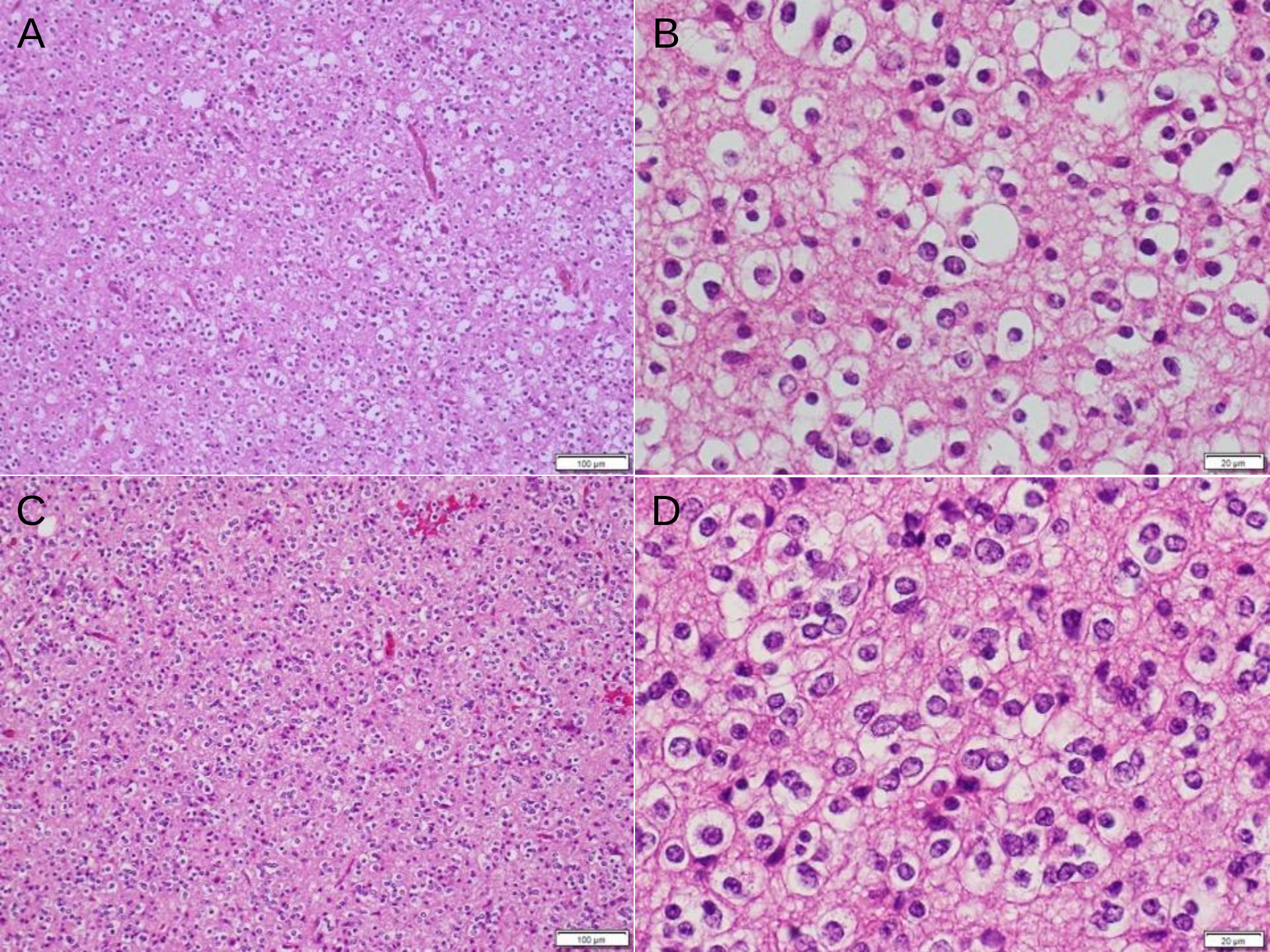
A. Sanger sequencing of the initial tumor presenting both C250T and C228T mutations in *TERT* promoter region.

B. Overview of primer designation.

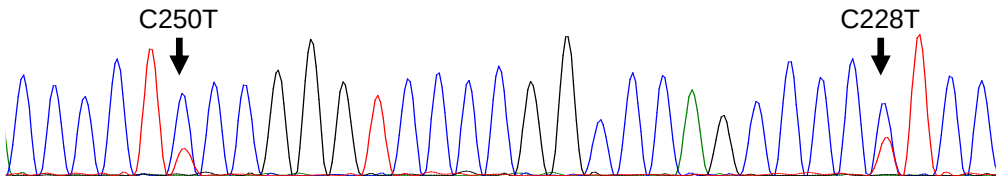
C. Sanger sequencing of initial tumor specimen using mutation-specific primers. Alleles with C250T only (upper), C228 only (middle), and both wild-types (lower) were detected.

D. Sanger sequencing of tumor specimen obtained at the second surgery presenting C250T and C228-wild-type.

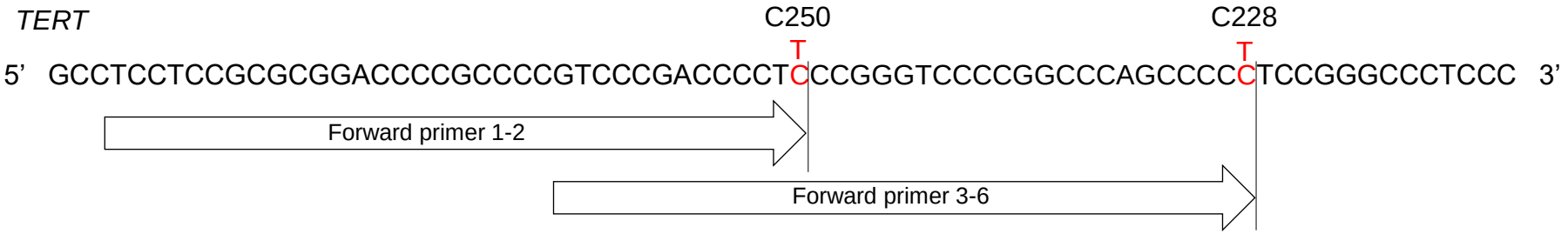

A
B
C
D
E


A
100 µm
B
20 µm
C
100 µm
D
20 µm

A

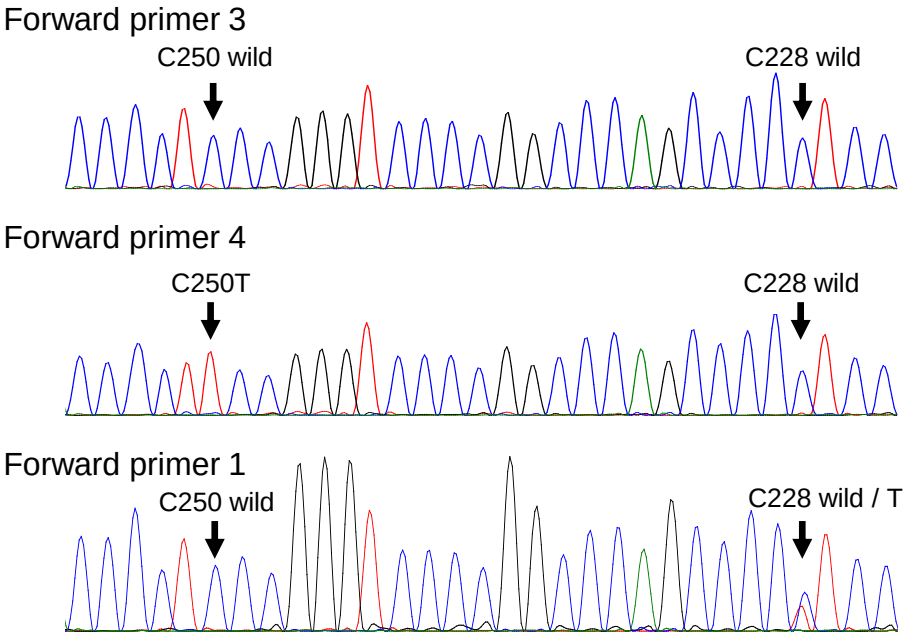


B



Forward primer 1 : TCCTCCGCGCGGACCCCGCCCCGTCCCGACCCCTC
Forward primer 2 : TCCTCCGCGCGGACCCCGCCCCGTCCCGACCCCTT
Forward primer 3 : GTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCC
Forward primer 4 : GTCCCGACCCCTTCCGGGTCCCCGGCCCAGCCCCC
Forward primer 5 : GTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCT
Forward primer 6 : GTCCCGACCCCTTCCGGGTCCCCGGCCCAGCCCCT

C



D

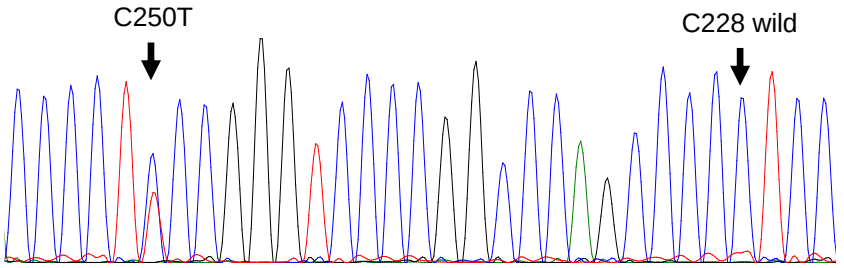


Table 1. Summary of sequencing primers used in this study.

Gene	Forward/reverse	Sequence (5 - 3)
IDH1	Forward	TGTGGAAATCACCAAATGGCAC
	Reverse	TACAAGTTGGAAATTTCTGGGC
IDH2	Forward	GGGAGCCCATCATCTGCAAAAA
	Reverse	ACAAGAGGATGGCTAGGCGA
TERT promoter (screening)	Forward	GGCCGATTCGACCTCTCT
	Reverse	CTCGCGGTAGTGGCTGC
TERT promoter (mutation-specific)	Forward 1	TCCTCCGCGCGGACCCCGCCCCGTCCCGACCCCTC
	Forward 2	TCCTCCGCGCGGACCCCGCCCCGTCCCGACCCCTT
	Forward 3	GTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCC
	Forward 4	GTCCCGACCCCTTCCGGGTCCCCGGCCCAGCCCCC
	Forward 5	GTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCT
	Forward 6	GTCCCGACCCCTTCCGGGTCCCCGGCCCAGCCCCT
	Reverse	CTCGCGGTAGTGGCTGCGCAGCAGGGAGCGCACGG

IDH, isocitrate dehydrogenase; TERT, telomerase reverse transcriptase

Table 2. Summary of reported cases of oligodendroglial tumors and diffuse gliomas with oligodendroglioma components presenting with the spatial and/or temporal heterogeneity of the TERT promoter mutations with common IDH mutation

Author	Age	Gender	Pathology	Tumor specimen 1			Tumor specimen 2		
				Description	IDH mutation	TERT promoter mutation	Description	IDH mutation	TERT promoter mutation
Aihara et al.[20]	68	F	AO	Initial, Gd-CE (-), AO	IDH1 R132H	C228T	Initial, Gd-CE (+), OL	IDH1 R132H	C250T
Aihara et al. [20]	47	M	AO	Initial, methionine PET high	IDH1 R132H	C228T	Initial, methionine PET low	IDH1 R132H	WT
Wilcox et al.[23]	30	M	OA	Initial, oligodendroglial region	IDH1 R132H	C250T	Initial, astrocytic region	IDH1 R132H	WT
Barresi et al.[21]	25	M	OA	Initial, oligodendroglial region	IDH2 R172M	C228T	Initial, astrocytic region	IDH2 R172M	WT
Nasrallah et al. [22]	29	F	OA	Initial, oligodendroglial region	IDH1 R132H	C228T	Initial, astrocytic region	IDH1 R132H	WT
Zhang et al.[24]	28	M	OL	Initial	IDH1 R132H	C228T	Recurrence without adjuvant therapy, OL	IDH1 R132H	WT
Kim et al.[19]	29	M	AO	Initial	IDH1 R132H	C228T	Recurrence after CRT	IDH1 R132H	WT
Present case	38	M	OL	Initial	IDH1 R132H	C250T and C228T	Recurrence without adjuvant therapy, AO	IDH1 R132H	C250T

AO, anaplastic oligodendroglioma; CRT, chemoradiotherapy; Gd-CE, gadolinium contrast enhancement; OA, oligoastrocytoma; OL, oligodendroglioma; PET, positron emission tomography; WT, wild type