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Discontiguous recurrences of IDH-wildtype glioblastoma share a common origin with the initial tumor and are frequently hypermutated

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Abstract

Glioblastoma is the deadliest primary brain tumor, largely due to inevitable recurrence of the disease after treatment. While most recurrences are local, patients rarely present with a new discontiguous focus of glioblastoma. Little is currently known about the genetic profile of discontiguous recurrences. In our institutional database, we identified 22 patients with targeted exome sequencing of pairs of initial and recurrent IDH-wildtype glioblastoma. Recurrences were classified as contiguous or discontiguous based on the presence or absence of T2 FLAIR signal connection to the initial site of disease on MRI. Exome analysis revealed shared driver and passenger mutations between discontiguous recurrences and initial tumors, supporting a common origin. Discontiguous recurrences were more likely to be hypermutated compared to contiguous recurrences ($p=0.038$). Analysis of 2 glioblastoma cases with discontiguous recurrence at a collaborating institution also exhibited hypermutation. In conclusion, discontiguous glioblastoma recurrences share a common origin with the initial tumor and are more likely to be hypermutated than contiguous recurrences.

Keywords IDH WT glioma, Glioblastoma recurrence, Multicentric, Discontiguous, Hypermutation

Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor with a median survival of approximately 15 months [1]. The lethality of GBM is closely linked with its near universal recurrence, often associated with increased aggression and therapeutic resistance [2]. While most GBMs recur locally, 10–13% of recurrences are considered distant [3, 4], and several groups have reported on molecular characteristics of these recurrent tumors [5–8]. Developmentally, both local and distant recurrences are generally thought to result from glioma cell infiltration of surrounding brain tissue and are only separated by an arbitrary distance of travel [5, 8]. However, rare distant recurrences, here termed "discontiguous recurrences", lack any evidence of infiltrating disease on T2 FLAIR MRI connecting them to the initial tumor site. This is in contrast to the vast majority of recurrences, termed "contiguous recurrences", which remain in contiguity on T2 FLAIR MRI with the initial tumor site. Discontiguous GBM recurrences are also considered a form of multicentric GBM where the second tumor develops metachronously, separated by time and, for

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the purposes of this study, courses of treatment (Fig. 1) [9–13]. Traditionally, some viewed multicentric gliomas as separate, unrelated tumors arising de novo from distinct cell populations [14, 15]. Others, on the other hand, have proposed a common origin for these tumors [9, 11]. Modern sequencing techniques provide an opportunity to investigate these possibilities and look for molecular features that may distinguish contiguous and discontiguous recurrences after treatment of an initial GBM [7, 8, 16–19].

Over the past two decades, detailed molecular characterization of gliomas has greatly improved our understanding of the genetic heterogeneity of these tumors as well as the biological and prognostic significance of their common driver mutations [20–22]. For instance, glioblastoma classification now requires an Isocitrate Dehydrogenase (IDH)-wildtype status, a distinction that more accurately captures the clinical behavior of these tumors [23]. Serial sequencing of gliomas has demonstrated that driver mutations present at the initial stage are generally retained at recurrence, maintaining the clonal architecture as tumor cells infiltrate and disperse within the brain, even to distant sites in the opposite hemisphere [5, 18]. Hypermutation, defined as 10 mutations/MB or higher, is a phenomenon observed in a number of recurrent gliomas, and this is thought be related to alkylating chemotherapy in the setting of DNA mismatch repair deficiency [24, 25]. This phenomenon has been observed in a large percentage of recurrent IDH-mutant gliomas treated with alkylating agents [18, 24], including an association with distant recurrences [26]. However, the incidence of hypermutation in recurrent IDH-wildtype GBM is much lower [8, 18, 19], and its association with discontiguous recurrence is unknown.

To better understand the distinguishing genetic characteristics of discontiguous recurrences, we identified 22 recurrent IDH-wildtype GBMs including 3 discontiguous recurrences in our database of patients with sequenced initial and recurrent tumors. and identified additional discontiguous GBM recurrences at a collaborating institution. Here, we report on our analyses and findings.

Materials and methods

Patient selection and classification

Patients were identified within our institutional database of brain tumors with targeted exome sequencing. Only IDH-wildtype GBM patients with sequenced recurrent tumors were included in the study. Patients provided

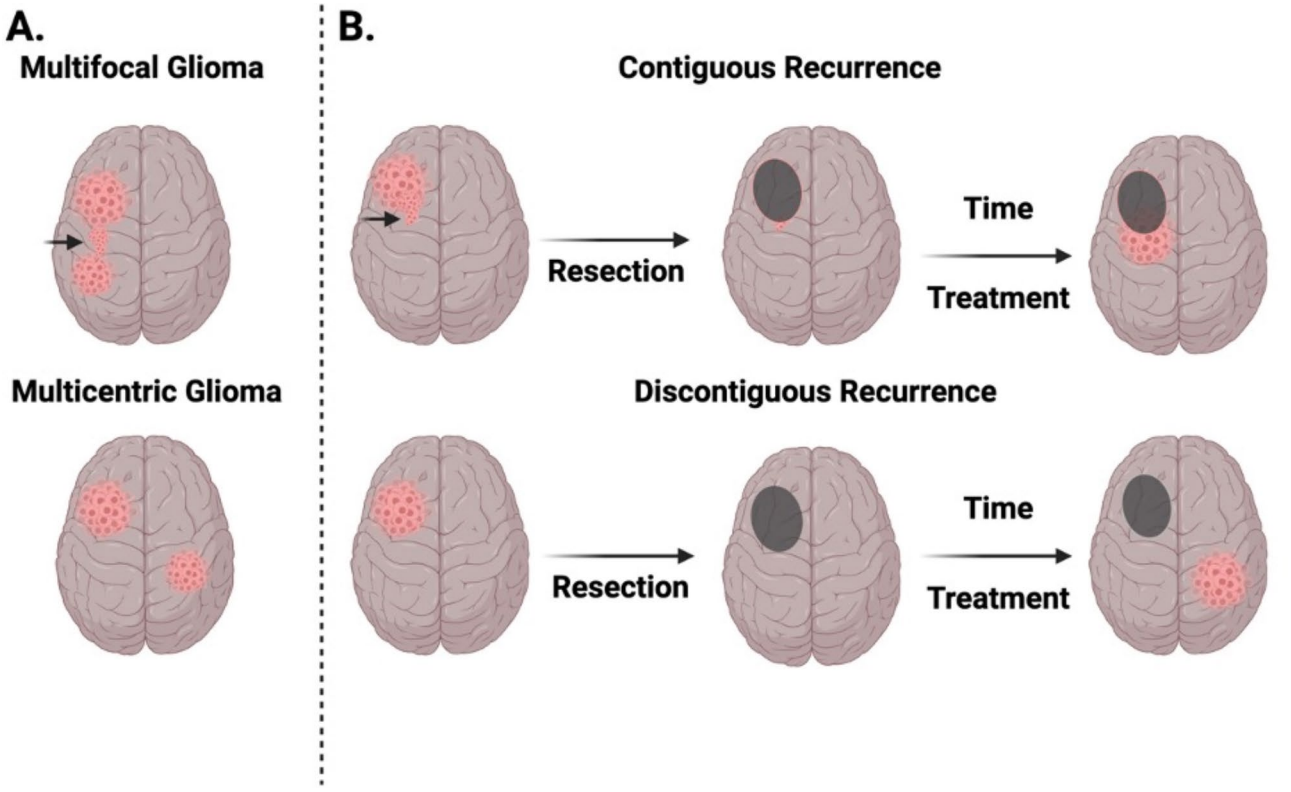

Fig. 1 Schematic of glioma recurrence patterns. (**A**) Multifocal gliomas typically present with concurrent discreet foci of tumor connected by infilitrating disease (arrow, top) while multicentric gliomas present with concurrent discreet foci of disease not linked by detectable infiltiration (bottom). (**B**) Foci of contiguous disease recurrence are connected to the initial tumor site via infiltrating disease (top), whereas discontiguous recurrences show no evidence of infiltration linking the two sites (bottom)

informed consent for research under an IRB approved protocol. All patients with sequencing results for an initial IDH-wild-type GBM and at least one recurrence were included. Patient MRI T1 post contrast and T2 FLAIR sequences were reviewed at the time of the initial diagnosis as well as recurrence. MRI sequences were analyzed for T2 FLAIR contiguity between lesions or areas where lesions arise. Intervening MRI images were also reviewed to clarify any points of ambiguity. Recurrent tumors were classified as discontiguous if they lacked contiguous T2 FLAIR signal on MRI connecting them to the site of the initial tumor. The site of the discontiguous tumor was screened for any evidence of early disease at initial presentation, excluding a synchronous multicentric presentation of disease. Using our radiological criteria, our collaborators at University Hospital Dresden identified additional patients with discontiguous recurrences with sequencing for tumor mutational burden in the recurrent tumor.

Tumor samples and sequencing

Tumor specimens were surgically obtained at Baylor College of Medicine affiliated hospitals. Targeted exome sequencing for cancer related genes (Supplementary Table 1) was conducted via hybrid capture next-generation sequencing (NGS) by Tempus Labs Inc. and annotated for single nucleotide variants, indels and translocations. Tumor mutational burden (TMB) measures the quantity of somatic mutations, of any pathogenicity, including benign, carried in a tumor as the number of single nucleotide protein-altering mutations per million coding base pairs. Expressed fusion transcripts were detected with whole transcriptome RNA-Seq in an unbiased fashion. Patients had the option to consent to germline sequencing of a normal sample. For the University Hospital Dresden samples, the sequencing methodologies and bioinformatics pipelines utilized in the MASTER (Molecularly Aided Stratification for Tumor Eradication Research) program and the validation methodologies have been thoroughly described elsewhere [27].

Statistics

Percentage of private mutations and tumor mutational burden values were compared using Mann-Whitney U test. Two-sided Fisher's exact test was used to compare proportions of hypermutated tumors within contiguous and discontiguous recurrences. The 95% confidence interval for the estimated overall hypermutation rate in other studies was determined using the binomial distribution equation with confidence interval = p +/- $z^*(\sqrt{p(1-p)} / n)$ and z = 1.96. The statistical comparison of this figure against the hypermutation rate in our discontiguous tumors was also based on the binomial distribution.

Results

Patient characteristics

A total of 22 patients were identified within our institutional database with targeted exome sequencing of pairs of initial and recurrent IDH-wildtype glioblastoma. All patients were initially treated with maximal safe resection followed by standard temozolomide chemoradiation. Each patient subsequently developed a recurrence that was resected between 3 months and 56 months (median of 9.7 months) after the initial surgery, and 3 of these were discontiguous recurrences (Table 1). MRI images revealed supratentorial location for the initial tumor in these 3 patients and infratentorial (Patient 1) or contralateral hemisphere (Patients 2 and 3) location for the discontiguous recurrence (Fig. 2). The recurrent tumors in the remaining 19 patients were classified as contiguous.

Discontiguous recurrences are clonally related to the initial tumor

The mutational landscape of common GBM pathways in the 3 discontiguous recurrences and their initial tumors is represented in Fig. 3A (full mutation list in Supplementary Table 1). All 3 of these tumors and/or their recurrences had molecular signatures commonly associated with GBM including TERT promoter mutations, EGFR mutations and amplifications, and loss of CDKN2A/2B

Table 1 Characteristics of patients from our institution with discontiguous recurrence

Patient	Age/sex	Tumor	Location	Pathology	Initial treatment	Resection interval	Mutational burden
P1	80 Female	T1	Left fronto-parietal	IDH-WT GBM, MGMT methylated	Gross total resection Concurrent TMZ chemoradiation 3 cycles adjuv. TMZ	1.8 years	1.7 m/MB
		T2	Right cerebellar	IDH-WT high-grade astrocytoma			13.7 m/MB
P2	48 Female	T1	Right parietal	GBM	Gross total resection Concurrent TMZ chemoradiation 6 cycles adjuv. TMZ	4.5 years	2.6 m/MB
		T2	Left frontal	IDH-WT anaplastic astrocytoma			53.7 m/MB
P3	69 Male	T1	Right temporo-occipital	IDH-WT GBM, MGMT unmethylated	Gross total resection Concurrent TMZ chemoradiation 8 cycles adjuv. TMZ	1 year	2.6 m/MB
		T2	Left temporal	IDH-WT GBM, MGMT unmethylated			2.1 m/MB

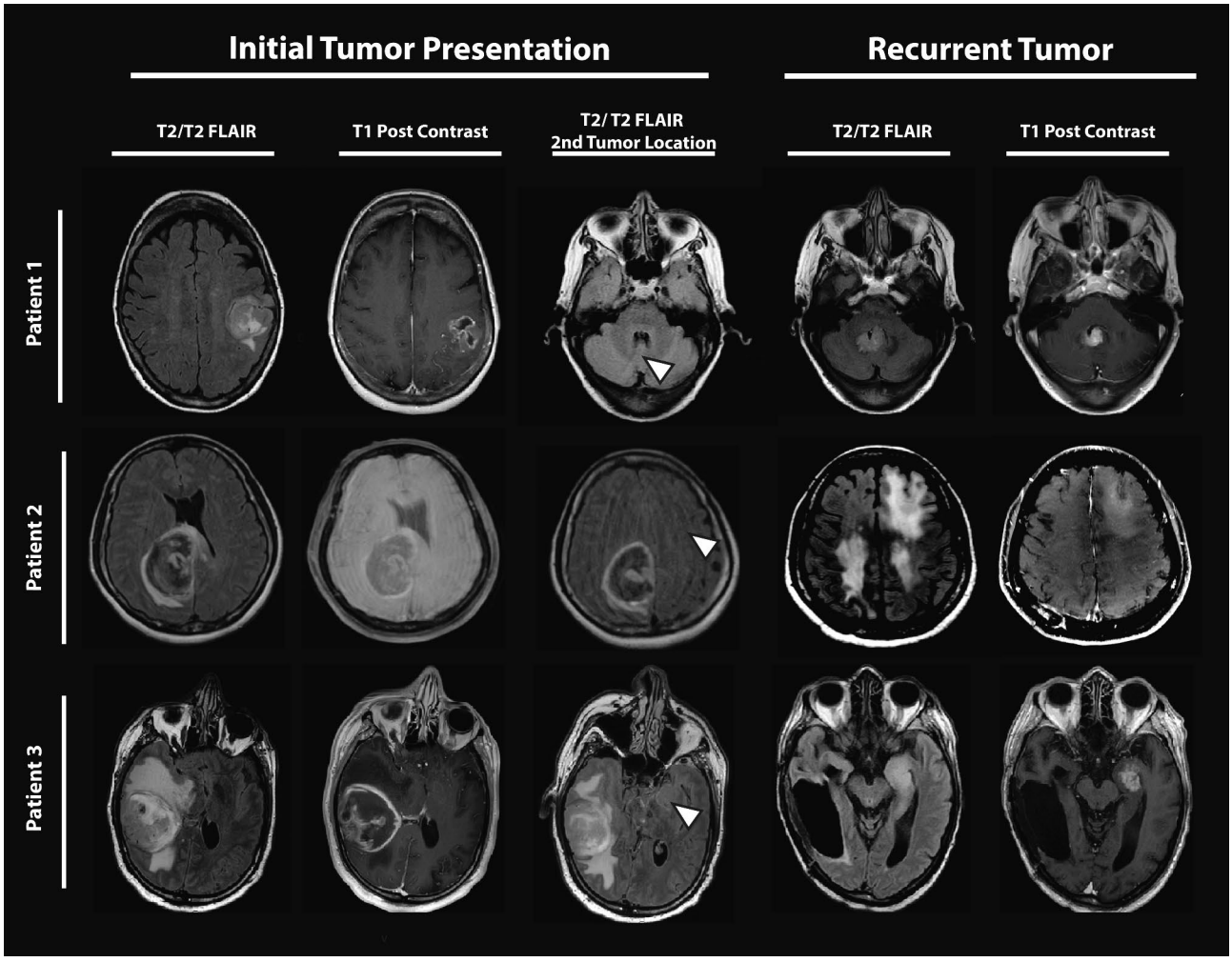

Fig. 2 Brain MRI findings in patients with discontiguous IDH-wildtype GBM recurrence. Pre-operative MRI at initial presentation shows enhancing disease at the site of the initial tumor (left 2 panels) but no sign of disease at the future location of the recurrnt tumor (middle panel). A follow up MRI (right 2 panels) shows development of a discontiguous recurrence at this location

[22, 28–30]. Additionally, all of these tumors lacked IDH1/2 mutations.

All 3 pairs of tumors with discontiguous recurrence had identical mutations, including several variants of unknown significance, shared within each pair. For instance, Patient 1 had identical NOTCH1 and IKZF1 mutations, Patient 2 had identical GATA4 and PTPRD mutations, and Patient 3 had identical RPL5, FCGR2A, SLIT2, and KMT2C mutations in both the initial tumor and the discontiguous recurrence (Fig. 3B). The sharing of identical mutations among tumor pairs is indicative of a clonal relationship and sharing of a common tumor cell ancestor.

Discontiguous recurrences are frequently hypermutated

In addition to shared mutations, each tumor in a pair also contained private mutations, amplifications, copy number variations, or chromosomal rearrangements not seen in the other tumor. The proportion of private mutations among discontiguous recurrences (91%) was higher than that seen in contiguous recurrences (39%) but the difference was not statistically significant (Fig. 3C). The high proportion of private mutations in discontiguous recurrences was suggestive of hypermutation, and this was confirmed in Patient 1 and Patient 2, whose recurrence TMB was 13.7 m/MB and 53.7 m/MB, respectively. Concordant with this, both recurrent tumors contained private mutations in MSH2, a mismatch repair (MMR) gene associated with hypermutation, as well as mutations in other genes involved in DNA damage repair or MMR including BRCA2, MLH1, ATM, and ATR. In contrast, among the contiguous recurrence cohort of 19 tumors, only 1 recurrence had TMB > 10 m/MB (Fig. 3D). Fisher's exact test demonstrated that the rate of hypermutation among discontiguous recurrences of IDH-wildtype GBM

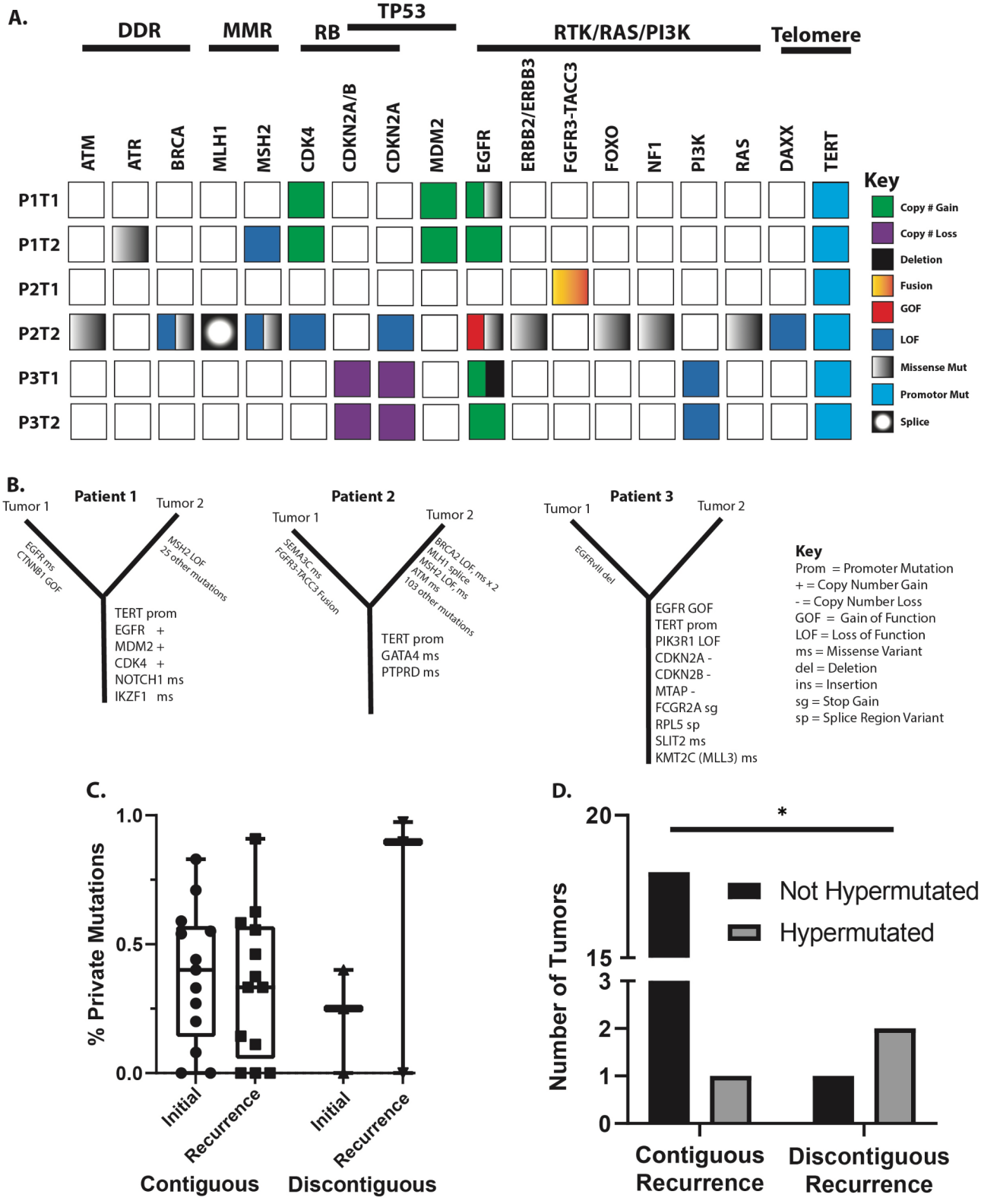

Fig. 3 Molecular findings in patients with discontiguous IDH-wildtype GBM recurrence. (**A**) The molecular landscape of the initial and recurrent tumors shows shared and private alterations of a number of common pathways in glioma. (**B**) Representation of tumor clonal evolution depicting shared and private mutations within the initial and recurrent tumors. (**C**) Comparison of the percentage of private mutations in contiguous and discontiguous recurrences. (**D**) The rate of hypermutation in discontiguous recurrences is significantly higher than contiguous recurrences (* two-sided Fisher's exact $p = 0.038$)

was significantly higher than that of contiguous recurrences ($p = 0.038$, Fig. 3D).

Corroboration in additional samples and comparison with published studies

To supplement the small number of discontiguous recurrences in our cohort, we sought additional discontiguous IDH-wildtype GBM recurrences from our collaborating institution, the Carl Gustav Carus University Hospital, to assess for hypermutation. Upon reviewing radiographic images of IDH-wildtype GBM patients with sequencing data, two cases of discontiguous recurrence were identified (Table 2). Initially presenting with supratentorial GBM, these patients subsequently developed contralateral or infratentorial discontiguous recurrences within 10–14 months (Fig. 4A). Tumor recurrence samples from both Patient 4 and Patient 5 underwent whole exome sequencing as part of the MASTER program. The recurrences were found to be hypermutant, with TMB of 245 m/MB and 39 m/MB, respectively, supporting the association noted in our patient cohort. With these two patients added to our cohort, discontiguous recurrences had a higher TMB compared to contiguous recurrences ($p = 0.0047$, Fig. 4B) and a higher rate of hypermutation ($p = 0.0023$, Fig. 4C).

We also compiled data from 3 prior studies reporting on hypermutation among IDH-wildtype GBM recurrences. While these studies did not distinguish between contiguous and discontiguous recurrences, our analysis of their data (Fig. 4D) provides an estimate of the overall hypermutation rate among all IDH-wildtype GBM recurrences (0.10, 95% confidence interval 0.06–0.14). This figure was significantly lower than the hypermutation rate among our exclusive group of discontiguous recurrences (4 out of 5 tumors, $p = 0.00044$), supporting an association of discontiguous GBM recurrence with hypermutation.

Discussion

We report on two main findings in this study. The first finding pertains to the clonal relationship between discreet instances of GBM that appear radiologically to have arisen independently within the brain. Independence in this context refers to a lack of a radiographically evident path of infiltrative or otherwise traveling cancer cells connecting the two intra-axial tumor sites. While a number of authors have favored distinct development paths for these tumors [14, 15, 31], termed "multicentric glioma", others support common origins [7, 8, 17, 32]. Stepwise accumulation of all the mutations and genetic changes that transform a normal cell into a glioblastoma cell is a rare event, which explains the low incidence of these tumors among the population (lifetime risk < 0.5%). Coincidence of a second glioma in the same individual developed fully independently of the first tumor would be expected to be exceptionally rare. Therefore, the reported 5–14% incidence of multicentric glioma among glioma patients implies the sharing of one or more driver mutations, supporting a common origin hypothesis [9, 33, 34]. While our data does not distinguish between the possibilities, it is conceivable that in a multicentric tumor, one or more tumor-initiating cells migrate from one tumor site to a novel site without leaving a radiologically visible connection on T2/FLAIR MRI. We, however, deem this possibility less likely as the process through which gliomas infiltrate the brain parenchyma is expected to leave a diffuse trail of tumor progeny and tissue edema on MRI. The second possibility is that the mutations shared between the anatomically distinct tumors were inherited or acquired de-novo early in life and distributed within the brain in a mosaic pattern via dispersion of tumor precursor cells. This possibility is suggestive of an earlier clonal separation between the two tumors, and consistent with this, several studies have noted mosaic distribution of various de-novo mutations including driver mutations within the brain and other organs [35]. These possibilities can pertain to both synchronous and metachronous presentation of multicentric glioma, and a detailed assessment of these possibilities is outside the scope of this study.

The second main finding in our study is the association of discontiguous recurrences of IDH-wildtype GBM with hypermutation, a phenomenon more commonly observed in IDH-mutant glioma recurrences [8, 26, 36]. While hypermutation is fairly uncommon in contiguous recurrences of IDH-wildtype GBM (estimated at around 10%), 2 out of 3 discontiguous recurrences in our cohort and an additional 2 discontiguous recurrences at a

Table 2 Characteristics of patients from outside institution with discontiguous recurrence

Patient	Age/sex	Tumor	Location	Pathology	Initial treatment	Resection interval	Mutational burden
P4	41 Female	T1 T2	Left parietal Right temporal	IDH-WT GBM, MGMT unmethylated IDH-WT GBM, MGMT unmethylated	Gross total resection Concurrent TMZ chemoradiation 6 cycles adjuv TMZ	1.2 years	N.A. 245 m/MB
P5	20 Male	T1 T2	Right frontal Right cerebellar	IDH-WT GBM, MGMT unmethylated IDH-WT GBM, MGMT unmethylated	Gross total resection Concurrent TMZ chemoradiation 6 cycles adjuv. TMZ	10 months	N.A. 39 m/MB

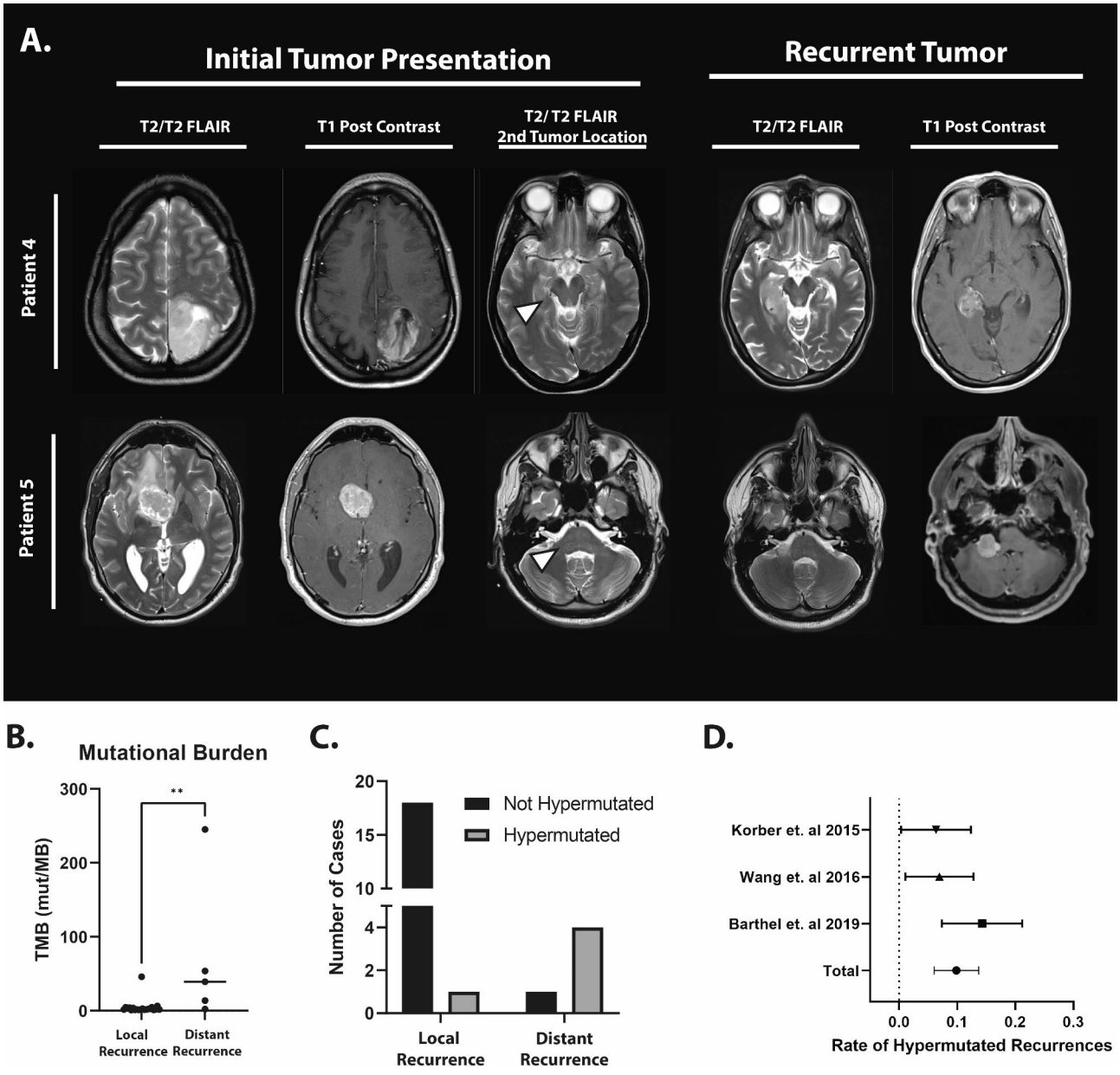

Fig. 4 Corroboration in additional samples and comparison with published studies. (**A**) Pre-operative MRI at initial presentation of two patients from our collaborating institution shows enhancing disease at the site of the initial tumor (left 2 panels) but no sign of disease at the future location of the recurrnt tumor (middle panel). A follow up MRI (right 2 panels) shows development of a discontiguous recurrence at this location. (**B** and **C**) When combined with our cohort, discontiguous recurrences have a significantly higher tumor mutation burden (** two-sided Mann-Whitney U $p = 0.0047$) hypermutation rate (** two-sided Fisher's exact $p = 0.0023$) than contiguous recurrences. (**D**) Overall hypermutation rate in recurrent IDH-wildtype GBM is estimated at 0.10 (95% confidence interval 0.06–0.14) using our data and previously published studies

collaborating institution were found to be hypermutated. Interestingly, a study of IDH-mutant low grade gliomas that transformed to high grade in recurrence found that hypermutated tumors were more likely to be distant recurrences [26]. Similarly, while these hypermutated tumors generally contain disabling mutations in MMR genes, it is not clear from our data whether these mutations occurred early after clonal separation and dispersion of tumor precursor cells, or at a later time, perhaps during the adjuvant treatment course or as a result of radiation. It is likely that exposure to alkylating chemotherapy in the context of MMR deficiency is responsible for the development of hypermutation [37]. In terms of discontiguous gliomagenesis, we hypothesize that at the time of diagnosis of the initial tumor, the discontiguous focus of tumor with several shared mutations is likely in a precancerous state, not visible radiologically, and possibly lacking one or more key driver mutations. These

driver muttions are likely gained in the course of adjuvant treatment or later, and MMR deficiency and the resultant hypermutation can also aid in this process. Of particular clinical interest, select studies reported cases of hypermutated GBM responding to immune checkpoint inhibition [38, 39]. While it has been speculated that these hypermutated tumors could benefit from immunotherapy, there are currently no successful clinical trials of checkpoint inhibition in hypermutated GBM [37, 38, 40]. Nevertheless, our observation of increased hypermutation in discontiguous GBM recurrences warrants larger collaborative studies to validate this finding and investigate its biological mechanisms.

The main limitation of this study is the small number of discontiguously recurrent GBMs. These recurrences are uncommon, possibly in part due to the short survival of IDH-wildtype GBM patients. Additionally, while there are ongoing efforts to more routinely biopsy recurrent tumors [41], many are currently not biopsied, depriving the clinicians and researchers of potentially valuable molecular insights. Nevertheless, the high prevalence of hypermutation among these recurrences was sufficient to produce a statistically significant association despite the small numbers in our institutional cohort. Since we did not have contiguous GBM recurrence data with molecular information from our collaborating institution, those discontiguous recurrences were treated as a separate cohort, which nevertheless support our main findings. Lastly, our available data cannot address the possibility that hypermutation may be more frequent within distant areas of contiguous recurrences. A future study can assess hypermutation within spatially distinct areas of contiguous GBM recurrenes and determine whether distance from the primary resection cavity is associated with the frequency of hypermutation.

Conclusion

Our analysis of tumor data from patients with recurrent IDH-wildtype GBM demonstrated that discontiguous recurrences are clonally related to the initial tumor and are more likely to be hypermutated. Future studies may be able to shed light on the time of clonal separation and spread of tumor precursor cells as well as the mechanisms behind the higher prevalence of hypermutation among these recurrences.

Abbreviations

GBM	Glioblastoma
FLAIR MRI	Fluid attenuated inversion recovery magnetic resonance imaging
IDH	Isocitrate dehydrogenase
TMB	Tumor mutational burden
NGS	Next generation sequencing
MASTER	Molecularly aided stratification for tumor eradication research
MMR	Mismatch repair

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40478-024-01900-1.

Supplementary Material 1

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Author contributions

Author Contributions: All authors have seen and approved of the manuscript. AJ, JJM, and MFM conceptualized the study. MFM, AJ, JJM, TJ, IE, and SG analyzed the data. MFM wrote the initial draft of the manuscript. AJ, JJM, GR, and TJ provided critical revisions.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Patients provided informed consent for research under an IRB approved protocol.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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