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ORIGINAL REPORT

Evidence for Sequenced Molecular Evolution of *IDH1* Mutant Glioblastoma From a Distinct Cell of Origin

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ABSTRACT

Purpose
Mutation in isocitrate dehydrogenase 1 (*IDH1*) at R132 (*IDH1*^{R132MUT}) is frequent in low-grade diffuse gliomas and, within glioblastoma (GBM), has been proposed as a marker for GBMs that arise by transformation from lower-grade gliomas, regardless of clinical history. To determine how GBMs arising with *IDH1*^{R132MUT} differ from other GBMs, we undertook a comprehensive comparison of patients presenting clinically with primary GBM as a function of *IDH1*^{R132} mutation status.

Patients and Methods
In all, 618 treatment-naive primary GBMs and 235 lower-grade diffuse gliomas were sequenced for *IDH1*^{R132} and analyzed for demographic, radiographic, anatomic, histologic, genomic, epigenetic, and transcriptional characteristics.

Results
Investigation revealed a constellation of features that distinguishes *IDH1*^{R132MUT} GBMs from other GBMs (including frontal location and lesser extent of contrast enhancement and necrosis), relates them to lower-grade *IDH1*^{R132MUT} gliomas, and supports the concept that *IDH1*^{R132MUT} gliomas arise from a neural precursor population that is spatially and temporally restricted in the brain. The observed patterns of DNA sequence, methylation, and copy number alterations support a model of ordered molecular evolution of *IDH1*^{R132MUT} GBM in which the appearance of mutant IDH1 protein is an initial event, followed by production of p53 mutant protein, and finally by copy number alterations of *PTEN* and *EGFR*.

Conclusion
Although histologically similar, GBMs arising with and without *IDH1*^{R132MUT} appear to represent distinct disease entities that arise from separate cell types of origin as the result of largely nonoverlapping sets of molecular events. Optimal clinical management should account for the distinction between these GBM disease subtypes.

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INTRODUCTION

Glioblastoma (GBM), also known as grade 4 astrocytoma, is the most aggressive intrinsic brain tumor in adults and continues to be associated with extremely poor outcomes.[1] Evidence to date indicates that the cells of origin for GBM may be either neural stem cells or their more differentiated progeny.[2-4] Most GBMs arise with no prior clinical history of a precursor lesion and are referred to as primary or de novo GBMs. A minority of GBM cases, known as secondary GBMs, develop from lower-grade astrocytomas or oligodendrogliomas and bear different genomic abnormalities than primary GBM.[5-7] Despite recent studies suggesting that molecular subsets of GBM differ in response to current treatments,[8,9] standard treatment for all patients with primary GBM[10] is a regimen combining radiation and temozolomide. The recent identification of R132 mutations in isocitrate dehydrogenase 1 (*IDH1*^{R132MUT}) in the majority of low-grade gliomas and secondary GBMs, with relative exclusion from primary GBMs, implicates *IDH1*^{R132MUT} as a defining marker and key oncogenic event for GBMs

Table 1. Summary of Patient Cohorts

Cohort	Source Institution	Tumor Type	Sample Type	No. of Patients	Ratio of Males to Females	No. of $IDH1^{R132MUT}$ Tumors	Mean Age (years)	Range of Ages (years)	Figure	Reference
A	UCLA, KPLA	Primary GBM	Newly diagnosed*	389	1.5	19	56.8	24-90	1A-B, 2A, 3A-C, 4A; Appendix A1A-A1C, A2B, A3D-A3E, A4A-A4C	N/A
B	MDACC	Primary GBM	Newly diagnosed	70	1.7	8	54.6	20-80	1A, 2A, 3A, 3C, 4A; Appendix A1A, A3E, A4A, A4C	N/A
C	UCSF, MDACC	Primary GBM	Newly diagnosed*	98	1.7	22	46.3	19-82	2A-B, 3A, 3C, 4A; Appendix A1A, A3E, A4A	Phillips et al[17]
D	UCSF	Primary GBM	Newly diagnosed	29	1.9	0	56.0	27-76	2A, 3A, 3C, 4A; Appendix A3E, A4A	Chen et al[18]
E	UCSF, MDACC	Primary GBM	Newly diagnosed	14	N/D	0	11.7	2-17	3A, 3C; Appendix A4A	Schiffman et al[19]†
F	UKE	Primary GBM	Newly diagnosed*	18	1.3	0	65.3	32-85	2A, 3A, 3C, 4A; Appendix A1A, A3E, A4A	Günther et al[20]†
G	UCSF, MDACC	AA	Newly diagnosed	77	1.3	42	38.5	7-75	2A, 3C, 4A; Appendix A2A-A2B, A3E, A4A	Phillips et al[17]†
H	UCLA, KPLA	Grades II and III diffuse glioma	Newly diagnosed	158	1.1	103	40.1	15-79	3C, 4A; Appendix A3A-A3C, A3E	N/A
I	Various	Grades II to IV diffuse glioma	Newly diagnosed and recurrent	105	N/D	37	N/D		4A	N/A

NOTE. Samples from newly diagnosed patients were obtained before treatment. Tumor type is according to WHO criteria.
Abbreviations: AA, anaplastic astrocytoma; GBM, glioblastoma; $IDH1^{R132MUT}$, mutation in isocitrate dehydrogenase 1 (*IDH1*) at R132; KPLA, Kaiser Permanente Los Angeles; MDACC, MD Anderson Cancer Center; N/A, not applicable; N/D, not done; UCLA, University of California at Los Angeles; UCSF, University of California at San Francisco; UKE, Universitätsklinikum Hamburg-Eppendorf.
*Includes matched recurrent specimens for a subset of cases.
†Cohort includes additional specimens not described in original publication.

that evolve from lower-grade glioma.[11-16] Herein, we sought to develop a detailed portrait of untreated GBMs arising with $IDH1^{R132MUT}$ with the aim of gaining insights into the manner in which $IDH1^{R132MUT}$ gliomas develop and to determine whether $IDH1^{R132MUT}$ GBM is a distinct disease entity.

PATIENTS AND METHODS

The focus of our investigation is 618 patients with newly diagnosed untreated primary (de novo) GBMs (cohorts A through F) and 235 patients with newly diagnosed untreated lower-grade diffuse gliomas (cohorts G and H). Patients with GBM included two cohorts compiled for this investigation (cohorts A and B), plus patients associated with previously reported studies[17-20] (cohorts C through F). Sequence analysis of $IDH1^{R132}$ and $p53^{R273}$ included 105 additional samples of diffuse glioma (cohort I). A summary of patient cohorts is provided in Table 1. Additional details regarding Methods and patient cohorts are provided in the Data Supplement.

RESULTS

IDH1^{R132MUT} *GBMs Are Phenotypically Distinct*

To characterize $IDH1^{R132MUT}$ and $IDH1^{R132}$-wild type ($IDH1^{R132WT}$) GBMs in the absence of confounding treatment effects, we pursued a comparison within treatment-naive patients with primary GBM. From 618 de novo GBMs, we identified 49 $IDH1^{R132MUT}$ tumors, all of which occurred in adults. Within adult patients, we confirmed that $IDH1^{R132MUT}$ GBMs manifest longer overall survival[12,13,16,21] (Appendix Fig A1A, online only) and showed more frequent promoter methylation of O^6-methylguanine–DNA methyltransferase (*MGMT*)[8,21,22] as illustrated in Figure 1A. Histologic characterization of a sampling of $IDH1^{R132MUT}$ and $IDH1^{R132WT}$ GBMs demonstrated similar levels of cell proliferation on the basis of MIB-1 staining (Data Supplement), but revealed a lesser extent of necrosis in $IDH1^{R132MUT}$ GBMs (Fig 1A) and a nonsignificant trend toward less frequent occurrence of vascular abnormalities (Fig 1A). Consistent with a previous report,[12] examination of an expanded series of samples revealed a statistically significant, albeit modest, increase in the percentage of cells with oligodendroglial morphology in $IDH1^{R132MUT}$ GBMs (Fig 1A). To examine whether tumors in our series of GBMs harbored the co-deletion of chromosome arms 1p/19q commonly observed in oligodendrogliomas, we assessed DNA copy number alterations by using array comparative genomic hybridization in a series of samples and found that a minority of $IDH1^{R132MUT}$ GBMs (two of eight) and none of the $IDH1^{R132WT}$ GBMs (zero of 13) displayed 1p/19q co-deletion (Appendix Fig A1B). There was a striking difference between the two GBM subsets regarding loss of chromosome 10; this alteration was absent in all $IDH1^{R132MUT}$ GBMs but was present in all but one sample of $IDH1^{R132WT}$ GBM (Appendix Fig A1B).

By using previously defined parameters,[23] we examined available preoperative cranial magnetic resonance images. Consistent with our histologic findings, detection of necrosis was less frequent in $IDH1^{R132MUT}$ GBMs; moreover, $IDH1^{R132MUT}$ GBMs exhibited more frequent non-enhancing tumor component, larger size at diagnosis, lesser extent of edema, and increased prevalence of cystic and diffuse components (Fig 1B and Appendix Fig A1C). In addition, the $IDH1^{R132MUT}$ GBMs demonstrated greater frequency of contact with brain ventricles, although interpretation of this finding may be confounded by the larger size of $IDH1^{R132MUT}$ GBMs (Appendix Fig A1C). Overall, the radiographic and histologic features that distinguish $IDH1^{R132MUT}$ GBMs resemble characteristics of lower-grade gliomas and are consistent with a less aggressive clinical course.

Restricted Gene Expression of IDH1^{R132MUT} *GBMs*

By examining transcriptional signatures of both the newly diagnosed high-grade astrocytomas in this investigation and The Cancer Genome Atlas (TCGA) primary GBM data set, we found that the majority of $IDH1^{R132MUT}$ tumors express the Proneural[17] subtype signature (Fig 2A and Appendix Fig A2A, online only). This Proneural signature has previously been reported as a positive prognostic indicator[17] and is substantially similar to the TCGA Proneural signature associated with $IDH1^{R132MUT}$ GBMs that resembles the signature of oligodendroglia.[9] A minority of $IDH1^{R132MUT}$ GBMs possessed the Proliferative signature, and none possessed the Mesenchymal signature associated with angiogenesis and poor outcome[17] (Fig 2A and Appendix Fig A2A). In contrast, $IDH1^{R132WT}$ GBMs displayed all

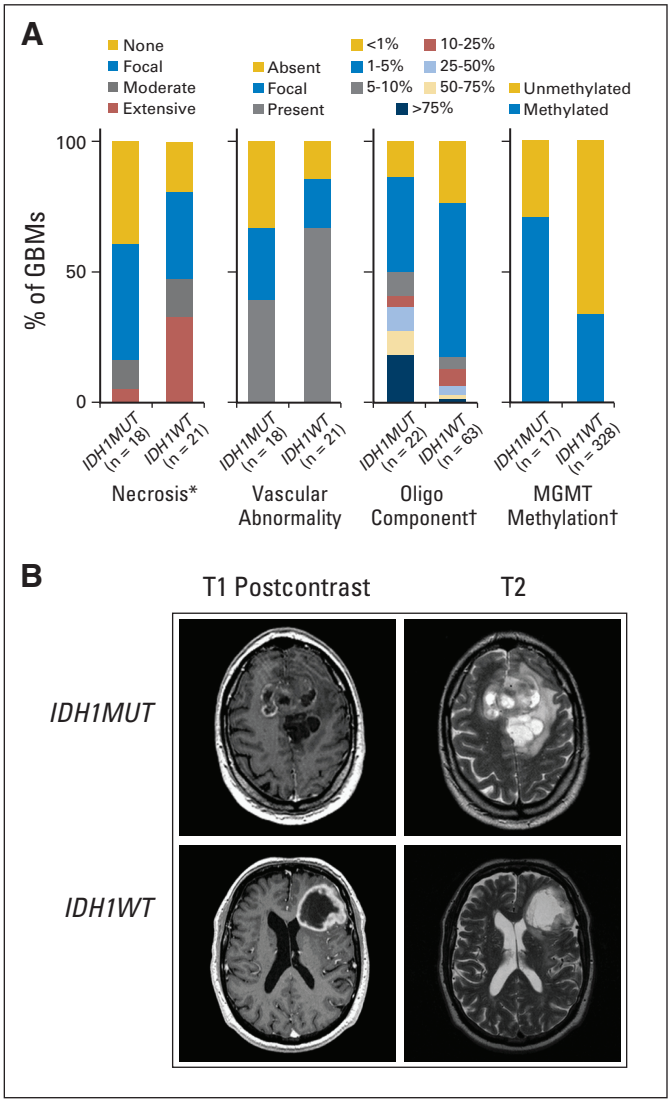

Fig 1. Glioblastomas (GBMs) with mutations in isocitrate dehydrogenase 1 (*IDH1*) at R132 ($IDH1^{R132MUT}$; *IDH1MUT*) are phenotypically distinct from $IDH1^{R132}$-wild type ($IDH1^{R132WT}$; *IDH1WT*) GBMs. (A) Histologic assessment of $IDH1^{R132MUT}$ and $IDH1^{R132WT}$ GBMs reveals decreased necrosis (*$P < .05$) and oligodendroglial content (†$P < .005$), with a trend toward decreased vascular abnormalities ($P < .075$) for $IDH1^{R132MUT}$ GBMs (Data Supplement). Frequency of O^6-methylguanine–DNA methyltransferase (*MGMT*) promoter methylation is increased in $IDH1^{R132MUT}$ GBMs (†$P < .005$; Data Supplement). (B) Representative pretreatment contrast-enhanced T1-weighted images (left) with corresponding T2-weighted images (right) of a patient with $IDH1^{R132MUT}$ (*IDH1MUT*; upper panel) and a patient with $IDH1^{R132WT}$ (*IDH1WT*; lower panel).

three signatures, with a preponderance of the Mesenchymal subtype. Strikingly, evaluation of gene expression subtype in matched sample pairs from high-grade astrocytomas obtained at initial diagnosis and after recurrence (Appendix Fig A2B) showed that all $IDH1^{R132MUT}$ tumors maintained their original subtype, although several $IDH1^{R132WT}$ GBMs shifted to the Mesenchymal subtype. Thus, $IDH1^{R132MUT}$ tumors differ not only in their presentation but also in their pattern of disease progression and do not share the propensity of $IDH1^{R132WT}$ GBMs to adopt a Mesenchymal phenotype.

By using agreement of differential expression (AGDEX)[24] to compare expression profiles of the human tumors with a published embryonic mouse forebrain gene expression data set,[25] we found that global expression profiles of $IDH1^{R132WT}$ GBMs resemble mouse neural stem cells, and $IDH1^{R132MUT}$ GBMs resemble lineage-committed neural precursors (AGDEX, +0.147; $P < .004$; Data Supplement). A separate hierarchical clustering analysis that used the genes most differentially expressed between $IDH1^{R132MUT}$ and $IDH1^{R132WT}$ GBMs reveals similarity of $IDH1^{R132MUT}$ samples to normal fetal or adult brain parenchyma and similarity of $IDH1^{R132WT}$ GBMs to cultured adult neural stem cells[26] (Fig 2B). Because both fetal and adult brain samples are enriched for differentiating or mature neural cell types, these findings underscore the greater similarity of $IDH1^{R132MUT}$ GBMs to lineage-committed neural cells than to stem cells.

IDH1^{R132MUT} *GBMs Are Spatially and Temporally Restricted*

Tabulating the location of $IDH1^{R132MUT}$ and $IDH1^{R132WT}$ GBMs, we found a striking predominance of frontal lobe involvement of $IDH1^{R132MUT}$ GBMs that contrasts with the more widespread distribution of $IDH1^{R132WT}$ GBMs (Fig 3A). Regardless of histologic subtype, $IDH1^{R132MUT}$ gliomas displayed a nearly identical percentage of frontal lobe involvement (Appendix Figs A3A-A3C, online only).

Overlay of tumor areas from a series of all available $IDH1^{R132MUT}$ GBMs with digitized pretreatment magnetic resonance images confirmed the high frequency of frontal lobe involvement, whereas overlay of a random sampling of $IDH1^{R132WT}$ tumors failed to demonstrate any frequently involved regions (Appendix Fig A3D). By performing a voxel-wise Fisher's exact test to isolate the area of differential involvement, we found that $IDH1^{R132MUT}$ GBMs were distributed at increased frequency in the area of the frontal lobe surrounding the rostral extension of the lateral ventricle (Fig 3B).

By examining tumor genotype as a function of age for both GBMs and grade 3 astrocytoma (anaplastic astrocytoma), we found that the relative frequency of $IDH1^{R132MUT}$ tumors rises sharply in the third decade of life and decreases in the fourth or fifth decade (Fig 3C). Thus, relative to $IDH1^{R132WT}$ tumors, $IDH1^{R132MUT}$ tumors appear to arise at greatest frequency within a more restricted time period. Interestingly, within adult GBMs, a significant difference in sex ratios was seen as a function of $IDH1^{R132}$ status, consistent with previous reports of trends for a greater fraction of female patients with secondary versus primary GBM[27,28] (Appendix Fig A3E).

IDH1^{R132MUT} *GBMs Show Preponderance of Template Strand Mutation in* IDH1 *and Coding Strand Mutation in* p53

In agreement with previous reports,[13,16,29] our data showed a higher frequency of *p53* mutation in $IDH1^{R132MUT}$ versus $IDH1^{R132WT}$ high-grade astrocytomas (Appendix Fig A4A, online only). Consistent with the well-documented propensity for C>T mutation at cytosine phosphate guanine (CpG) sites,[30] data from our sample set showed that the most common mutations in both *p53* and *IDH1* are at Arg residues encoded by the codon CGT ($IDH1^{R132}$ and $p53^{R273}$; Figs 4A and 4B). For both $IDH1^{R132}$ and $p53^{R273}$, C>T mutations on template and coding strands will result in substitutions of His or Cys, respectively. By sequencing an expanded series of grades 2 to 4 gliomas for these codons in *IDH1* and *p53*, we found, within $IDH1^{R132MUT}$ gliomas, a marked and unexpected contrast between the prevalence of Cys and His substitutions in the two proteins ($P <$ .001 with Fisher's exact test; Fig 4A). Given that the probability for

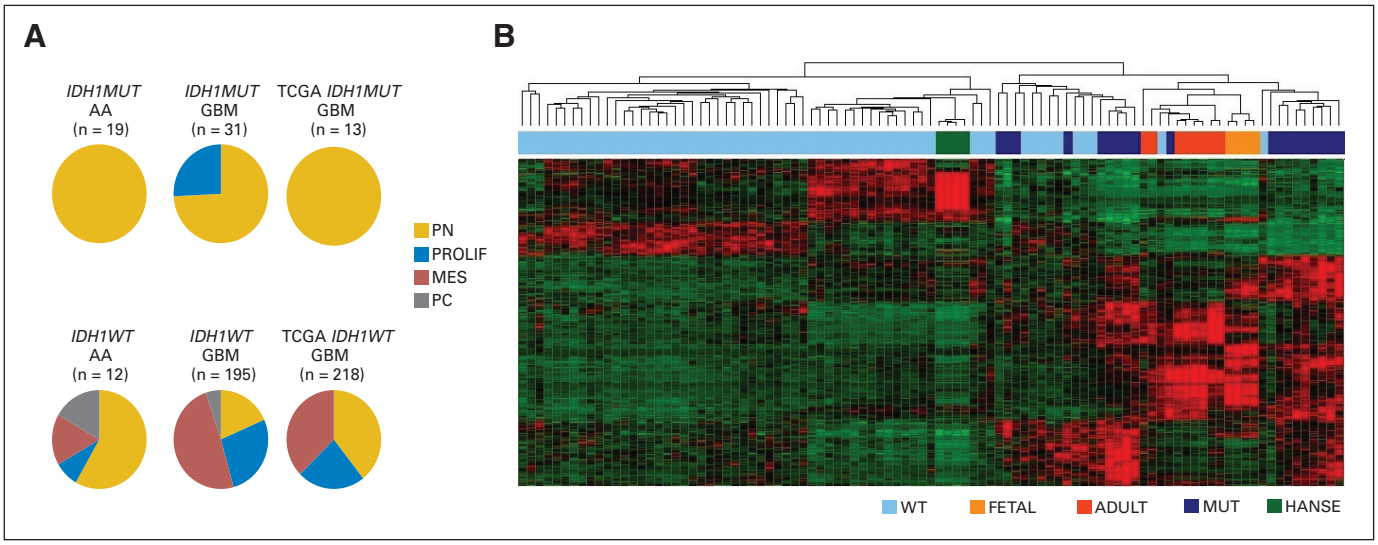

Fig 2. Glioblastomas (GBMs) with mutations in isocitrate dehydrogenase 1 (*IDH1*) at R132 (*IDH1*^{R132MUT}; *IDH1MUT*) are restricted from Mesenchymal gene expression and show greater similarity to transcriptional signatures of brain cells than to those of neural stem cells. (A) Gene expression analysis reveals a robust positive association of *IDH1*^{R132MUT} high-grade astrocytomas with the Proneural signature and an absolute exclusion of the Mesenchymal gene signature from *IDH1*^{R132MUT} tumors. Subtypes are labeled as follows: Proneural (PN), Proliferative (PROLIF), and Mesenchymal (MES). PC denotes poorly classified tumors. Pie charts depict tabulated data for all tumors assigned to subtype. AA, anaplastic astrocytoma (grade 3 astrocytoma) cohort G (Data Supplement); GBM, cohorts A-D, and F (Data Supplement); TCGA, newly diagnosed GBM samples from The Cancer Genome Atlas (TCGA) data set (Cancer Genome Atlas Research Network: Nature 455:1061-1068, 2008). (B) Heatmap of hierarchical clustering of GBMs in cohort C based on gene list derived from most variable genes between *IDH1*^{R132MUT} and *IDH1*^{R132}-wild type (*IDH1*^{R132WT}; *IDH1WT*) GBMs (Data Supplement). *IDH1*^{R132WT} GBMs (WT) show similarity to human adult neural stem cells for experimentation (HANSE), whereas *IDH1*^{R132MUT} GBMs (MUT) show similarity to fetal/adult brain tissue (FETAL, ADULT).

mutation is highest for C>T mutations, we deduce that the mutation pattern observed in tumors with mutations in both *IDH1*^{R132} and *p53*^{R273} is most likely to have occurred by strong selection for *IDH1* and *p53* mutations on the template versus on the coding strands, respectively (Fig 4B). These findings suggest that if both *IDH1*^{R132H} and *p53*^{R273C} mutations occur as C>T mutations in a nonproliferating cell, mutant IDH1 protein will be expressed immediately, whereas mutant p53 protein will not occur until after DNA replication. The predominance of *p53*^{R273C} in the *IDH1*^{R132MUT} tumors contrasts with the preference for *p53*^{R273H} in the *IDH1*^{R132WT} tumors ($P < .005$ with Fisher's exact test).

IDH1^{R132MUT} *GBMs Demonstrate CpG Island Methylator Phenotype, Focal* EGFR *Amplification, and Focal* PTEN *Loss*

To identify other molecular aberrations that may cooperate with *IDH1*^{R132MUT} in gliomagenesis, we performed a CpG island methylation profiling analysis on a subset of *IDH1*^{R132MUT} and *IDH1*^{R132WT} GBMs and found a distinct pattern of CpG island hypermethylation that was detected in all GBMs and lower-grade gliomas with *IDH1*^{R132MUT} but was absent from nearly all *IDH1*^{R132WT} gliomas (Appendix Fig A4B). The methylation pattern in *IDH1*^{R132MUT} GBMs shows similarity to the recently reported CpG island methylator phenotype (CIMP) found to be closely associated with *IDH1*^{R132MUT} gliomas[31] and adds to the previous suggestion that these coordinated methylation changes occur early in the development of *IDH1*^{R132MUT} tumors.

Array comparative genomic hybridization analysis and/or fluorescent in situ hybridization revealed lower frequencies of *PTEN* loss and fewer instances of *EGFR* amplification in *IDH1*^{R132MUT} GBMs versus *IDH1*^{R132WT} GBMs (Appendix Fig A4C; Data Supplement). A more robust finding from fluorescent in situ hybridization, however, was that, within cases showing any evidence of cells with *PTEN* loss or *EGFR* amplification, *IDH1*^{R132MUT} cases showed a significantly lower percentage of affected cells (Appendix Figs A4C and A4D). The focality of copy number alterations for *EGFR* and *PTEN* in the *IDH1*^{R132MUT} GBMs points to the likelihood that these are late events in the evolution of these tumors.

DISCUSSION

The development of novel therapeutic regimens for human malignancies, particularly those involving targeted therapy, is greatly facilitated by methods for identifying clinically meaningful disease subsets. To gain greater understanding of the utility of *IDH1*^{R132MUT} as a marker in diffuse glioma, we have conducted a comprehensive analysis of the features of newly diagnosed cases of primary GBMs arising with and without *IDH1*^{R132MUT}. Our results indicate that, although histologically similar, *IDH1*^{R132MUT} and *IDH1*^{R132WT} GBMs differ in their demographic, anatomic, phenotypic, epigenetic, and genomic presentation and follow a different clinical course, supporting a model of two disease entities that most likely arise from separate cell types of origin as the result of largely nonoverlapping sets of molecular events (Fig 5). Increased understanding of the cell of origin and molecular evolution of *IDH1*^{R132MUT} GBM may aid in development of therapeutic strategies for this tumor type by yielding insights into the biology of these lesions and facilitating the development of animal models.

Our analysis demonstrates that the phenotypic features that distinguish *IDH1*^{R132MUT} from *IDH1*^{R132WT} GBMs include better outcome, predominance of frontal lobe location, presentation and

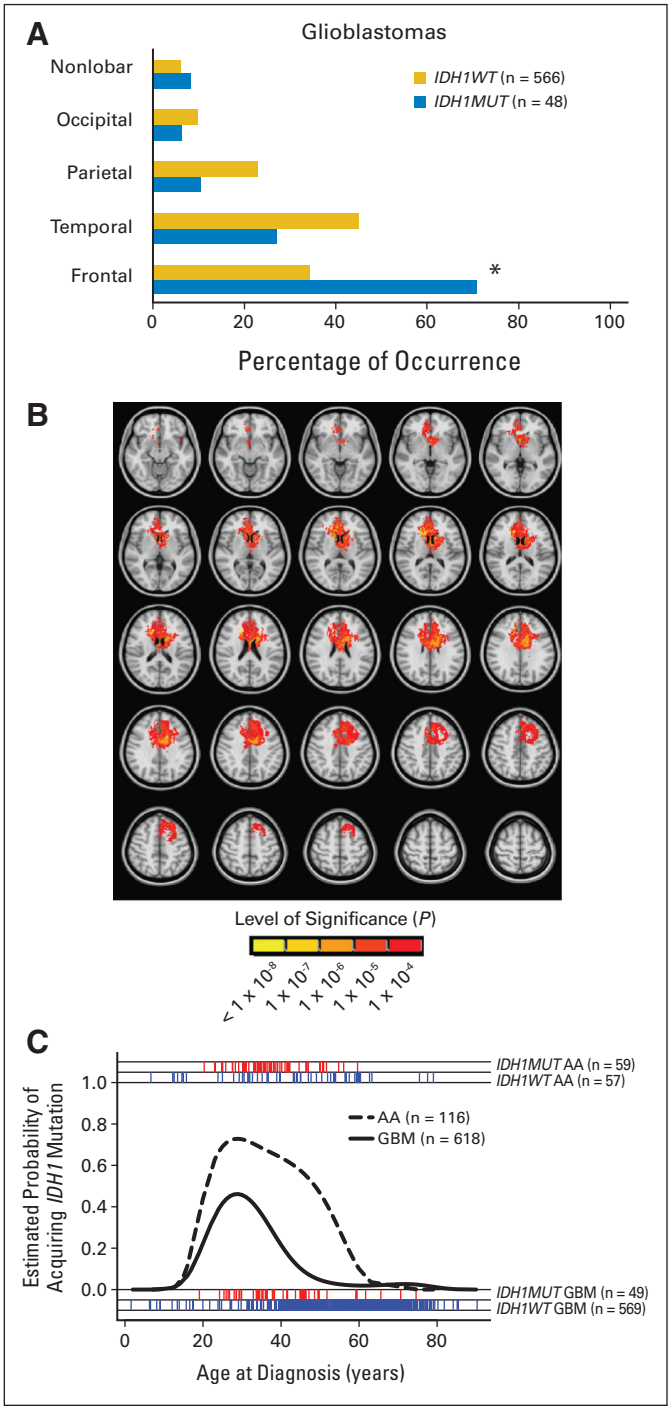

Fig 3. Spatial and temporal restriction of gliomas arising with mutations in isocitrate dehydrogenase 1 (*IDH1*) at R132 ($IDH1^{R132MUT}$; *IDH1MUT*). (A) $IDH1^{R132MUT}$ de novo glioblastomas (GBMs) occur more frequently in the frontal lobe compared with $IDH1^{R132}$-wild type ($IDH1^{R132WT}$; *IDH1WT*) GBMs (*$P <$ .001, two-tailed Fisher's exact test, with Bonferroni correction for multiple comparisons; Data Supplement). (B) Area of differential involvement (ADIFFI) analysis of axial magnetic resonance images. Voxels that are more frequently involved in $IDH1^{R132MUT}$ GBMs can be seen to cluster in the frontal lobe near the rostral extension of the lateral ventricle. All voxels with $P < .0001$ for differential involvement are depicted, as indicated in the color-coded bar. (C). $IDH1^{R132MUT}$ GBMs are temporally restricted. Logistic regression curves show the probability of $IDH1^{R132MUT}$ occuring in anaplastic astrocytomas (AAs; grade 3 astrocytoma) and GBMs as a function of age. The red ($IDH1^{R132MUT}$) and blue ($IDH1^{R132WT}$) tick marks show actual ages of patients (Data Supplement).

maintenance of Proneural expression signature, lesser extent of necrosis and edema, presence of non–contrast-enhancing component, and greater oligodendroglial content. These phenotypic findings, along with our genomic and epigenetic observations, confirm and extend earlier reports that $IDH1^{R132MUT}$ GBMs are distinguished by features associated with lower-grade diffuse glioma and support the contention that all $IDH1^{R132MUT}$ GBMs arise by evolution from lower-grade $IDH1^{R132MUT}$ gliomas, regardless of clinical history.[12,14,15,29,31] Differences in demographics (age and sex) and tumor location in patients with $IDH1^{R132MUT}$ and $IDH1^{R132WT}$ GBM add to the features that suggest different etiologies of the two disease entities.

A key finding of this investigation is the discovery of a constellation of restricted phenotypic, spatial, and temporal features of $IDH1^{R132MUT}$ GBMs that is consistent with origin from a non–stem-cell neural precursor pool. By revealing the exclusion of Mesenchymal signature as an absolute feature of $IDH1^{R132H}$ gliomas, our results extend earlier reports of an association between *IDH1* mutation and Proneural gene expression.[9,32] This observation, and the closer resemblance of $IDH1^{R132MUT}$ GBM transcriptional signatures to those of brain tissue rather than of stem cells suggests that $IDH1^{R132MUT}$ GBM cells do not retain the capability to generate progeny with a broad range of transcriptional profiles.

Although the restriction of $IDH1^{R132MUT}$ GBM gene expression signatures may be a consequence of the actions of $IDH1^{R132MUT}$, taken with the spatial and temporal homogeneity of $IDH1^{R132MUT}$ GBM presentation, this finding suggests that the cell of origin for $IDH1^{R132MUT}$ GBMs is a neural precursor population with limited differentiation potential that is most abundant during a specific stage and location in forebrain development. Our area of differential involvement analysis demonstrates a strong propensity for $IDH1^{R132MUT}$ gliomas to occur in the frontal lobe, specifically in the area surrounding the rostral extension of the lateral ventricles, indicating this region as a likely location of the cell of origin for many $IDH1^{R132MUT}$ gliomas. Consistent with our findings, predominance of frontal lobe location has been reported in oligodendrogliomas with 1p/19q co-deletion,[33,34] a tumor type now known to carry *IDH1* mutation at high frequency.[13,14,29] Previous studies[13,15,16,21,35,36] indicate that although the median age of patients with $IDH1^{R132MUT}$ GBM is younger than that for $IDH1^{R132WT}$ GBM, $IDH1^{R132MUT}$ is rare in the pediatric population. By using logistic regression to overcome the limitations of patient sampling, we were able to determine that the relative probability of a tumor harboring $IDH1^{R132MUT}$ abruptly increases around age 20 and begins to decrease a decade later. This raises the possibility that the cell type of origin for $IDH1^{R132MUT}$ gliomas is most abundant and permissive during a limited developmental time window, possibly coinciding with remodeling of prefrontal cortex in adolescence.[37,38] Our findings that Proneural gene expression and increased oligodendroglial histology are associated with $IDH1^{R132MUT}$ GBMs are consistent with an oligodendroglial progenitor cell type of origin, and several studies lend support for oligodendroglial progenitor cells as a cell type of origin for glioma.[39-44]

Given the strong correlation we confirmed between CIMP and *IDH1* mutation, we propose that CIMP is also an early and critical event in the development of $IDH1^{R132MUT}$ gliomas. Recent studies[45,46] indicating that *IDH1* mutation acts to inhibit a class of alpha-ketoglutarate–dependent enzymes, including proteins that catalyze histone demethylation and hydroxylation of methylated DNA, support the possibility that *IDH1* mutation can initiate

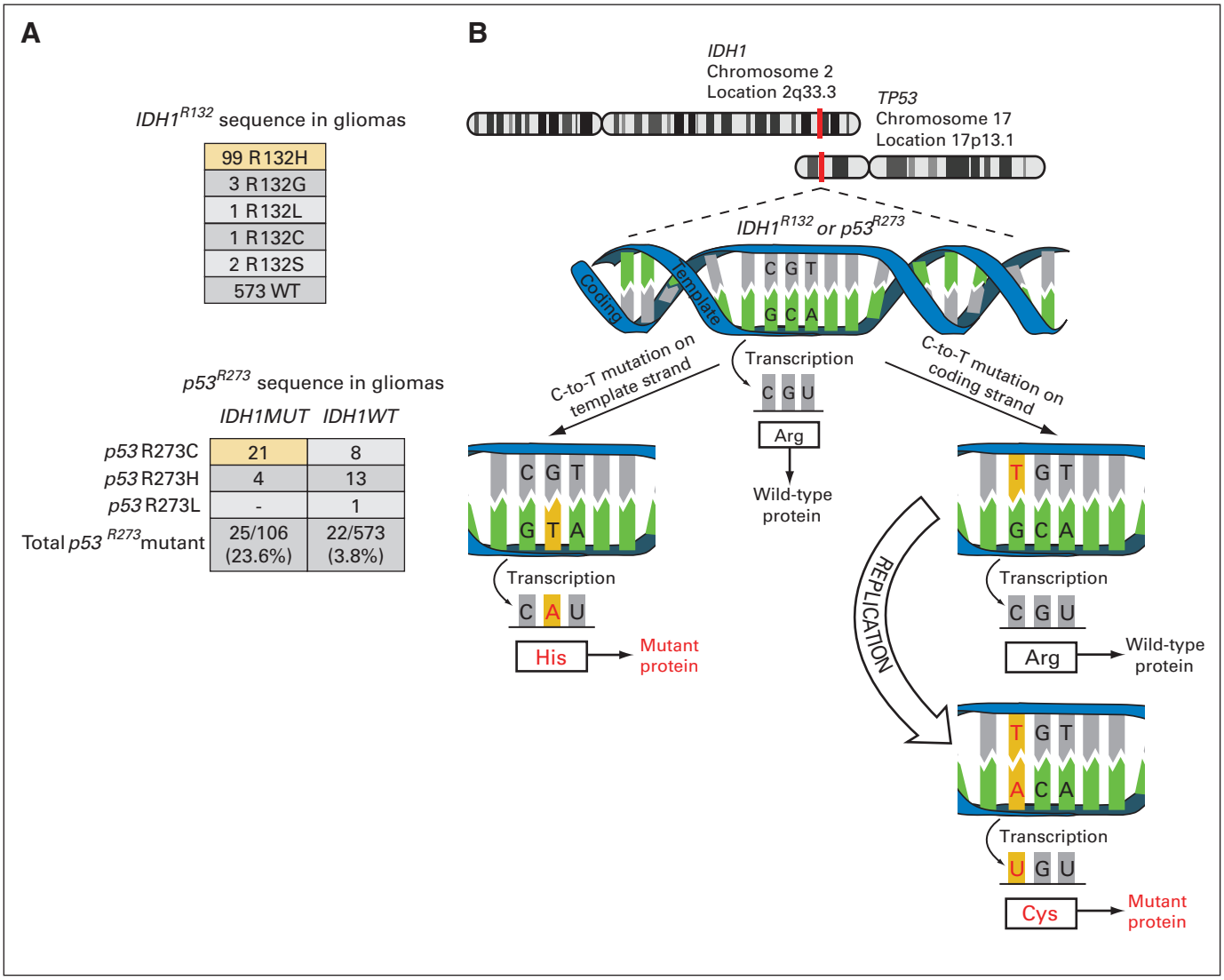

Fig 4. Evidence for ordered appearance of aberrations in isocitrate dehydrogenase 1 (*IDH1*) mutant glioblastoma (GBM). (A) Top: Frequencies of sequences found at $IDH1^{R132}$ show that the predominant substitution is His (yellow box). Bottom: Among gliomas with mutations at $IDH1^{R132}$ (*IDH1MUT*) and $p53^{R273}$, a predominance of Cys substitution at $p53^{R273}$ is seen (yellow box); for $IDH1^{R132}$-wild type (*IDH1WT*) tumors, the preferred substitution at $p53^{R273}$ is His. (B) Schematic diagram showing that *IDH1* mutation on the template strand and *p53* mutation on coding strand can select for the expression of IDH1 mutant protein before p53 mutant protein if replication is delayed.

oncogenesis by inducing an epigenetic block to differentiation in a specific population of CNS cells poised at a particular developmental state. This hypothesis explains the homogeneity in presentation of $IDH1^{R132MUT}$ gliomas and suggests the possibility that these lesions might show sensitivity to therapeutic regimens with differentiating agents.

Several investigations[13,16,18] demonstrate that most $IDH1^{R132MUT}$ astrocytomas harbor mutations of *p53*, and one study[12] revealed instances in which *IDH1* mutation was apparent before the appearance of *p53* mutation. Our findings reveal the existence of a mechanism that is capable of ensuring sequential appearance of IDH1 mutant protein before p53 mutant protein, regardless of the order in which the mutations occur. Specifically, our sequencing data on *IDH1* and *p53* provides evidence for strand asymmetry of CpG mutations,[47] demonstrating a strong preponderance of presumed C>T mutations on template versus coding strand for $IDH1^{R132}$ and $p53^{R273}$, respectively.

Although mutation of *IDH1* on the template strand permits immediate translation of IDH1 mutant protein, mutation of $p53^{R273}$ on the coding strand allows the appearance of p53 mutant protein only after DNA replication. Thus, in a quiescent cell, the pattern of mutations we observed at high frequency in *IDH1* mutant glioma will result in appearance of IDH1 mutant protein to be followed by p53 mutant protein only after a cycle of DNA replication. The preferred substitutions seen in gliomas harboring mutations at both $IDH1^{R132}$ and $p53^{R273}$ stand in stark contrast to the substitution pattern in cancers with mutations in only one of these genes. Specifically, for $IDH1^{R132}$, the preferred substitution in acute myeloid leukemia is Cys,[48-50] and for $p53^{R273}$, the predominant substitution is His[51] (including the $IDH1^{R132WT}$ gliomas in this study). Our finding of the frequent co-occurrence of $IDH1^{R132H}$ and $p53^{R273C}$ suggests that sequential appearance of IDH1 mutant protein before p53 mutant protein may be critical for formation of *IDH1* mutant gliomas.

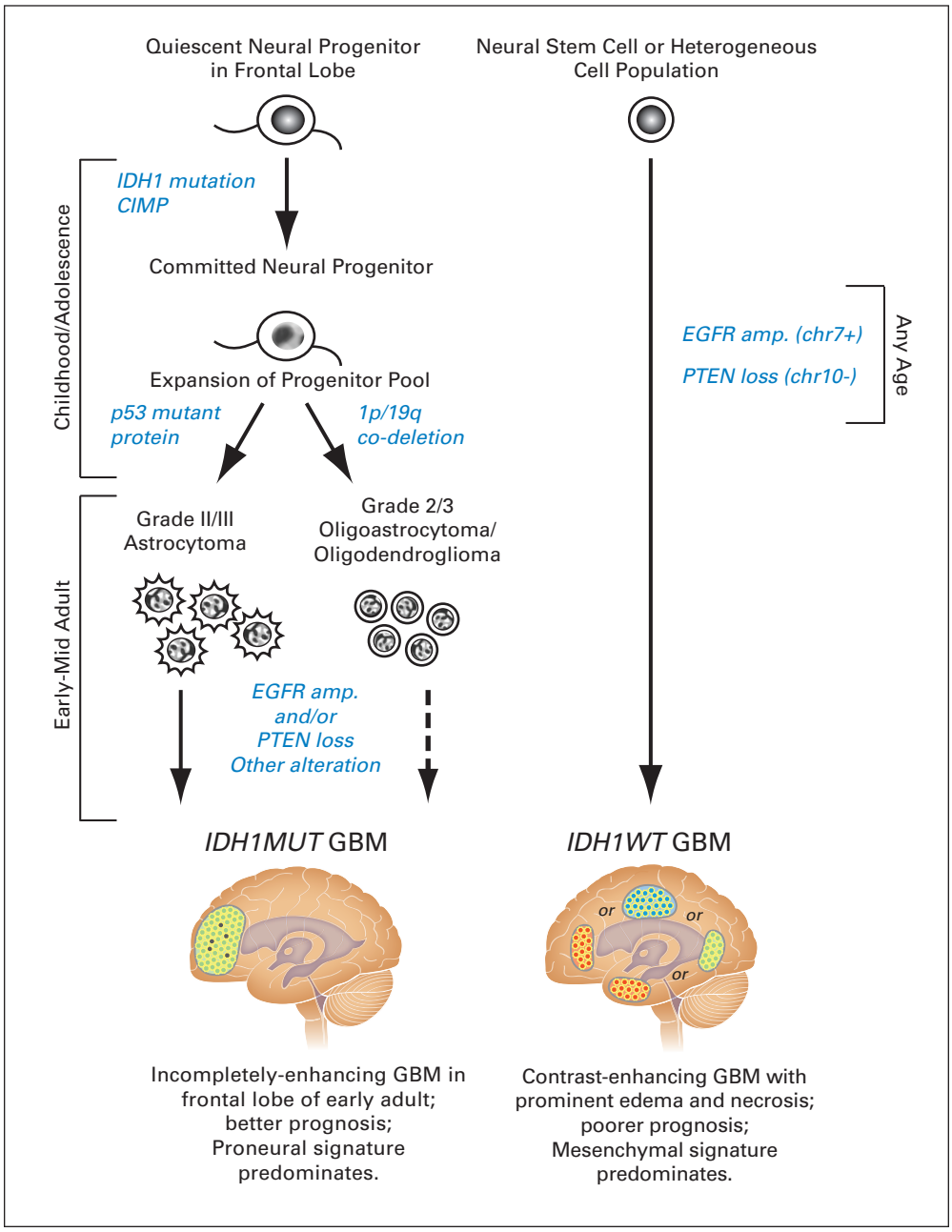

Fig 5. Model comparing glioblastomas (GBMs) arising with and without mutations in isocitrate dehydrogenase 1 (*IDH1*) at R132. We propose that the *IDH1*^{R132MUT} GBM pathway (left) is initiated by the occurrence of *IDH1* mutation and resultant CpG island methylator phenotype (CIMP) in a quiescent neural progenitor residing in the frontal lobe. Although *p53* mutation can be present during this time, expression of p53 mutant protein ensues only after expansion of this progenitor pool during late adolescence or early adulthood. According to the proposed model, glioma formation along the *IDH1*^{R132MUT} pathway requires the ordered appearance of IDH1 mutant protein and CIMP, followed by p53 mutant protein (or 1p/19q co-deletion[11]). Tumors along this pathway arise from a spatially and temporally restricted neural progenitor population and most frequently maintain a Proneural gene expression signature. Transformation to *IDH1*^{R132MUT} GBM requires *EGFR* amplification (amp), *PTEN* deletion, or other genomic alterations. In contrast, in the *IDH1*^{R132WT} GBM pathway (right), *EGFR* amplification, and *PTEN* loss frequently act in concert to drive GBM formation from a cell population that maintains the ability to adopt a Mesenchymal gene expression signature. chr7+, chromosome 7 gain; chr10−, chromosome 10 loss. *IDH1MUT*, *IDH1*^{R132MUT}; *IDH1WT*, *IDH1*^{R132WT}.

Our analysis suggests a model in which *IDH1*^{R132MUT} GBMs arise in a stepwise fashion as the result of a series of sequenced molecular alterations that cooperate with normal developmental events (Fig 5 and Data Supplement). We propose that although *IDH1*^{R132MUT}, induction of CIMP, and *p53*^{R273MUT} often occur in a quiescent neural stem cell, tumors arise only from lineage-committed progeny following a wave of proliferation related to forebrain maturation that triggers appearance of mutant p53 protein and loss of cell cycle control. This oncogenic process results in a low-grade glioma that subsequently acquires additional genomic alterations that promote malignant transformation to GBM. Previous studies[11,14,52] provide strong evidence that *PTEN* loss via loss of chromosome arm 10q is an event that occurs during transition to secondary GBM. Our cytogenetic observations of focal *EGFR* amplification and *PTEN* loss in *IDH1*^{R132MUT}

GBMs are consistent with the proposal that these events occur during the evolution of lower-grade *IDH1*^{R132MUT} glioma to GBM.

In our model, the production of IDH1 mutant protein is the initial event in an orchestrated process that leads to the stepwise emergence of a distinct GBM entity that has a less aggressive clinical course than other GBMs. In contrast to the heterogeneous presentation of most GBMs, *IDH1*^{R132MUT} GBMs arise at high frequency in early adult life as frontal lobe lesions with a constellation of radiographic, histologic, and transcriptional features that relates these lesions to the lower-grade diffuse gliomas from which we contend they arise. This investigation adds to a growing body of data that suggests that histologically similar brain tumors may represent distinct disease entities arising as a result of vulnerability of different stem-cell and progenitor populations to particular oncogenic alterations.[24,53,54] *IDH1*^{R132MUT} glioma may be an especially interesting example of the dependence of oncogenesis on normal developmental processes, because the cell of origin we propose may be uniquely abundant in human brain.

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