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Evidence for age-related contributions of DNA damage and epigenetics in brain tumorigenesis

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Abstract

Glioblastoma (GBM) is a highly malignant primary brain tumour displaying rapid cell proliferation and infiltration. GBM primarily occurs at older age; however, younger populations have also been affected. In GBM and other cancers, genetic and epigenetic alterations promote tumorigenesis causing increased cell proliferation and invasiveness. This investigation explored epigenetic events as contributing factors, especially in gliomas that arise in patients aged 40-60 years. Furthermore, DNA damage in tumours with respect to age was assessed. Archival fixed tissues from 88 cases of glioblastoma and adjacent non-malignant tissues were tested. Global methylation and DNA damage were measured using ELISA detection of 5-methyl cytosine and 8-hydroxy guanine, respectively. *IDH* mutations and *CDKN2* promoter hypermethylation were analysed by pyrosequencing. Tumour tissue was hypomethylated compared with non-malignant tissue ($P = .001$), and there was a trend towards increased methylation with increasing age. There was a significant increase in DNA damage in patients older than forty years compared with those aged forty years or younger ($P = .035$). *CDKN2* promoter methylation levels followed the age trends of global methylation in this patient group. Patients younger than 60 had more frequently mutated *IDH* ($P = .004$). Conclusions: The data support the potential of epigenetic factors in promoting tumorigenesis in younger patients, while increased DNA damage contributes to tumorigenesis in the older patients.

KEYWORDS

Brain cancer, DNA injury, epigenetics, methylation

1 | INTRODUCTION

According to the American Brain Tumor Association, gliomas account for 25% of new primary brain tumour diagnoses and about half of these are glioblastoma multiforme (GBM). Primary GBM and paediatric GBM arise directly from neural stem cells, while secondary GBM goes through stages of astrocytoma.[1] Astrocytoma, the tumour of astrocytic glial cells, is a common type of primary central nervous system neoplasm. Diffuse infiltrating astrocytomas are graded into low-grade diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma (grade IV) in the order of increasing malignancy. Temporal heterogeneity and spatial heterogeneity displayed by these neoplasms appear to affect therapeutic response and relapse. The extensive knowledge that has accumulated about the molecular characteristics of GBM and astrocytoma has raised the possibility for predictable application of new, as well as classical, therapeutic

strategies. Platform trials such as the GBM Adaptive Global Innovative Learning Environment (GBM AGILE; estimated study completion date 2024) have assessed potential biomarkers to confirm effective therapies based on overall survival.[2]

Several tumorigenic pathways have been associated with GBM. Accumulation of DNA lesions as a result of exposure to various DNA damaging agents is generally accepted as one cause of malignancy in neural cells. The DNA damage response in GBM, especially tumour stem cells, promotes tumorigenesis and causes resistance to therapeutic treatment by removing genotoxic lesions caused by alkylating agents.[3] Genetic and epigenetic mechanisms involved in repair of damaged DNA present an increased risk in tumorigenesis and poor outcome in GBM.[4]

The study of oncogenic drivers in different tumour types in specific patient populations contributes to improved therapeutic design and treatment. Genomic studies have expanded knowledge of genetic drivers in gliomas that support tumour classification by histopathology.[5] Outcome studies linking diagnoses with genomic profiles show significant differences based on tumour biomarkers such as *IDH1/2*, *H3F3A*, *BRAF*, *CDKN2A*, and *TERT*.[6] Copy-number abnormalities such as deletions of phosphatase and tensin homolog (*PTEN*) and the alpha thalassaemia/mental retardation X-linked chromatin remodeler (*ATRX*) are found in a significant percentage in primary and secondary glioblastoma.[7] Copy-number changes of the *PTEN* gene have been implicated in tumorigenesis. Deletion/truncation of the myb proto-oncogene-like 1 (*MYBL1*) has also been implicated in tumour formation. Truncated *MYBL1* has been observed to be a recurrent event in diffuse paediatric low-grade gliomas. [8] Fewer recurrent gene copy-number variants have been observed in paediatric low-grade gliomas than adult low-grade gliomas. In contrast to gene-limited variants, an integrated analysis of a thousand paediatric low-grade gliomas showed that rearrangement-driven tumours were diagnosed at a younger age compared with those with genetic variants.[9]

Biomarkers of ageing based on epigenetic data or 'epigenetic clocks' link developmental and maintenance processes to biological ageing.[10] Epigenetic mechanisms such as down-regulation of tumour suppressor genes by promoter methylation have been recognized as contributory to the malignant cell phenotype. It has been hypothesized that epigenetic mechanisms are involved in the decline of DNA repair in brain.[11] A meta-analysis of paediatric high-grade glioma and diffuse intrinsic pontine glioma identified histone-mutant subgroups, while histone wild-type subgroups with particular methylation profiles more closely resembled lower-grade tumours.[12] Genomic aberrations increase with age, highlighting the infant population as biologically and clinically distinct. Uncommon pathway dysregulation is seen in small subsets of tumours, further defining the molecular diversity of the disease, opening up avenues for biological study and providing a basis for functionally defined future treatment stratification. Studies of epigenetic changes in glioblastoma have focused on glioma CpG island methylator phenotype (G-CIMP) and O-6-methylguanine-DNA methyltransferase (*MGMT*), confirming the inactivation of specific genes through methylation. Correlative analyses have demonstrated survival advantages in subtypes of GBM conferred by G-CIMP phenotype and *MGMT* promoter methylation as a predictive biomarker for treatment response in classical subtype GBM.[13,14] GBM is particularly associated with poor prognosis in elderly patients. Increased survival has been observed in patients aged 70 years or younger treated with temozolomide (TMZ) chemotherapy and standard radiotherapy, regardless of *MGMT* methylation status in these patients.[15] Structural chromosome rearrangements can occur as a result of genome instability caused by epigenetic events. High rates of *MGMT* promoter methylation have been reported in oligodendrogliomas and astrocytomas of lower grade, in which they may correlate with deletions on chromosomes 1 and 19 (1p/19q) and isocitrate dehydrogenase (*IDH*) mutations.[16]

Mutations in the *IDH* genes (*IDH1* and *IDH2*) result in generation of an altered gene product, 2-hydroxyglutarate, which contributes to tumorigenesis by altering numerous cellular responses, including genome-wide epigenetic changes that characterize G-CIMP. Genome-wide analysis studies identified specific somatic mutations of isocitrate dehydrogenase (*IDH*) in 70% of secondary glioblastoma.[17] *IDH1* mutations were associated with the G-CIMP phenotype in secondary glioblastoma and recurrent GBM after treatment.[18] *IDH* mutations were also associated with methylation of *MGMT* in glioblastoma.[19]

A protein with potential role in prognosis in glioblastoma is cyclin-dependent kinase inhibitor 2A (*CDKN2A)* located in the *INK4* locus (9p21.3). *CDKN2A* encodes p16, a tumour suppressor protein that works by inhibiting the cell progression through the G1 cell cycle checkpoint.[20] In normal cells, p16 protein expression increases with age and is considered responsible for mounting cellular senescence resulting in phenotypic manifestations of ageing. Decreased expression of p16 in high-grade gliomas has been reported, suggesting a role of its loss in aberrant survival of tumour cells.[21] GBM studies related to *CDKN2A* were not always conclusive, however. Studies regarding promoter methylation of *CDKN2A* in gliomas found low rates of methylation in astrocytoma and variant among different grades of gliomas.[22] A significant negative correlation between *CDKN2A* promoter methylation and age has been observed with younger patients (<30 years old) having a higher frequency of methylated *CDKN2A* promoter than older patients.[23] Another study conducted on DNA methylation in mice, however, found significantly increased gene promoter methylation with ageing, indicating the loss of expression due to accumulated methylation in the ageing brain.[11]

To test a model of age-related differences in brain tumorigenesis, epigenetic events (genomic methylation and

demethylation), DNA damage, *PTEN* deletion, *IDH* mutation and *MGMT* methylation status in GBM were investigated with different ages of onset. Further, epigenetic regulation of *CDKN2* (p16) and DNA damage was assessed in these tumours.

2 | MATERIALS AND METHODS

Archival tissue samples from 88 patients with confirmed GBM (grade IV) and anaplastic astrocytoma (grade III [24]) were utilized. This study was approved as an exempt archival investigation by the Rush University Medical Center Institutional Review Board (IRB L01122004).

DNA and RNA were extracted from formalin-fixed glioma sections embedded in paraffin and used alongside previously extracted DNA from clinical studies. The cases ranged from ages 19-79 years at diagnosis (Table 1). In addition, nine samples of adjacent non-malignant tissues uninvolved from the tumour were included for normal controls. The nucleic acids isolated from these tissues were archived at −80°C and stored at −20°C for the duration of the study.

2.1 | Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assays (ELISAs) were used to determine global methylation and DNA damage of patient samples relative to positive controls. Global methylation was determined using MethylFlash Methylated DNA Quantification Kit (Epigentek) according to the manufacturer's instructions. The non-competitive assay detects 5-methylcytosine (5-mC) using specific antibodies and colorimetric detection with enzyme-linked secondary antibodies. Quantification of the methylated fraction of the DNA was performed by reading the absorbance on a SpectraMax plate reader (Molecular Probes) at 450 nm. Positive and negative controls supplied by the manufacturer were included in the procedure. Absorbance data were entered in the following equation to calculate the relative amount of 5-mC:

$$5-\mathrm{mC}\% = \frac{(Sample\ OD - ME3\ OD) \div S}{(ME4\ OD - ME3\ OD) \times 2 \div P} \times 100\%$$

where OD is optical density obtained from the microplate reader, ME3 is the supplied negative control, ME4 is the supplied positive control, S is the amount of input sample DNA in ng, and P is the amount of input positive control (ME4) in ng.

DNA damage was quantified using EpiQuik 8-OHdG DNA Damage Quantification Direct Kit (Epigentek) in a similar manner as described above. This colorimetric procedure detects the presence of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a representative indicator of oxidative DNA damage. In this assay, 8-OHdG was quantified from the absorbance readings at 450 nm. Manufacturer-supplied positive and negative controls were included in the procedure. The relative amount of 8-OHdG was calculated using the following formula:

$$8-\mathrm{OHdG}\% = \frac{(Sample\ OD - NC\ OD) \div S}{(PC\ OD - NC\ OD) \div P} \times 100\%$$

where OD is the optical density obtained from the microplate reader, NC is the negative control, PC is the positive control, S is the amount of input sample DNA in ng, and P is the amount of input positive control in ng.

2.2 | *CDKN2* promoter methylation

Methylation at CpG sites within the −64 to −40 (A of ATG of the start of translation = +1) region of the *CDKN2* promoter was analysed by pyrosequencing of bisulphite-converted DNA. For this procedure, DNA was bisulphite-converted using EZ DNA Methylation Kit (Zymo Research), according to the manufacturer's protocol. This treatment converts unmethylated cytosine (C) to uracil (U), while methylated cytosine remains unchanged.

Bisulphite-converted DNA (5 μL) was amplified using Hot Start Taq Master Mix Red (Dot Scientific Inc) on a Veriti

TABLE 1 Overview of study sample demographic data

	Number	Percent	<60 years	>60 years
Gender				
Males	43	48.9%	27	16
Females	45	51.1%	34	11
Tumour type[a]				
GBM	49	56.3%	28	21($P = .007$)
Astrocytoma	38	43.7%	32	6
Age				
≤60 years	60	68.2%		
>60 years	28	31.8%		

[a]One tumour was reclassified as astrogliosis.

Thermal Cycler (Applied Biosystems-Life Technologies) according to the package instructions. Every PCR run included a reagent blank. Amplification of the *CDKN2* region was confirmed by gel electrophoresis, using a 2% agarose gel. Methylation analysis of the *CDKN2* promoter was carried out on a PyroMark Q24 pyrosequencer (Qiagen) following the manufacturer's instructions.

2.3 | Clinical testing

MGMT promoter methylation and *IDH* mutation results were collected from clinical test reports. *MGMT* promoter methylation analysis was performed at a commercial reference laboratory using methylation-specific PCR (MSP) of bisulphite-converted DNA *IDH* mutations were detected in-house using pyrosequencing.

2.4 | Statistical analysis

Statistics were performed on SPSS software (version 23) and Microsoft Excel. DNA damage, global methylation and *CDKN2* promoter methylation data were analysed as non-parametric variables based on histogram observation. Continuous variables of age, relative DNA damage, relative global methylation and *CDKN2* promoter methylation levels were dichotomized or otherwise grouped for the Kruskal-Wallis test analysis. Ages were dichotomized into ≤60 year and >60 year groups. To further observe the distribution of genomic methylation, DNA damage and mean *CDKN2* promoter methylation across all ages, samples were grouped in age categories (≤40, 41-60 and >60 years). The chi-square analyses were used to compare categorical values of *MGMT* promoter methylation (yes/no) and of *IDH* gene mutations (yes/no) as dichotomized data.

3 | RESULTS

3.1 | Genomic methylation

Genomic methylation in high-grade gliomas with regard to age, type and gender was assessed. Methylation (5-mC) relative to internal control was measured by ELISA.

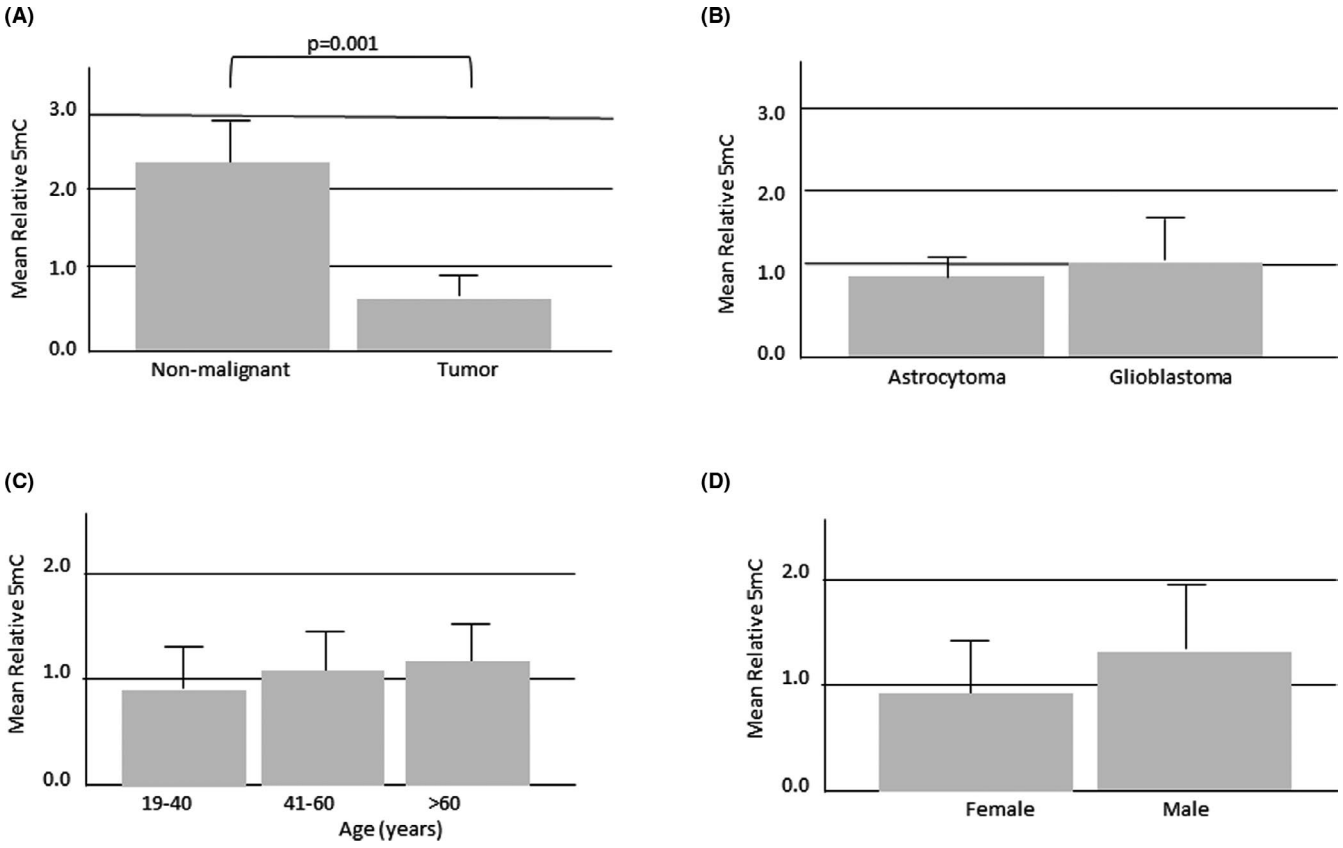

FIGURE 1 A, DNA methylation quantified by ELISA. Tumour tissue was hypomethylated compared with non-malignant tissue (N = 81, N = 14, respectively). B, There was no significant difference in global methylation in astrocytoma vs GBM ($P = .241$). Genomic DNA methylation vs age C, and gender D, in 107 glioma tumour samples showed a trend of increased methylation was observed with increasing age. There was no significant difference in genomic methylation with regard to gender, although males had slightly higher methylation levels ($P = .270$)

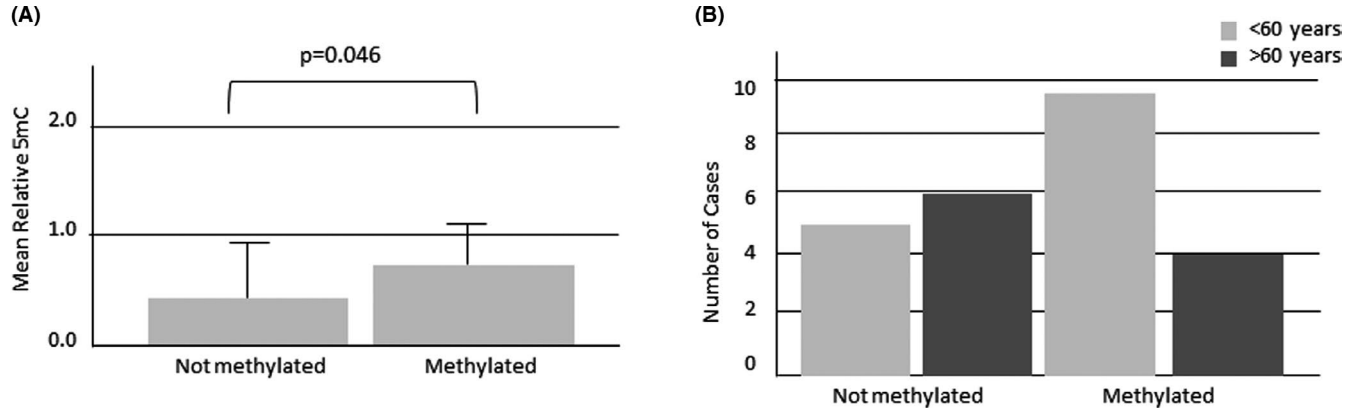

FIGURE 2 *MGMT* promoter methylation was previously analysed by methylation-specific PCR for clinical assessment. *MGMT* data were available from 32 cases. A, Genomic DNA methylation was increased in the presence of *MGMT* promoter hypermethylation. B, Methylated *MGMT* was present in 62.5% of those aged ≤60 compared with 45% of those aged >60

A Mann-Whitney statistic revealed a significant decrease in global DNA methylation in tumours compared with non-malignant brain tissue ($P = .001$; Figure 1A). Methylation levels were similar with regard to tumour type ($P = .241$) with decreased 5-mC in the ≤60 cases of both tumour types (astrocytoma, $P = .077$; and GBM, $P = .264$; Figure 1B).

A trend of increased methylation was observed with increasing age (Figure S1A). Methylation levels were highest in patients older than 60 (mean rank 41.62) and lowest in patients younger than 40 (mean rank 33.48; Figure 1C). Statistical analysis of the correlation between genomic methylation and gender showed no significant difference between genders ($P = .270$; Figure 1D).

3.2 | *MGMT* promoter methylation

MGMT promoter methylation is routinely assessed for treatment strategy in glioma.[23] Gliomas resistant to alkylating agents can be sensitized to temozolomide chemotherapy through promoter-mediated epigenetic silencing of the *MGMT* gene. *MGMT* promoter methylation is particularly relevant for elderly patients (>60 years), who may have decreased tolerance for combined aggressive chemoradiation. Data from clinical *MGMT* promoter methylation tests performed by methylation-specific PCR were used for comparisons in this patient group. *MGMT* promoter methylation frequency was similar in astrocytoma and GBM ($P = .815$). Comparison of global methylation to *MGMT* promoter methylation status revealed an increase in genomic methylation associated with *MGMT* hypermethylation ($P = .046$; Figure 2A). In this patient group, however, *MGMT* promoter hypermethylation was present in 62.5% of younger (≤60 years) cases compared with 45% of cases (>60 years; Figure 2B).

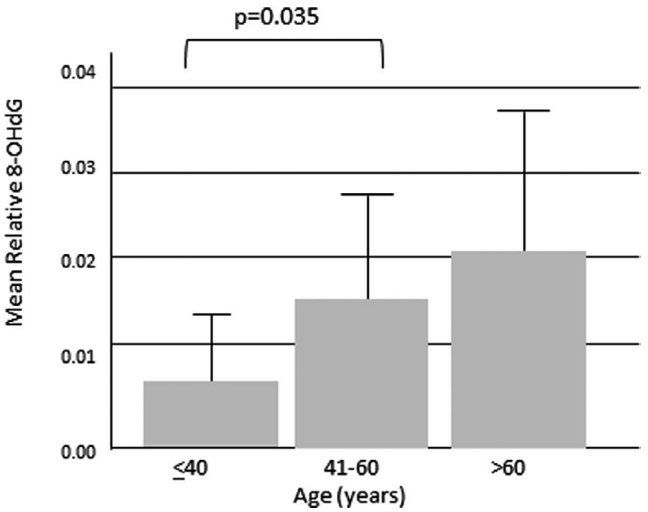

FIGURE 3 DNA damage vs. age. Average relative DNA damage increased with age in 76 samples tested (N = 21, 28 and 27 samples per group, respectively). A Mann-Whitney test showed a statistically significant difference between the ≤40 group and 41-60 group

3.3 | DNA damage

Oxidative DNA damage was measured by the presence of 8-OHdG in the different age groups. There was increased DNA damage in older patients (>60) compared with the younger group (≤60), but the difference did not reach significance. Further division of the cases into three age groups showed a trend to higher DNA damage with increasing age (Figure S1B). A Mann-Whitney test showed a statistical significance difference between the <40 group and 41-60 group ($P = .035$; Figure 3).

When DNA damage was compared between non-malignant and tumour tissue, no difference in the degree of DNA damage was observed between the two types of tissue in this patient group, nor was there any relationship between DNA damage and gender ($P = .407$). There was no

relationship between DNA damage and global methylation ($P = .386$). DNA damage was marginally higher in cases with methylated *MGMT* ($P = .113$).

3.4 | *CDKN2* promoter methylation

Promoter methylation levels of the *INK4* locus and DNA damage may accumulate in ageing tissues, including brain. As with *MGMT* methylation and DNA damage, the analysis of average *CDKN2* promoter methylation showed no significant difference between tumour and non-malignant tissue ($P = .440$) nor between tumour types (astrocytoma vs GBM, $P = .706$).

CDKN2 promoter methylation trended higher with age (Figure 4A). This is consistent with previous observations of *CDKN2* promoter methylation in lung cancer.[25,26] Cytosine positions −53 to −59 showed the greatest age-related effect (Figure 4B). Interestingly, methylation of the cytosine position −49 showed the greatest trend with increasing age (Figure S2).

3.5 | *IDH* mutations

Mutations in *IDH* have been identified in over 70% of lower-grade gliomas and secondary glioblastoma and are associated with a more favourable prognosis. Assessment of *IDH* mutation status may assist in tumour classification and provide prognostic information. *IDH* mutations were more frequently found in astrocytoma (48.1%) vs GBM (15.2%; $P = .021$), consistent with known mutation frequencies in these tumour types: GBM (~5%–10%) and astrocytomas (~50%-70%). Analysis of *IDH* mutations with age showed a significant correlation between the two age groups (0-60, >60) with the younger group presenting preponderantly with a mutated *IDH* ($P = .004$; Figure 5A). DNA damage and global methylation showed no significant difference with regard to

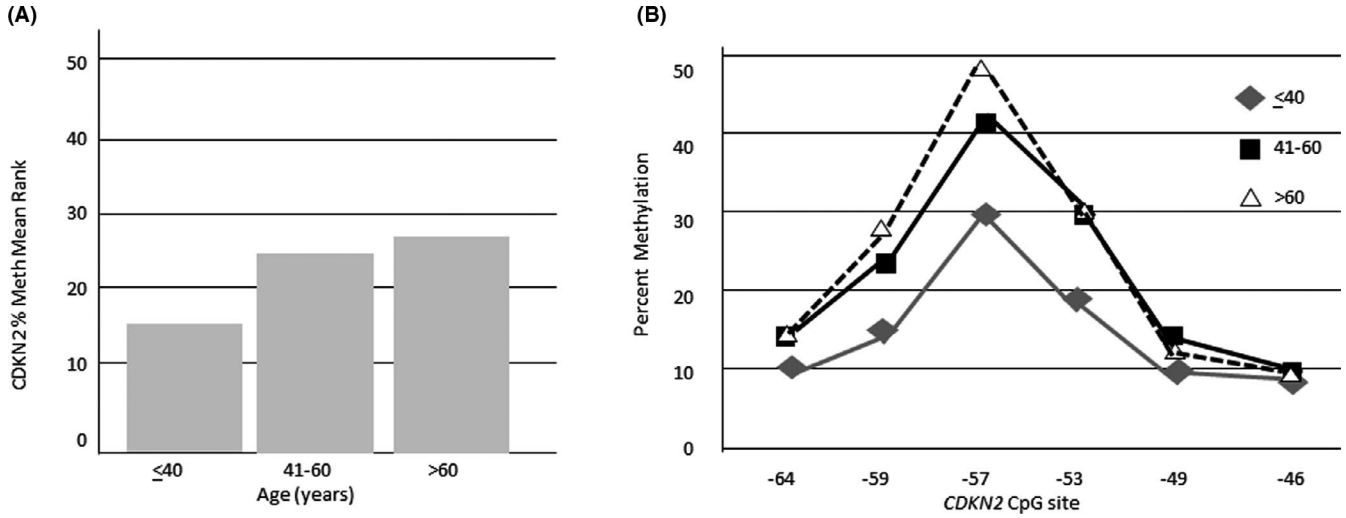

FIGURE 4 A, *CDKN2* promoter methylation vs age in 41 glioma tumour cases (A; N = 10, 16 and 13 per group, respectively). Non-parametric analysis shows that *CDKN2* promoter methylation levels follow the age trends of global methylation and DNA damage in this patient group (Kruskal-Wallis mean rank 13.40, 20.39 and 20.95, respectively). B, Methylation levels across the promoter sites have the same pattern in different age groups

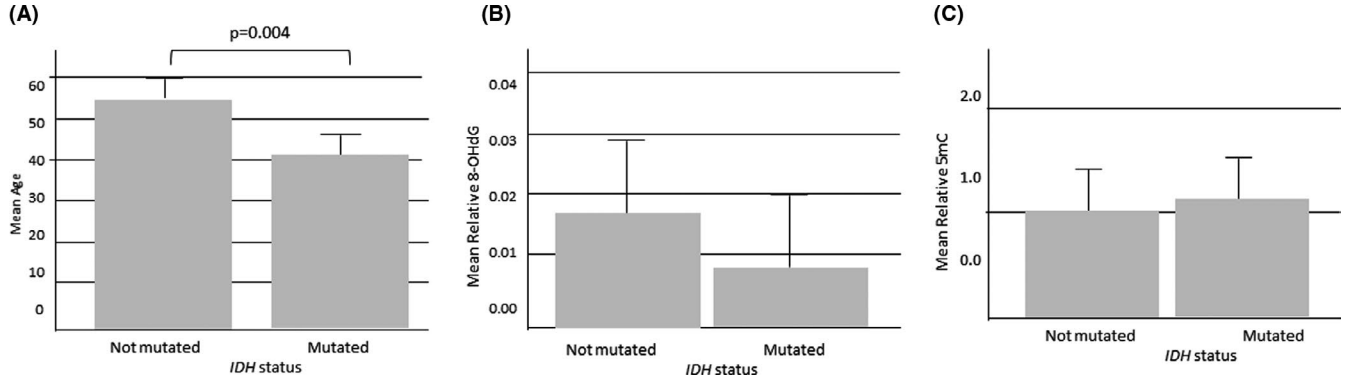

FIGURE 5 Age A, DNA damage B, and global methylation C, vs. *IDH* mutation status. *IDH* mutation status measured for clinical assessment was available for 66 cases. 'Yes' mutation included all *IDH1* R135 codon mutations

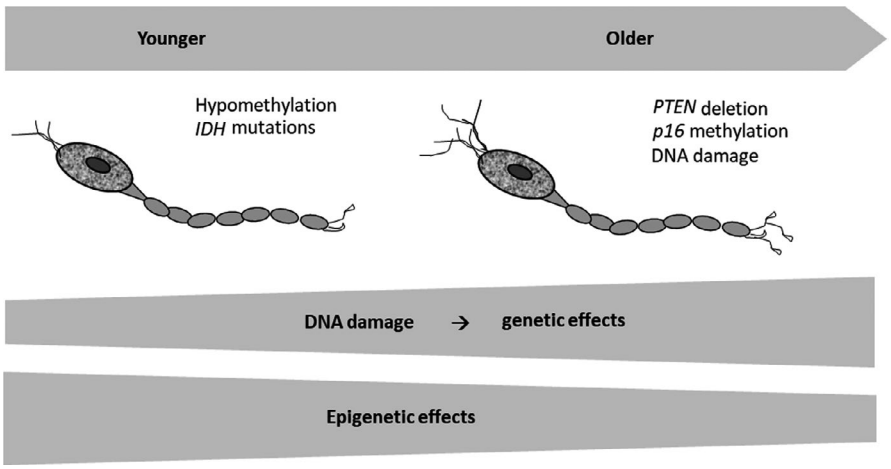

FIGURE 6 Model of epigenetic vs genetic events with respect to age. Younger tumours showed epigenetic events such as genomic hypomethylation, which can lead to chromosomal instability and increased *IDH* variants. DNA damage and genetic mutations such as *PTEN* loss of heterozygosity tended to be higher in the older group

IDH mutation status (Figure 5B, C; $P = .330$ and $P = .222$, respectively).

3.6 | *PTEN* deletions

PTEN acts as a 3'-specific phosphatidylinositol phosphatase inhibiting the PI3K pathway. Deletions of 10q23 occur frequently in glioblastoma and other cancers. In the current patient group, *PTEN* deletions were found in less than 10% of cases ≤40 years compared with 60% of cases older than 40 (N = 11 and 25, respectively; $P = .013$). FISH studies have shown frequent 10q23/*PTEN* deletion in patients older than 45 years ($P = .034$) with the median age of patients harbouring *PTEN* deletions significantly higher than those with the retained status ($P = .019$; Figure 6).[24]

4 | DISCUSSION

This study addressed genetic and epigenetic characteristics of brain cancer with regard to patient age. The tumour types included astrocytoma and glioblastoma multiforme. Comparison of genomic methylation, DNA damage and *CDKN2* promoter methylation was generally similar in the two tumour types. *IDH* mutations, which may inhibit demethylation of DNA (leading to hypermethylation), were more frequent with younger age in this study. This may have been related to 40% of the younger (1-60 years) group being astrocytoma, compared with 20% of the older group (chi-square, $P = .050$). *MGMT* promoter methylation frequency was higher in the younger age group.

Genetic abnormalities can result from exposure to chemical or physical agents that damage DNA. The results showing increased oxidative DNA damage with older patient age at diagnosis might be expected if DNA damage accumulates with time. The higher presence of DNA damage in older cases (Figure S1B) increases the opportunity for genetic abnormalities in these cases compared with the younger cases. The malignant phenotype might arise in the older cases due to the accumulating mutation load and abnormalities in tumour suppressor genes such as the *PTEN* deletion. In the current patient group, *PTEN* deletions were observed more frequently in older cases compared with younger cases ($P = .013$). *PTEN* deletions have been found frequently in GBM and associated with shorter survival in patients older than 45 years ($P < .05$).[27] This supports the clinical importance of loss of *PTEN* in elderly patients.

Genomic hypomethylation and local hypermethylation of CpG islands have been observed in a variety of tumour types.[28] With regard to tumorigenesis, these events may precede genetic changes.[29] Epigenetic events such as DNA hypomethylation may promote chromosomal abnormalities as those seen in young brain tumours. Chromosomal abnormalities such as deletions in chromosomes 1 and 19 (1p/19q deletion) that were not included in the current study have been associated with better therapeutic response and patient survival in oligodendrogliomas.[30] In a study of anaplastic oligodendrogliomas, 1p/19q deletions were significantly more frequent in younger patients than in older patients (>60 years of age).[31]

The genomic methylation assessment of glioblastoma in this patient group revealed significant hypomethylation in high-grade glioma compared with non-malignant tissue. Lower levels of methylation seen in glioblastoma samples were weighted into the younger group with increasing methylation levels with advancing age (Figure S1A). A global hypomethylated state associated with glioma risk was observed in a previous study in leucocytes.[32] Although regression analysis of increased genomic methylation levels with increasing age did not reach significance by regression analysis, the trend is consistent with hypomethylation in the younger age group, These data provide further evidence regarding the role of genomic hypomethylation in glioblastoma, especially in young patients.

MGMT methylation is frequently observed in glioblastoma and has been correlated with the G-CIMP phenotype.[14] *MGMT* encodes a repair enzyme that removes alkyl groups from DNA. The loss of this alkyl removal when the *MGMT* promoter is hypermethylated enhances the therapeutic activity of alkylating agents such as temozolomide compared with tumours with hypermethylation of the *MGMT*. Approximately half of the patient group studied here (56%) had *MGMT* promoter methylation.

In the current study, *MGMT* methylation was associated with increased overall genomic methylation. Although the role of *MGMT* promoter methylation in brain cancer is most well known as a therapeutic target, its contribution to the tumour cell malignancy has not been determined. With regard to age, *MGMT* promoter methylation was similar in the younger (1-60) and older >60 groups despite hypomethylation in the younger cases. Since both comparisons (age vs *MGMT* promoter methylation and genome methylation vs *MGMT* promoter methylation) approached significance, data from additional samples are required to confirm these associations.

Alpha-ketoglutarate, the product of isocitrate dehydrogenase, is required for enzymes that remove methyl groups from cytosine residues in DNA (demethylation).[33] *IDH* mutations can lead to decreased demethylation and are associated with transcriptional silencing. Recurrent H3F3A mutations affecting two critical amino acids of histone H3.3 (K27 and G34) have been suggested to define epigenetic subgroups of GBM with distinct global methylation patterns that are mutually exclusive with *IDH1* mutations. Three further epigenetic subgroups were observed to be enriched for adult GBM genetic events.[34] In the current study, *CDKN2* promoter methylation trended higher with age (Figure S2). The observations from analysis of *IDH* mutations and the correlation between age, hypermethylation and *CDKN2* expression suggest an epigenetic pathway in glioma tumorigenesis. A classical model would show an increased promoter methylation due in part to the mutated *IDH* (decreased demethylation). Promoter hypermethylation would subsequently lead to lower expression of p16 protein; however, no significant decrease in *CDKN2* expression measured by qRT-PCR (nor *CDKN2* promoter methylation) was observed with *IDH* mutations. This might be explained by a coincidental occurrence; the mutated *IDH* could be affecting a variety of genes.

There are admitted limitations to the current study. Comparisons of malignant tissue with 'normal brain' tissue were confounded by the limited availability of normal brain tissue. The non-malignant tissues used in this study were adjacent to the tumour tissue in the paraffin samples. Therefore, comparison with non-malignant tissue is subject to a 'field effect'. The tumour can influence the surrounding 'non-malignant' area, or the non-malignant area may be in a precancerous state. Such effects could potentially skew the statistical analysis. Since the results of global methylation show a significant difference between the malignant and non-malignant tissues, the field effect is not complete for all aspects of the malignant epigenetics. The 'premalignant' tissue might share particular characteristics with the tumour cells, while not yet showing the genomic hypomethylation state.

Tumour and tissue heterogeneity might also be considered. Different gene mutations have been associated with GBMs in separate anatomic compartments, possibly reflecting different cellular origins.[35] The current analyses were performed on tumours occurring in specific brain locations. The heterogeneity of cell types present in the different regions of the brain may present distinct methylation profiles and respond differently to stress factors that would induce DNA damage.

The observations in this patient group suggest a model of the role genetic and epigenetic events in the brain cancer cells (Figure 6). Increased DNA damage in the older cases can lead more to genetic abnormalities such as mutations and gene deletions. In contrast, cells from younger cases without as much DNA damage may suffer from hypomethylation leading to chromosomal instability and loss or greater demand for DNA repair, exacerbated by other epigenetic events such as specific *MGMT* promoter hypermethylation. Such tumour suppressor gene promoter hypermethylation in the context of intergenic hypomethylation is commonly seen in tumour cells compared with normal cells.[35,36]

5 | CONCLUSIONS

The data support a theory that brain cancer may develop along different pathways in younger vs older populations. While DNA damage accumulates with age, leading to tumorigenic gene mutations, young tissue may be more subject to epigenetic events, leading to genomic instability and aberrant gene expression. Although numerous studies focus on the impact of accumulated DNA damage on tumour suppressor genes, further more precisely controllable studies on the direct effects of genomic hypomethylation and gene promoter hypermethylation and *IDH* mutations in cell lines may provide better understanding of the framework of molecular mechanisms involved in these epigenetic systems.

CONFLICTS OF INTEREST

The authors have no conflicts of interest. The authors have no financial interests in any company or institution that might benefit from their publication nor any other potential competing interests of a personal nature that might be considered relevant to their publication.

AUTHORS' CONTRIBUTIONS

AT and LB performed conceptualization, contributed to methodology and reviewed and edited the manuscript. AT

performed data curation and formal analysis, underwent investigation and wrote the manuscript. LB contributed to funding acquisition.

ETHICAL APPROVAL

This study was approved by the Rush University Medical Center Institutional Review Board as a study of archival investigation from de-identified tissue samples (IRB L01122004).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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