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Defining the molecular features of radiation-induced glioma: A systematic review and meta-analysis

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Abstract

Background. Cranial radiation therapy is essential in treating many pediatric cancers, especially brain tumors; however, its use comes with the risk of developing second malignancies. Cranial radiation-induced gliomas (RIGs) are aggressive high-grade tumors with a dismal prognosis, for which no standard therapy exists. A definitive molecular signature for RIGs has not yet been established. We sought to address this gap by performing a systematic review and meta-analysis of the molecular features of cranial RIGs.

Methods. A systematic review of the literature was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Articles and case reports that described molecular analyses of cranial radiation-induced high-grade gliomas were identified and evaluated, and data extracted for collation.

Results. Of 1727 records identified, 31 were eligible, containing 102 unique RIGs with molecular data. The most frequent genetic alterations in RIGs included *PDGFRA* or *TP53* mutations, *PDGFRA* or *CDK4* amplifications, and *CDKN2A* deletion, along with 1q gain, 1p loss and 13q loss. Of note, mutations in *ACVR1*, *EGFR*, *H3F3A*, *HIST1H3B*, *HIST1H3C*, *IDH2*, *SMARCB1* or the *TERT* promoter were not observed. A comparative analysis revealed that RIGs are molecularly distinct from most other astrocytomas and gliomas and instead align most closely with the pedGBM_RTK1 subgroup of pediatric glioblastoma.

Conclusions. This comprehensive analysis highlights the major molecular features of RIGs, demonstrates their molecular distinction from many other astrocytomas and gliomas, and reveals potential genetic drivers and therapeutic targets for this currently fatal disease.

Key Points

- A comprehensive meta-analysis of the molecular features of radiation-induced glioma.
- Radiation-induced gliomas are genetically distinct from most other brain tumors.
- Radiation-induced gliomas share many genetic features with pedGBM_RTK1 pediatric glioblastoma.

Importance of Study

Investigations into the genetic features of radiation-induced gliomas (RIGs) are few and are limited by the small number of cases in each study. Consequently, a definitive molecular signature for RIGs has not yet been established, highlighting a gap in the knowledge of potentially actionable drivers of this aggressive disease. To our knowledge, this is the most comprehensive systematic review and meta-analysis of the genetic features of cranial RIGs. This study identified recurrent molecular alterations in these tumors and demonstrated that they are molecularly distinct from many other brain tumor types that they are commonly diagnosed and treated as. Despite most RIGs being diagnosed during adulthood, we identified that RIGs share the largest genetic overlap with the pedGBM_RTK1 subtype of pediatric glioblastoma, which may have implications for future clinical management. These findings reveal that molecular classification of RIGs may complement existing tools for pathological diagnosis of these tumors in the future.

Cranial radiation therapy is a key treatment modality for many pediatric cancers, particularly brain tumors where radiotherapy is routinely delivered to the brain or entire craniospinal axis. Cranial or craniospinal irradiation is associated with a myriad of significant long-term complications[1,2] including the development of second malignant neoplasms.[3–5] The cumulative risk of developing a brain tumor following cranial radiation therapy ranges from 0.5% to 2.7% at 15 years.[6] A recent study of 1294 medulloblastoma patients treated with radiation therapy between 1973 and 2014 reported that these patients developed second central nervous system (CNS) tumors at 40 times the rate expected in the general population.[4] Furthermore, several large cohort studies have demonstrated a direct correlation between the cumulative dose of radiation received and the risk of subsequent CNS tumor development.[5,7,8] This is particularly pertinent for young children, as those under 5 years of age are more susceptible to the development of radiation-associated gliomas compared with children receiving radiotherapy at a later age.[5]

Ionizing radiation directly damages DNA by inducing both single- and double-strand breaks, with the latter being the most deleterious.[9] Indirect DNA damage can also occur via radiolysis of water molecules which produces reactive oxygen species, in turn causing single-strand breaks and other alterations to DNA[10,11] (Figure 1). Imperfect repair of this damage can result in point mutations, gene fusions, large-scale deletions or translocations, all with the potential to activate oncogenes or inactivate tumor-suppressor genes. These changes are often associated with ongoing genomic instability and thus an increased risk of developing cancer.[9,11,12] In the case of radiation-induced second malignancies, genomic instability is thought to persist for multiple generations of cells over many years prior to oncogenic transformation, resulting in a significant latency period between the exposure event and the development of radiation-induced cancer.[12]

A seminal study by Cahan et al[13] established a widely accepted set of criteria that define radiation-induced malignancies. These are: (1) the tumor must arise within the irradiated field, (2) a sufficient latency period must have passed between the time of irradiation and the development of the second tumor (measured in years), (3) the second tumor must be histologically distinct from the primary tumor, and (4) the patient must have no genetic history of cancer predisposition (eg, Li-Fraumeni Syndrome or Neurofibromatosis).

The most common radiation-induced CNS neoplasms following radiation treatment for childhood cancer are gliomas and meningiomas.[5,8,14] The survival rates for patients that develop glioma following cranial radiation treatment are far poorer compared with those that develop meningioma, with a 5-year relative survival rate of just 4% for radiation-induced gliomas (RIGs) compared with 77%–84% for radiation-induced meningiomas.[6,15] For this meta-analysis, we have focused on the more aggressive cranial RIGs.

A comprehensive epidemiological meta-analysis of patients diagnosed with RIG[16] showed that the most frequent primary tumors were hematological malignancies (35%), followed by medulloblastoma (13%) and pituitary adenoma (12%). The median overall survival following RIG diagnosis was just 11 months, highlighting the aggressive nature of these cancers. The median overall latency of disease onset is approximately 9 years between radiotherapy for the primary lesion and diagnosis of the RIG.[5,17] Over 50% of RIGs are diagnosed during adulthood,[16] and arise more frequently in patients who received radiotherapy early in life.[4,5,17]

Several reviews have comprehensively detailed the epidemiological and clinical aspects of RIGs.[5,14,16,17] In contrast, few investigations have described the molecular features of these tumors. Those that do exist are limited by the small number of cases available for analysis, with 1 study reporting that RIGs are genetically similar to pilocytic astrocytoma (PA),[18] while another study suggests they are analogous to primary adult glioblastoma (GBM).[19] Recently DNA methylation analysis has enhanced traditional diagnostic methods to significantly improve the accuracy of brain tumor classification.[20] Despite this, a definitive molecular signature for RIGs has not yet been established, highlighting a gap in the knowledge of potentially actionable drivers of this extremely aggressive disease. Indeed, there are currently no clear

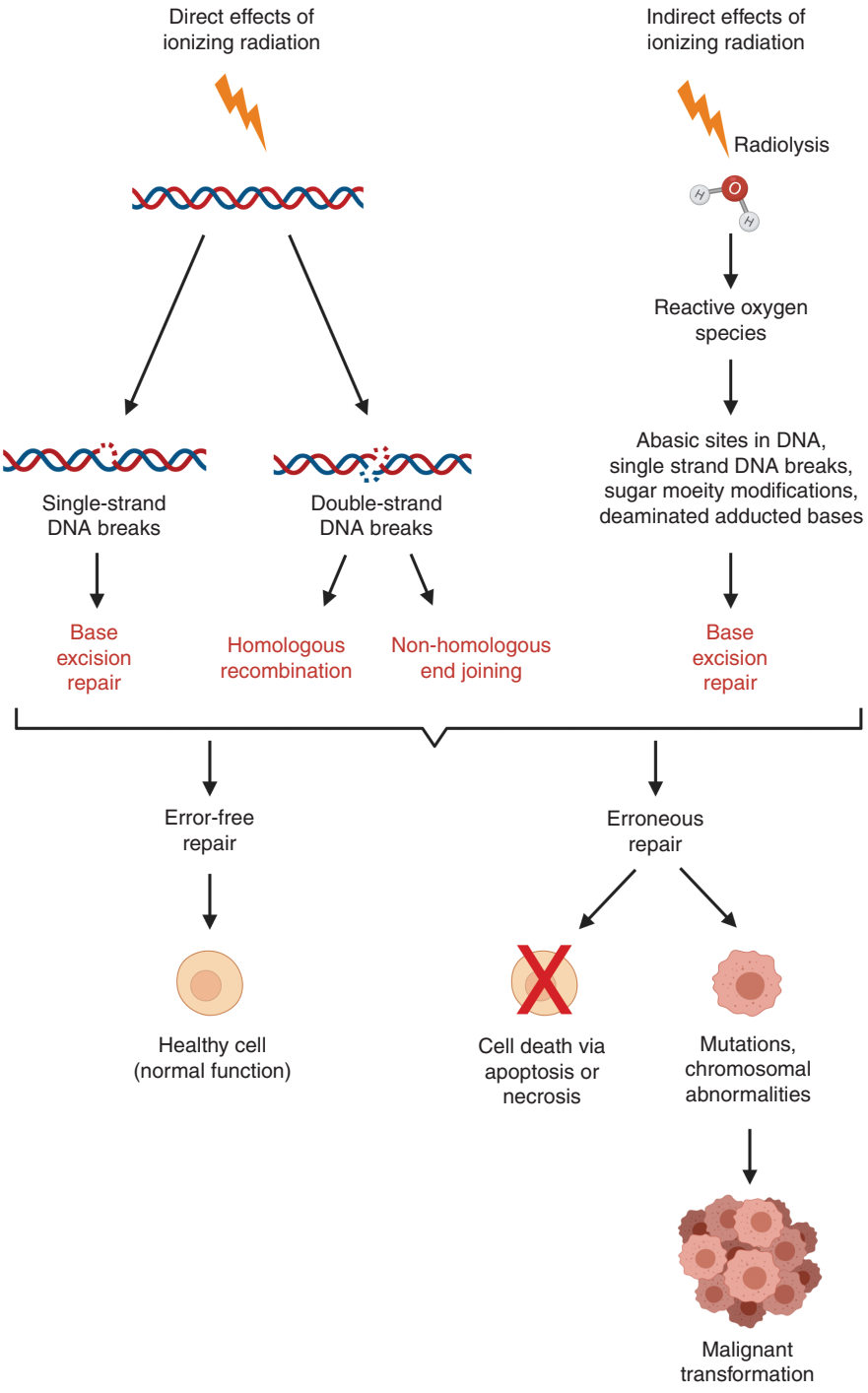

Figure 1. Mechanisms of DNA damage caused by ionizing radiation. Ionizing radiation damages DNA both directly and indirectly. Depending on the type of damage caused, cells will attempt to repair DNA lesions by base excision repair, homologous recombination or non-homologous end-joining. Successful repair results in a healthy cell with normal function whereas unsuccessful repair may result in cell death or the accumulation of mutations or chromosomal abnormalities, potentially leading to malignant transformation.

diagnostic criteria for RIGs and, as a consequence, no consistent or optimal treatment regimen has been defined. With this in mind, we have performed a systematic review and meta-analysis of the genetic features of cranial RIGs reported in the literature to identify recurrent changes that may be characteristic of this disease.

Methods

Systematic review and meta-analysis

A systematic search of the literature (from database inception up to April 7, 2021) was performed using PubMed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.[21] The terms used in the search were "radiation-induced" or "radiation-associated" or "treatment-induced" or "treatment-associated" each in combination with "glioma" or "astrocytoma" or "glioblastoma" or "ependymoma." Titles and abstracts of articles and case reports written in English were screened for cases of radiation-induced high-grade gliomas (HGGs, WHO grade III and IV) located in the human brain. Records describing cases with a genetic predisposition to cancer development or where the second glioma may have been a relapse of the initial tumor were excluded. The full-text versions of these articles were obtained in their entirety and examined for analysis of molecular features for inclusion in this study. The reference lists of eligible articles were also examined for further records not obtained through the database search. Only peer-reviewed articles were eligible for inclusion, with unpublished data excluded.

All individual cases were examined for the satisfaction of Cahan's criteria,[13] detailed in Table 1. The molecular data available from all eligible RIG cases in the literature were examined and compiled. All data were independently studied by 3 reviewers (J.P.W., M.H., and R.E.), who assessed the eligibility of each case and extracted data from the publications. Cases without information on family history of genetic cancer predisposition or matched germline DNA were included where available clinical evidence (latency, location of RIG, and/or distinction from primary malignancy) was suggestive of the second tumor being a RIG. Details on individual case exclusions and a brief description of analysis techniques employed by the primary sources are detailed in Supplementary Figure 1.

t-Distributed Stochastic Neighbor Embedding (*t*-SNE) analysis

Raw IDAT files from both reference set (https://academic.oup.com/neuro-oncology/article/23/1/34/5948536?login=true) and reported patient-derived xenograft (PDX) sample and from both array types (450k or EPIC) were loaded into the R environment (version 4.0.1) using the minfi package (version 1.21.4). CpG site probes present on both arrays have been selected; sample signal intensities have been normalized, filtered, and $\log_2$-transformed (limma package version 3.30.11). Unsupervised t-SNE analysis was performed on the top 10 000 most variable (row variance) CpG probes applying the Rtsne package (version 0.15), with the following parameters: pca = F, max_iter = 2500, theta = 0, perplexity = 35.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism software, version 8. Comparison of means for latency, anatomical location, and recurrent genetic alterations was performed using a 1-way ANOVA with a nonparametric Kruskal–Wallis test.

Results

The Profile of Recurrent Genetic Alterations in RIGs Is Distinct From Most Other Astrocytomas or Gliomas

Database searching identified 3006 records. After duplicates were removed, 1696 records were screened, from which 155 full-text articles were obtained. A further 31 records were identified from searching the reference lists of eligible articles and full-text versions of these obtained. A total of 152 reports were excluded as they did not contain molecular analysis, 2 reports were excluded as the RIG described may have been a relapse of the initial tumor,[51,52] and 1 report was excluded as it had not been peer-reviewed.[53] A final total of 31 reports were included, describing 102 unique cases of high-grade cranial RIGs with molecular data (Figure 2).

To identify recurrent themes and ensure confidence in interpretations, only genetic alterations that were tested in at least 10 unique RIGs are reported here (summarized in Table 2), with all remaining analyzed data available in Supplementary Table 1. Herein, the frequency of each genetic alteration in RIGs is expressed both as a percentage (%) and as a fraction of the total number of cases tested for that alteration (denoted as n/n).

PDGFRA was the most frequently altered gene in cranial RIGs, with amplification of this gene observed in 48% of tumors (10/21), and 44% (7/16) of cases harboring *PDGFRA* mutations. Another frequently altered gene was *TP53*, with 47% (14/30) of cases harboring mutations and 14% (2/14) demonstrating *TP53* deletion. Deletion of *CDKN2A* (often also including *CDKN2B*) was reported in 46% (13/28) of tumors, and *CDK4* amplification was also frequent (40%; 4/10).

Three out of 10 RIGs with sequence data demonstrated mutations in *ATRX*. In contrast, ATRX protein was detected via immunohistochemistry in 100% of samples tested (12/12 cases). Loss of ATRX protein strongly correlates with *ATRX* mutation by immunohistochemistry.[54,55] Although certain missense *ATRX* mutations may result in positive staining in a small percentage of cases,[55,56] immunohistochemistry for ATRX is routinely used histopathologically as a surrogate for *ATRX* mutation in the absence of genetic data. The immunohistochemistry results compiled here are suggestive of a very low mutation rate in this gene in cranial RIGs, in contrast to the sequencing results. To reduce the risk of bias, we have included results from both techniques in this analysis, resulting in an overall *ATRX* mutation rate of 14% (3/22).

Molecular alterations in other oncogenes or tumor-suppressor genes known to be associated with human glioma have also been reported for cranial RIGs including *PTEN* deletion (16%; 4/25 of cases tested) or mutation (5%; 1/22), amplification of *EGFR* (13%; 4/31) or *MYCN* (10%; 1/10), and mutations in *PIK3CA* (25%; 3/12), *NF1* (11%; 2/19), *BRAF* (5%; 1/19) or *IDH1* (2%; 1/47). An

Table 1. Satisfaction of Cahan's Criteria[13] for Radiation-induced Glioma Cases with Molecular Data Examined in This Study

Reference	Year	Genetic Predisposition	Latency Postradiation Treatment (Years)	RIG Occurred in an Irradiated Field	Distinct From Primary Malignancy (Primary Tumor Diagnosis)
Tada[22]	1997	No family history of genetic predisposition, no germline p53 mutation (other germline predispositions not tested for).	10	Yes	Yes (suprasellar germ cell tumor)
Matsumura[23]	1998	No family history of genetic predisposition. Germline DNA not tested.	8	Yes	Yes (subependymal giant cell astrocytoma)
Brat[24]	1999	No family history provided. Germline DNA not tested.	5-23	Yes	Yes (Hodgkin's disease, pituitary adenoma, rhabdomyosarcoma, craniopharyngioma, ALL, pineal tumor, ependymoma, lymphoblastic lymphoma)
Yang[25]	2005	No family history of genetic predisposition, and no TP53 mutation in the primary MB (TP53 mutation present in the RIG). Germline DNA not tested.	10	Yes	Yes (MB)
Berman[26]	2007	Satisfaction of Cahan's criteria stated.	9	Yes	Yes (arteriovenous malformation)
Donson[18]	2007	No family history provided. Germline DNA not tested.	3–15	Yes	Yes (Burkitt's lymphoma, MB, pilocytic astrocytoma, ALL, ependymoma)
Romeike[27]	2007	No family history of genetic predisposition. Germline DNA not tested.	7–14	Yes	Yes (MB, ALL)
Gessi[28]	2008	No family history of genetic predisposition, and no TP53 mutation in the primary MB or germline DNA (TP53 mutation present in the RIG).	8	Yes	Yes (MB)
Salvati[29]	2008	Satisfaction of Cahan's criteria stated for all cases. No family history of genetic predisposition. Germline DNA not tested.	6–26	Yes	Yes (MB, cavernous angioma, tinea capitis, cutaneous hemangioma, scalp hemangioma, ALL)
Sasayama[30]	2008	No family history of genetic predisposition, no germline p53 mutation (other germline predispositions not tested for).	28	Yes	Yes (MB)
Garcia-Navarro[31]	2009	No family history provided. Germline DNA not tested.	8	Yes	Yes (pineal germinoma)
Kamide[32]	2010	Satisfaction of Cahan's criteria stated. No family history provided. Germline DNA not tested.	29	Yes	Yes (MB)
Paugh[33]	2010	No family history provided. Germline DNA not tested.	Not described	Yes	Yes (ALL, germinoma, ependymoma, MB)
Ohba[34]	2011	No family history of genetic predisposition. Germline DNA not tested.	4	Yes	Yes (meningothelial meningioma)
Khoo[35]	2012	Satisfaction of Cahan's criteria stated.	30	Yes	Yes (diffuse astrocytoma)
Mascelli[36]	2012	No family history provided. Germline DNA only analyzed for IDH1/2 mutation.	7	Yes	Yes (sellar/suprasellar craniopharyngioma)
Ahmed[37]	2014	No family history provided. Germline DNA not tested.	10	Yes	Yes (ALL)
Nakao[38]	2017	Satisfaction of Cahan's criteria stated for all cases. No family history of genetic predisposition. Germline DNA not tested.	22–29	Yes	Yes (MB, craniopharyngioma, primitive neuroectodermal tumor, pituitary adenoma)

Table 1. Continued

Reference	Year	Genetic Predisposition	Latency Postradiation Treatment (Years)	RIG Occurred in an Irradiated Field	Distinct From Primary Malignancy (Primary Tumor Diagnosis)
Ng[39]	2017	No family history of Neurofibromatosis 1 or 2. Germline DNA not tested.	6	Yes	Yes (vestibular schwannoma)
Gits[19]	2018	Germline DNA tested for 2/3 cases (case #6 without germline DNA was reanalyzed with matched germline DNA available in Whitehouse et al[40])	4–12	Yes	Yes (MB)
Izycka-Swieszewska[41]	2018	No family history provided. Germline DNA not tested.	3–6.5	Yes	Yes (ALL)
Kajitani[42]	2018	No family history of genetic predisposition. Germline DNA not tested.	5–10	Yes	Yes (ALL)
Phi[43]	2018	Germline DNA only available for 4/5 patients.	4.3–10 years post primary diagnosis	Yes	Yes (MB)
Porter[44]	2018	No family history provided. Germline DNA not tested.	19	Yes	Yes (MB)
Wang[45]	2018	No family history of genetic predisposition. Germline DNA not tested.	8	Yes	Yes (MB)
Lopez[46]	2019	Satisfaction of Cahan's criteria stated for all cases. No family history of genetic predisposition. Germline DNA tested for 6/12 cases.	4–41	Yes	Yes (MB, intracranial germinoma, leukemia, Hodgkin's lymphoma, craniopharyngioma, pineocytoma)
Mucha-Malecka[47]	2019	Satisfaction of Cahan's criteria stated. Germline DNA not tested.	12	Yes	Yes (MB)
Biswas[48]	2020	No family history of genetic predisposition. One case of unilateral breast cancer in paternal grandmother.	5	Yes	Yes (ALL)
Smith[49]	2020	No family history provided. Germline DNA not tested.	Not described	Yes	Yes (MB)
Whitehouse[40]	2020	Satisfaction of Cahan's criteria stated. No family history of genetic predisposition. Germline DNA tested.	11	Yes	Yes (MB)
Woo[50]	2021	Satisfaction of Cahan's criteria stated.	6–23	Yes	Yes (nasopharyngeal carcinoma, primary intracranial germinoma)

ALL, acute lymphoblastic leukemia; GBM, glioblastoma; MB, medulloblastoma; RIG, radiation-induced glioma.

intragenic deletion in *BRAF* (5%; 1/19) and a *GTF2I-BRAF* fusion (5%; 1/19) were also reported.[46] The *MGMT* promoter was methylated in 28% (8/29) of cases tested for this alteration. Correspondingly, MGMT protein expression was low or undetectable in 40% (4/10) of samples tested by immunohistochemical techniques. Of note, none of the cranial RIGs examined harbored mutations in other genes frequently altered in HGG or other cancers, including *ACVR1* (0/12), *EGFR* (0/14), *H3F3A* (0/21), *HIST1H3B* (0/12), *IDH2* (0/16), *SMARCB1* (0/10) or the *TERT* promoter (0/11).

RIGs Demonstrate Unique Recurrent Chromosomal Alterations

The karyotypes of HGGs are often highly complex, with increasing complexity associated with increasing tumor grade.[57–59] A small cytogenetic study of 3 RIGs reported extremely complex karyotypes[18]; however, few studies have extensively examined broad chromosomal gains or losses in cranial RIGs. Paugh et al[33] performed the largest analysis to date examining 10 RIG samples, to which we have added seven more cases here[34,40,43,49] (Figure 3 and

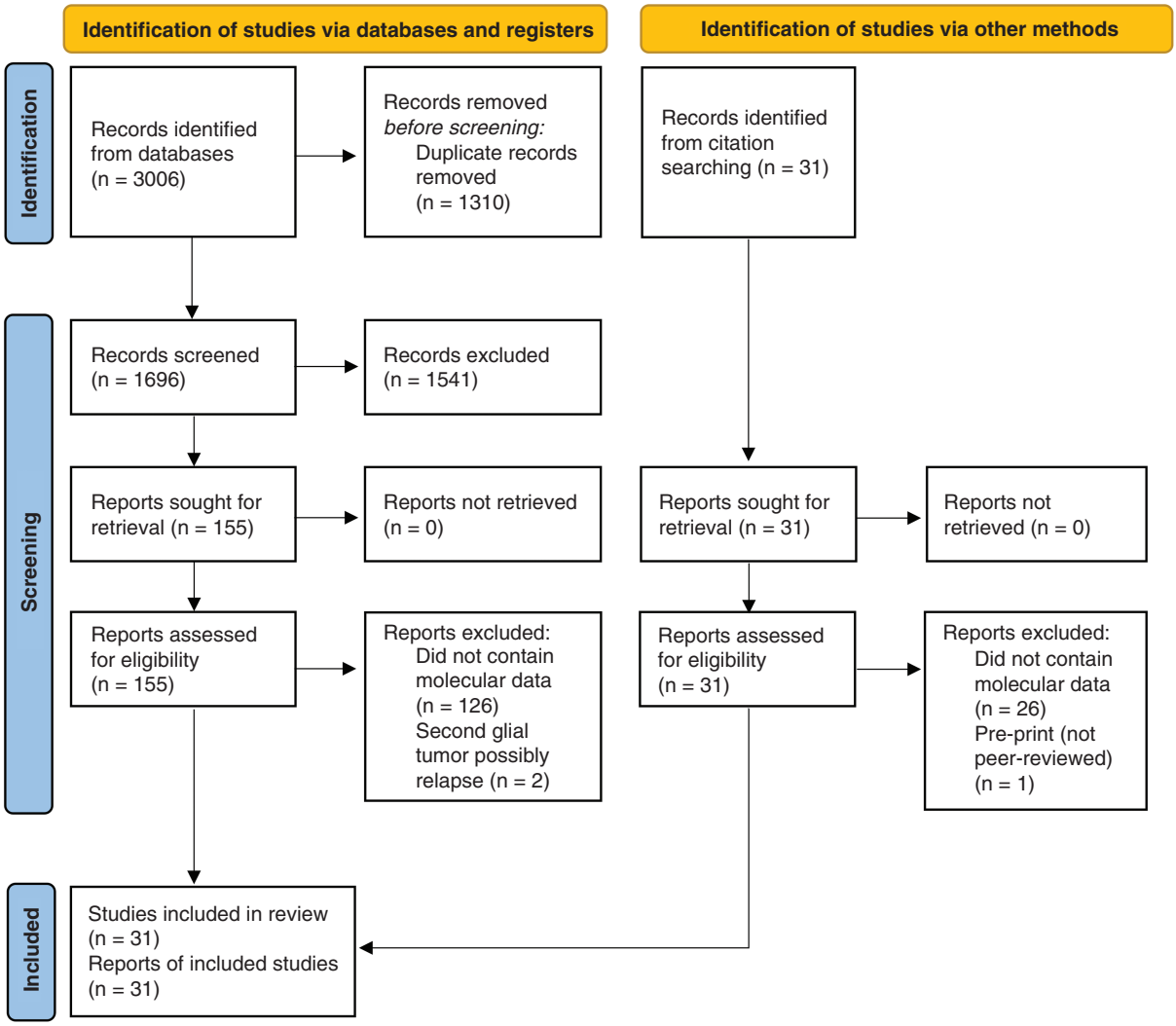

Figure 2. Flow chart of literature searching strategy detailing the report selection procedure as outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines.[21] A total of 31 reports were eligible for inclusion in this systematic review.

Supplementary Tables 2 and 3). Overall, the most frequent copy number changes observed in RIGs were loss of 13q (observed in 59% of samples reported), gain of 1q (53%), and loss of 1p (47%). This was followed by gain of 9q (35%), loss of 14q (35%) and loss of 14p (33%). Of these changes, it has been reported that gains of chromosome 1q and 9q and losses of 1p and 13q are significantly more frequent in RIGs compared with other pediatric and adult HGG.[33]

Anatomical Distribution of Recurrent Genetic Alterations and Latency Intervals of RIGs

Previously, glioma-associated genetic alterations have correlated closely with tumor type, age of onset and location of disease.[60] To determine whether this was also the case in RIG, we examined whether there was a correlation between RIG location and genetic features. The most frequent recurrent genetic alterations found in RIGs (*CDK4* amplification, *CDKN2A* deletion, *PDGFRA* amplification and/or mutation, or *TP53* mutation) were mapped to 3 brain regions: the cerebrum, cerebellum, or brainstem and diencephalon. Deletion of *CDKN2A* was most commonly observed in RIGs located in the brainstem and diencephalon region, while *TP53* mutations were most often found in cerebellar RIGs (Figure 4A), with many tumors harboring more than 1 genetic alteration (Supplementary Table 4). However, the anatomical location of RIGs harboring these recurrent genetic alterations was only reported for a small number of cases (n=14) which significantly limits the strength of this correlation and highlights the need for further research in this area.

We also investigated whether the most frequently observed genetic alterations correlated with time to RIG onset, although the latency interval of RIGs harboring these was only reported for a total of 18 unique cases. Using this limited data set we observed no correlation between disease latency and genetic alterations (Supplementary Table

Table 2. Summary of molecular alterations tested in at least ten unique radiation-induced glioma cases

Function	Gene	DNA Alterations			RNA Expression		Protein Expression		Total Unique cases	References
		Amplified		Mutation/Fusion/Promoter Methylation (*MGMT* only)	Low	High	Low	High		
Receptor	*ACVR1*	0/9	0/9	0/12	NR	NR	NR	NR	12	19,46
	EGFR	4/31	0/10	0/14	1/5	0/5	14/22	4/22	48	18, 24–28, 33, 40, 41, 46, 48, 49
	PDGFRA	10/21	0/18	7/16	0/5	5/5	2/7	3/7	32	18a, 19, 28, 33, 40, 43, 46
Signal transduction	*BRAF*	0/9	0/9	3/19	NR	NR	NR	NR	19	19, 38b, 42c, 46
	NF-1	0/18	0/18	2/19	1/1	0/1	NR	NR	19	33,40,46
	PIK3CA	0/9	0/9	3/12	NR	NR	NR	NR	12	19,40,46
	PTEN	0/9	4/25	1/22	0/1	0/1	0/4	4/4	32	18, 19, 24, 28, 40, 41, 43, 46
Cellular metabolism	*IDH1*	0/9	0/9	1/47	0/1	1/1	NR	NR	47	34d, 36, 38d,e, 39d, 40, 41d, 42, 43d, 46, 47d, 50
	IDH2	0/9	0/9	0/16	NR	NR	NR	NR	16	36, 38e, 42, 46
Cell cycle regulation	*CDK4*	4/10	0/10	0/10	NR	NR	NR	NR	10	46,49
	CDKN2A	0/10	13/28	0/10	NR	NR	1/3	2/3	31	19, 24, 33, 40, 42, 46, 49
Transcriptional regulation/ chromatin modification	*ATRX*	0/10	0/10	3/10	NR	NR	0/12	12/12	22	38, 39, 41, 42, 45, 46, 48
	H3F3A	0/9	0/9	0/21	NR	NR	NR	NR	21	19, 38f, 39f, 40, 42g, 46
	HIST1H3B	0/9	0/9	0/12	NR	NR	NR	NR	12	19,46
	MYCN	1/10	0/10	0/10	NR	NR	NR	NR	10	46,49
	SMARCB1	0/10	0/10	0/10	NR	NR	NR	NR	10	46,49
	TERT promoter	0/9	0/9	0/11	NR	NR	NR	NR	11	38,46
	TP53	0/13	2/14	14/30	0/1	0/1	20/42	12/42	61	18, 19, 22–28, 30, 31, 34, 37, 38, 40–43, 45, 46, 48, 49
DNA repair	*MGMT*	NR	NR	8/29 promoter methylated	NR	NR	4/10	6/10	33	28,29,32,35,39,41,44,45,50

Data expressed as the total number of tumors positive for the described alteration as a fraction of the total number of tumors tested for that alteration (n/n). Genes are grouped based on function.
NR, not reported.
[a]Pooled microarray data excluded from analysis as individual tumor data unavailable.
[b]Mutational status determined by BRAF V600E direct sequencing.
[c]Mutational status determined by BRAF V600E IHC.
[d]Mutational status determined by IDH1 mutant-specific antibody via IHC.
[e]Mutational status determined by direct sequencing.
[f]Mutational status determined by H3K27M IHC.
[g]Mutational status determined by K27M, G34R, G34V mutation detection by direct sequencing.

4) however, further data are required to confidently assess this relationship.

Given these limitations within the existing studies, we examined if there was any correlation between RIG location and disease latency interval using all the cases in this series where location information was available (n = 79 cases). We found no correlation between anatomical location and the time between radiation treatment and RIG development (Figure 4B; Supplementary Table 5). Again, the small number of cases in this series must be taken into consideration when drawing conclusions from these data.

DNA Methylation Analysis of RIGs and Associated PDX models

The assessment of global DNA methylation is rapidly becoming a widely used method to classify CNS tumors. The majority of RIG cases described in the literature were

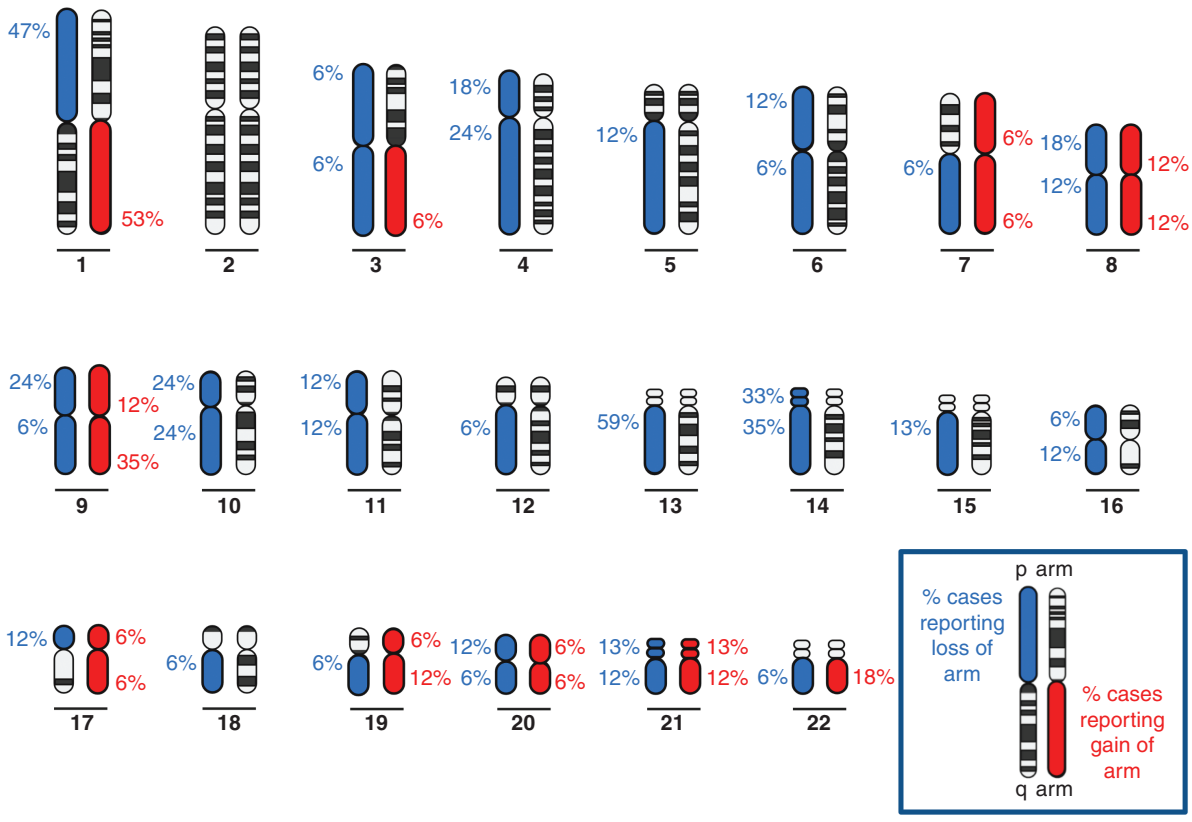

Figure 3. Frequency of gains and losses of autosomal chromosomal arms observed in cranial radiation-induced gliomas. Blue indicates loss and red indicates gain of chromosomal arms, with percentages of cases reporting gain or loss for each arm shown.[33,34,40,43,49]

published before the DNA methylation array was fully appreciated as a useful classification tool for CNS tumor diagnosis.[20] Indeed, only 2 recent studies performed methylation array on RIG cases,[40,49] which limits our ability to analyze these data in great detail. Smith et al[49] performed methylation clustering analysis using an online classification tool (www.molecularneuropathology.org, version 11b4) and reported that the RIG and the PDX derived from that tumor were most similar to the GBM, IDH-wild type, subclass midline (GBM, MID) methylation class. Of note, within the reference cohort,[20] there are no clearly defined RIG reference samples and a RIG-specific methylation profile has not been established. The GBM, MID methylation subclass describes tumors that are located in the midline and have a median age at diagnosis of 13 years. These tumors typically lack H3K27M mutations and frequently demonstrate amplification of *PDGFRA*, *CDKN2A/B* loss, and mutations in *FGFR1*[20].

Of the only other published RIG where methylation data are available, similar DNA methylation analysis was performed using the same web platform (www.molecularneuropathology.org, version 11b4); however, this tumor failed to cluster with any of the defined subclasses, as previously described.[40] Using DNA isolated from a PDX derived from this RIG, we repeated methylation-based clustering analysis using a more recently reported reference cohort of well-characterized astrocytic gliomas from both adults and children.[61] Analysis by *t*-SNE revealed that this RIG-derived PDX clustered with the pedGBM_RTK1 methylation subclass (Supplementary Figure 2). Tumors classified as pedGBM_RTK1 are characterized by frequent *PDGFRA* amplification, *TP53* mutation, and homozygous *CDKN2A* deletion and lack mutations in the *TERT* promoter or *EGFR* amplification.[62] Of note, this subclass is not described in the online methylation classifier used by Smith *et al*[49]. Upon comparison, there is substantial overlap between the GBM, MID and pedGBM_RTK1 methylation subclasses (David Jones, personal communication, July 26, 2021). Consequently, the next version of the molecular neuropathology classifier (version 12) will no longer include a GBM, MID subclass, but will instead include subclasses of pedGBM_RTK1 (David Jones, personal communication, July 26, 2021). Given this, it is likely that the RIG described in Smith et al[49] may correspond to a pedGBM_RTK1 subclass in this new version of the classifier.

Discussion

Historically, RIGs have been clinically diagnosed as diffuse intrinsic pontine glioma (DIPG),[19,40] anaplastic astrocytoma (AA),[24,30,31,36–38,41,46,47,63] or GBM[22–25,28,29,34,38,39,41,42,45,46,48,50] based primarily on histological

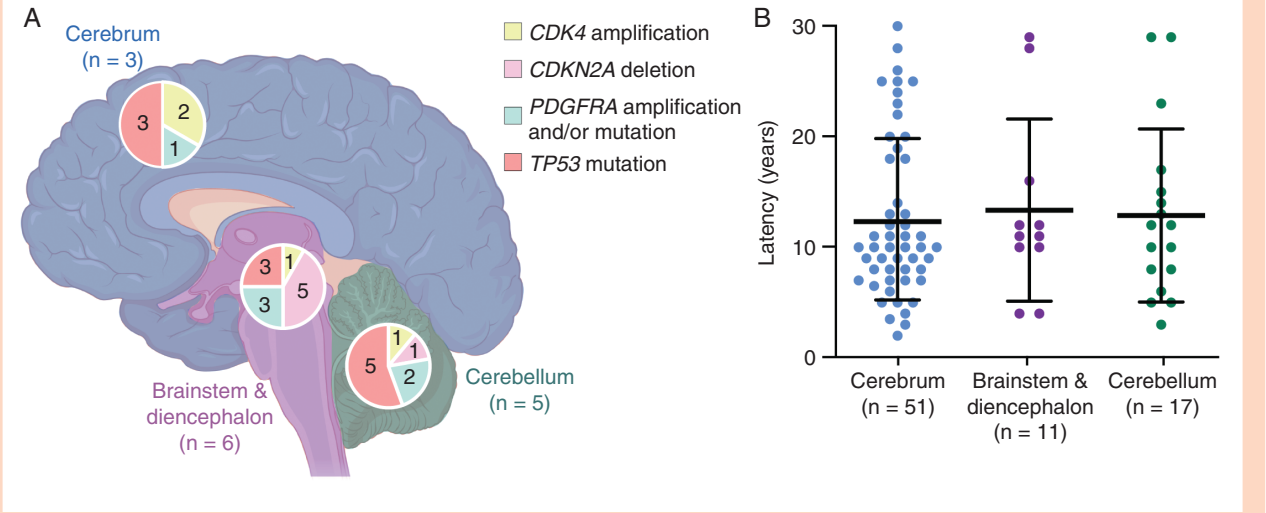

Figure 4. Relationship of recurrent radiation-induced glioma (RIG) mutations and disease latency with anatomical brain location. (A) RIGs with the indicated genetic alterations were grouped according to the locations shown (cerebrum, blue; brainstem and diencephalon, purple; or cerebellum, green). The number of tumors harboring each alteration (see key) are shown within each segment of the pie chart for each region, with the total number of tumors for each location (n) shown. Some tumors harbored multiple genetic alterations; data shown represent all reported genetic alterations for that location, and do not imply mutual exclusivity of genetic alterations within tumors.[19,22,25,28,40,46] (B) Scatter plot of latency intervals for RIGs arising in the three defined brain regions.[18,19,22,23,25–32,34–38,40–42,45–48,50] Bars show mean ± SD.

and/or radiographical characteristics. In very rare cases, the diagnosis of anaplastic ependymoma has also been reported.[35] RIGs have even been mistaken for recurrent medulloblastoma[43,49] or radiation necrosis.[63] By compiling molecular data from 31 publications describing RIG we strove to identify the most common genetic alterations in human cranial RIG. We found that the most frequent alterations in RIGs occurred in genes involved in cellular growth (*PDGFRA*), cell cycle regulation (*CDKN2A*, *CDK4*), DNA repair, and induction of apoptosis (*TP53*), with approximately half of the tumors tested for these changes demonstrating alterations in one or more of these genes.

In this study, we aimed to determine whether RIGs are molecularly similar to other brain tumor types or instead form their own distinct subgroup. As mentioned, RIGs have often been diagnosed as AA.[24,30,36,38,41,46,47,63] AAs are histologically WHO grade III diffuse HGGs with invasive and aggressive tendencies and arise most often in adults.[64] The most common WHO-defined variant is "Anaplastic astrocytoma, IDH-mutant".[65] These tumors are characterized by *IDH1/2* mutation, along with mutations in *TP53* and *ATRX*,[66–68] with an associated loss of ATRX protein.[69,70] Additionally, mutations in NOTCH pathway genes, and less frequently *PIK3CA*, *PIK3R1* and *DSG3* have been reported in AAs.[68] While we observed a high frequency of *TP53* mutations in RIGs and a small number of cases harbored mutations in *ATRX*, ATRX protein expression was detected in 100% of RIG cases by immunohistochemistry and mutations in *NOTCH* and *IDH1/2* were rare (Table 2; Supplementary Table 1), suggesting low concordance between the genetic profiles of these 2 tumor types.

A previous analysis of 5 RIGs suggested that their gene expression profile resembled PAs, with a 39% overlap in highly expressed genes reported between these 2 tumor types.[18] Of those genes, only *PDGFRA* was reliably overexpressed in RIGs from our analysis, with limited information available on expression levels of the other genes reported. A lack of *IDH1/2* mutations was the only other genetic similarity observed between these tumor types.[71] A hallmark feature of PAs is mitogen-activated protein kinase pathway dysregulation.[72] While alterations in *BRAF* were observed in a small number of RIG cases (16%; 3/19 tumors), the characteristic *KIAA1549:BRAF* fusion and other less frequent genetic alterations reported in PAs, such as *FGFR1* mutations or *NTRK2* fusions,[73–75] were not found (Supplementary Table 1). Our analysis suggests there are few genetic similarities between PA and RIGs. Indeed, Donson et al[18] suggested that the similar RNA expression profiles they observed may have been due to these tumors sharing a common precursor cell, particularly given the considerable differences in tumor grade and patient outcome between PA and RIG.

Due to disease location, radiological features and their aggressive nature, RIGs have also been clinically diagnosed as DIPG or brainstem glioma.[19,40] DIPGs are rapidly growing, diffuse gliomas arising in the brainstem, most commonly observed in children.[76] Nearly 80% of pediatric DIPG harbor a lysine 27 to methionine (K27M) mutation in histone H3[77–79], which led to the WHO reclassifying this tumor type as "diffuse midline glioma (DMG), H3 K27M-mutant" in 2016[65,80]. We specifically compared the recurrent genetic alterations reported for RIG with those reported for DIPG/DMG. Several genetic alterations were shared between DIPG/DMG and RIG, including a high frequency of *TP53* mutations and *PDGFRA* amplification, moderate frequency of *CDK4* amplification,[65] and lower frequencies of *IDH1/2, NF1*, and *PIK3CA* mutations, and *PTEN* deletion/mutation.[33,78,81–83] However, in contrast, all

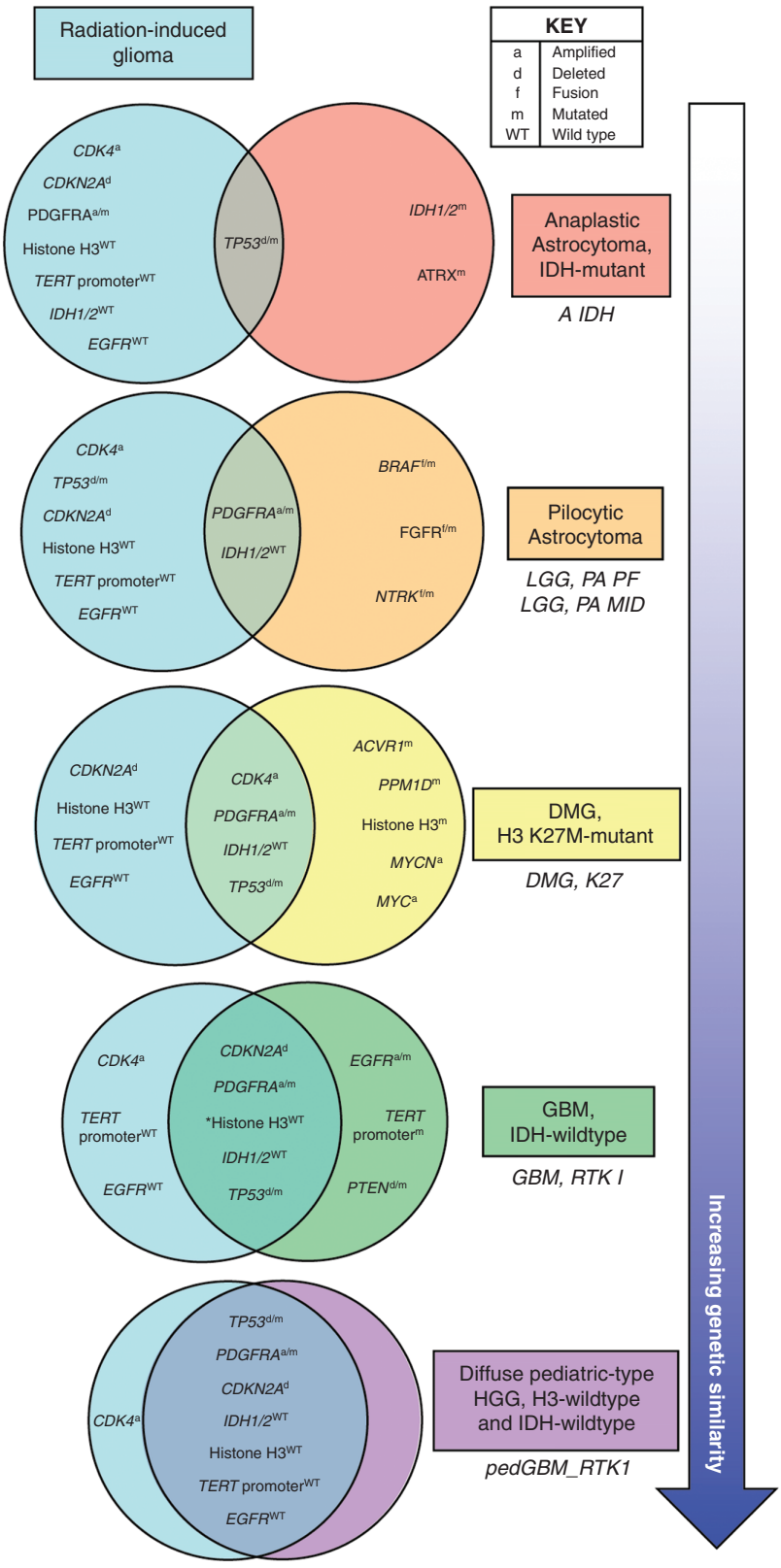

Figure 5. Radiation-induced gliomas (RIGs) are genetically distinct from most other astrocytoma and glioma brain tumor types and share the highest number of common genetic alterations with the pedGBM_RTK1 methylation subclass of pediatric glioblastoma. Major recurrent genetic features of RIGs were defined as occurring in more than 40% or cases and are compared here with hallmark genetic features of the indicated

RIGs lacked mutations in *ACVR1* or *PPM1D*, and few exhibited *MYC* or *MYCN* amplification (Table 2; Supplementary Table 1) previously described in DIPG/DMG. Most notably, the hallmark H3 K27M mutation that is now pathognomonic for DMG was not reported in any RIG within the cases examined here.[58,65,78,81,82] Furthermore, nearly half of the RIGs analyzed showed homozygous deletion of *CDKN2A*, which is almost never observed in DIPG/DMG, and 16% of RIGs harbored *BRAF* mutations that are absent in DIPG/DMG.[58,81,82] Our analysis demonstrates that while some key genetic alterations are shared between these tumor types, significant differences (most notably the lack of hallmark mutations) exist, rendering RIGs molecularly distinct from DIPG/DMG.

It has been proposed that pediatric RIGs share molecular similarities with adult primary GBMs.[19] GBMs are highly aggressive WHO Grade IV diffuse gliomas that can arise as a primary tumor (primary GBM) or result from progression of a lower-grade II/III glioma (secondary GBM)[84] and are most commonly wild type for IDH.[65] While our analysis showed some similarities between RIGs and adult primary GBMs, including a high frequency of *PDGFRA* amplification/mutation, *TP53* mutations, *CDKN2A* deletion, and absence of *IDH1/2* mutations,[85–87] a number of key differences were evident. Most notably, several hallmark alterations of adult primary GBM, such as *PTEN* mutation/deletion, *EGFR* mutation/amplification and *TERT* promoter mutation,[85–87] were either absent or only rarely observed in the cranial RIGs assessed in our analysis.

A large-scale study of mostly adult GBM samples identified 4 genetic subtypes of GBM: proneural (characterized by alterations in *IDH1*, *PDGFRA* and *TP53*), neural (no defining genetic alterations but overexpress neural markers), classical (often harbor *EGFR* amplifications/mutations and *CDKN2A* homozygous deletion) and mesenchymal (commonly demonstrate *NF1* mutations, some with concurrent *PTEN* mutations).[88] Sturm et al[89] later built on this work using combined pediatric and adult samples and defined a total of 6 epigenetic and biologic subgroups of GBM. Of these, the RTK I subgroup can be considered most closely aligned to RIGs from our analysis. This subgroup is generally typified as having wild-type *IDH1*, *PDGFRA* amplification, and *CDKN2A* deletion, with around half of the samples also harboring alterations in *TP53*. Mutations in *H3F3A* are also absent from this methylation subclass.[89]

From our comparisons thus far, it is evident that the genetic features of RIG are most closely aligned to the WHO diagnostic classification GBM IDH-wild type, and more specifically the RTK I methylation subclass. The tumors that fell into this methylation subclass described by Sturm et al[89] occurred in both adult and pediatric populations, while the "GBM, IDH wild type, subclass RTK I" described in Capper et al[20] was solely made up of adult patients. In contrast, Korshunov et al[62] specifically investigated histone H3-/IDH1-wild-type pediatric GBM. This integrated analysis identified 3 distinct molecular subgroups of pediatric GBM designated pedGBM_MYCN (demonstrating a high frequency of *MYCN* amplification), pedGBM_RTK1 (enriched for *PDGFRA* amplification), and pedGBM_RTK2 (characterized by *EGFR* amplification). Similar to the GBM RTK I methylation subclass,[89] the pedGBM_RTK1 subpopulation has frequent *PDGFRA* amplification and *TP53* mutation, as well as a considerable proportion of tumors harboring homozygous *CDKN2A* deletion. Additionally, pedGBM_RTK1 tumors do not exhibit mutations in the *TERT* promoter or amplifications of *EGFR* and have a comparable frequency of *MYCN* amplification and *PTEN* loss to RIGs examined in our analysis. Thus, the features of this subgroup closely correlate with recurrent genetic alterations of the RIGs compiled here, and our data show that a RIG PDX model (TK-RIG915) clusters with the pedGBM_RTK1 subgroup by methylation profiling. Preliminary data from others support this correlation, with 26 out of 36 gliomas that developed after therapy clustering with the pedGBM_RTK1 subgroup by methylation array.[90] In summary, despite most RIGs being diagnosed in adulthood,[16] our comparisons of cranial RIGs with multiple other types of glioma revealed that these tumors most closely resemble the pedGBM_RTK1 molecular subgroup of pediatric gliomas (depicted in Figure 5).

Limitations

This analysis used data reported from a limited number of published cases, rather than from a comprehensive large-scale genome-wide study using primary tumor tissue. As a result, our study was restricted to the genetic alterations reported in the original sources, with not all alterations tested across all samples, resulting in loss of power through missing information. Additionally, the limited number of samples precluded any meaningful correlative analysis of RIG location or latency and the underlying genetic features of these tumors. The correlation

WHO-defined CNS tumors[65] (colored boxes ordered by increasing similarity to RIG): IDH-mutant anaplastic astrocytoma,[65–68,91–93] pilocytic astrocytoma,[18,65,71,73–75,91,94] diffuse midline glioma (DMG), H3 K27M-mutant,[33,58,65,77–79,81–83] IDH-wild type glioblastoma (GBM).[65,85–89] The closest corresponding methylation subclass[20,62] is indicated beneath each WHO-defined tumor type in italics. Comparison of recurrent genetic features of RIGs with the pedGBM_RTK1 methylation subclass of pediatric GBM[62] is also shown. Being pediatric-specific, this methylation class does not align with a specific entity in the 2016 WHO diagnostic guidelines[65] therefore we have used a new entity proposed in the 2021 edition (diffuse pediatric-type high-grade glioma (HGG), H3-wild type and IDH-wild type).[95] Common astrocytoma/glioma genetic alterations that were only observed in a small proportion of RIGs (e.g. *ATRX* mutation (14%), *BRAF* mutation (10%), *BRAF* fusion (5%), *EGFR* amplification (13%), *IDH1* mutation (2%), *MYCN* amplification (10%), *PTEN* deletion (16%)) were considered infrequent and not deemed to be a defining characteristic of RIG. Asterisk indicates a genetic feature associated only with the GBM, RTK I methylation subclass and has not been described as a feature of GBM, IDH-wild type. Abbreviations for the methylation classes are as previously defined[20,62]: A IDH—IDH glioma, subclass astrocytoma; LGG, PA PF—low-grade glioma, subclass posterior fossa pilocytic astrocytoma; LGG, PA MID—low-grade glioma, subclass midline pilocytic astrocytoma; DMG K27—diffuse midline glioma H3 K27M mutant; GBM, RTK I—glioblastoma, IDH wild type, subclass RTK I; pedGBM_RTK1—pediatric glioblastoma enriched for *PDGFRA* amplification.

of recurrent RIG genetic alterations with those observed in the methylation subclass pedGBM_RTK1, and the positive association of the TK-RIG915 PDX with this methylation class, raises the question of whether other RIGs would cluster similarly using DNA methylation-based techniques. Furthermore, preliminary data by Lucas et al[90] corroborate our assessment, where they report that RIG DNA methylation profiles are similar to pedGBM_RTK1 methylation subgroup tumors. Additional research using a larger number of samples is essential to determine whether RIGs truly are genetically similar to pediatric GBM or if they represent a unique tumor subclass.

For the majority of cases, information on germline mutations predisposing to cancer and/or a family history of cancer was available. Where this information was not explicitly stated, cases were included based on the clinical evidence available; however, these cases are acknowledged as a limitation of this analysis as germline predisposition cannot be confidently excluded in these instances. Although Cahan's criteria state that cases with germline alterations predisposing to cancer should not be classified as RIGs, a more recent analysis reported that a high proportion of patients that developed glioma following therapy had frequent pathogenic germline alterations in DNA repair genes, including *BARD1*, *BRCA1*, *BRCA2*, *ATR*, and *PMS1*[96]. These data suggest that these patients may be at higher risk of RIG development, and as such may require increased surveillance following radiation therapy.

Finally, there is some controversy regarding radiation-induced tumors and the role of chemotherapy in contributing to their development. The combination of radiation and chemotherapy has been reported to have a synergistic effect on the development of treatment-induced gliomas[4,97] or result in a shorter latency period.[16] In contrast, others report statistically significant increases in the risk of glioma development with increasing radiation dose, but no further increased risk with the addition of chemotherapy.[5,8] Given these inconsistent findings in the literature, we cannot rule out the possibility that chemotherapy may have contributed to the effects of radiation in the initiation of the glioma cases we report here, despite satisfaction of Cahan's criteria in most cases. For this review, we have chosen to retain the more widely-used term "radiation-induced glioma", but acknowledge that broader terms such as "radiation-associated"[98] or "treatment-induced"[90] may prove to be more accurate should chemotherapy be shown to be a contributing factor in the development of these tumors in the future.

Future directions

Currently, a disease-specific treatment approach for RIG remains undefined and patients are treated in accordance with the broad treatment regimens assigned to the tumor type histologically and/or radiologically classified at the time of diagnosis (eg, AA, DIPG/DMG, GBM). However, our analysis reveals that the mutational and gene expression profiles of RIGs are distinct from most other astrocytomas and gliomas, aligning most closely with the pedGBM_RTK1 subgroup of pediatric GBM.

Given the paucity of molecular data currently available for RIGs, there is a clear need for research that further defines the molecular characteristics of RIG. This can only be achieved by increasing the number of samples comprehensively analyzed at the genetic, epigenetic and transcriptional level in a patient-specific manner, an opinion supported by other research groups.[90,99] This large-scale analysis will help the field conclusively determine if RIGs are molecularly distinct from, or should be considered synonymous with, the pedGBM_RTK1 tumors. Proposed plans to distinguish between pediatric and adult diffuse HGG in the next edition of the WHO classification of CNS tumors may assist further with a more accurate diagnosis of these tumors.[95]

The findings of this meta-analysis may have implications for future clinical management of this disease, particularly given that most RIGs are diagnosed in adulthood. Additionally, molecular classification of RIGs may complement existing tools for pathological diagnosis of these tumors in the future. Preclinical models such as genetically engineered mouse models of RIG susceptibility[100] and PDX models of RIG[40] can not only aid in our understanding of the biological pathways relevant to the development of these tumors but are also an essential tool in bridging the gap between potential therapeutic approaches and rational clinical trial design. Currently, these models are rare, highlighting the need to focus future efforts on their development to increase the number of relevant models available to the research community.

Given the rarity of these tumors, accurate characterization of RIGs moving forward will require a global collaborative effort from both the research and clinical communities to collectively advance the knowledge of the field. Indeed, the development of a global RIG registry would facilitate the centralized collection of relevant clinical, pathological and molecular data defining these rare tumors. Identifying the molecular features of these tumors will help us to understand the mechanisms that drive RIG initiation and progression, as well as potentially uncover therapeutic targets for this currently incurable disease.

Supplementary Material

Supplementary material is available at *Neuro-Oncology Advances* online.

Keywords

Radiation-induced glioma | molecular | pediatric | radiation | cancer

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Authorship Statement

Conceptualization: J.P.W. Data curation and analysis: J.P.W., M.H., A.F., M.K., R.E. Data interpretation and visualization: J.P.W., M.H., A.F., M.K., R.E., N.G.G. Funding acquisition: R.E., N.G.G. Methodology: J.P.W. Writing – original draft: J.P.W. Writing – critical review and editing: J.P.W., M.H., A.F., M.K., R.E., N.G.G.

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