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The epigenetics of brain tumors and its modulation during radiation: A review

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ABSTRACT:

The Brain tumor is the abnormal growth of heterogeneous cells around the central nervous system and spinal cord. Most clinically prominent brain tumors affecting both adult and pediatric are glioblastoma, medulloblastoma, and ependymoma and they are classified according to their origin of tissue. chemotherapy, radiotherapy, and surgery are important treatments available to date. However, these treatments fail due to multiple reasons, including chemoresistance and radiation resistance of cancer cells. Thus, there is a need of new therapeutic designs to target cell signaling and molecular events which are responsible for these resistance. Recently epigenetic changes received increased attention because it helps in understanding chromatin-mediated disease mechanism. The epigenetic modification alters chromatin structure that affects the docking site of many drugs which cause chemo-resistance of cancer therapy. This review centers the mechanism of how epigenetic changes affects the transcription repression and activation of various genes including Polycomb gene, V-Myc avian myelocytomatosis viral oncogene (MYCN). This review also put forth the pathway of radiation-induced reactive oxygen species generation and its role in epigenetic changes in the cellular level and its impact on tissue physiology. Additionally, there is a strong relationship between the behavior of an individual and environment-induced epigenetic regulation of gene expression. The review also discusses Transcriptome heterogeneity and role of tumor microenvironment in Glioblastoma. Overall, this review emphasis important and novel epigenetic targets that could be of therapeutic benefit, which helps in overcoming the unsolved chromatin alteration in brain cancer.

KEYWORDS: Epigenetics, Glioblastoma, Radiation, ROS

1. Introduction:

Primary brain tumors arise from the brain parenchyma cells and their adjacent structures, developing a heterogeneous group of benign and malignant tumors [1]. These tumors are responsible for high morbidity and mortality rate in both adults and children. According to statistics in 2017, studies showed that 23,880 cases reported for primary brain and spinal cord cancer in the United States, among which 3,560 cases were pediatric brain cancer [2]. The brain tumors arises mainly because of non-functionality of tumor suppresser genes on the chromosome. The tumor suppressor genes like *p53* take care about the cell cycle process and repair of genes defects [3]. There are also other causes of brain tumor like environmental factors also lead to further damage. Here in this review, we are concentrating on the epigenetic factors of brain tumor biology. Most of the therapies failed due to the unique genetic makeup of these brain cancers [4] and this unique genetic constitute is because of epigenetic changes. Epigenetic modifications are molecular changes that occur in histone or the DNA sequences without affecting the DNA sequence [5]. Most of the assertive brain cancers such as glioblastoma, medulloblastoma, ependymoma, atypical teratoid rhabdoid tumors (ATRT), diffuse intrinsic pontine gliomas (DIPG) and embryonic tumors with multilayered rosettes (ETMR) show somatic mutations and structural variations in the genetic makeup [5].

The epigenetic changes here was the loss of 5-methylcytosine (5mc), hypo-methylation at repetitive elements, over-expression of methyltransferase, methylation at CpG Island and chromatin remodeling. In fatal cancers, these changes occur at DNA repair genes like BRCA1 and BRCA2 or tumor suppressor genes like p53 and Rb that affect the protein expression. In this review, we focused on DNA and Histone modifications that affect the pathogenesis of various brain cancers and also the importance of irradiation in the epigenetical changes of brain tumor. Here we reviewed the topic by using research article

ranging from 1990-2019, we have tried to collect almost all research articles related to brain tumors and epigenetics.

2. Epigenetics of brain tumor:

Epigenetics is the change in the gene expression without affecting the actual DNA sequences [6]. Epigenetic modifications involve mainly DNA methylation, histone modification and microRNAs modification [6], which leads to various conditions like inflammation [7], cancer [4], neurological disease [8], cardiovascular disease and autoimmune diseases [9]. In gliomagenesis, mutations in regulatory genes including histone demethylases (JMJD1A and JMJD1B), Histone deacetylases (HDAC2, HDAC9) and histone methyltransferases (SET7, SETD7, MLL3, MLL4), methyl-CpG binding domain protein 1 (MBD1) are prominent histone changes that affects [9]. Hypermethylation of the CpG island promoter affects the cell cycle checkpoints and DNA repair mechanism, the metabolic process of carcinogen, cell-to-cell interaction, apoptosis, and angiogenesis [10]. Studies have reported that glioma patients have a greater survival rate whose tumor expressed a lower level of H3K18ac i.e. <74% of tumor cells on contradictory astrocytoma patients have reduced survival rate when the acetylation of H3K9 is lesser than 88% [11]. In pediatric brain tumor, H3.3K27me upregulates the level of PLAG1, LIN28B, and PLAGL1, which are referred to as stem cell-associated genes such and reduced expression of these genes inhibited tumor cell growth [12]. Further H3.3K27M mutation also decreases H3K27 methylation and increases H3K27 acetylation, which affects the H3.3K27M–containing nucleosomes leading to heterochromatin silencing through increased production of bromodomain-containing protein 1 (BRD1) and bromodomain-containing protein 4 (BRD4). A prominent mutation in pediatric glioma is valine or arginine (G34/V) replaced by glycine 34 in histone H3: this is associated with DNA hypomethylation [13] interfering the transcription of MYCN gene [14] and similarly alternate of lysine 27 by methionine in the Histone H3 protein contributes to

tumorigenesis (15). In medulloblastoma, there is an interaction between H3K27ac and BRD4-DNA (Bromodomain Containing 4 sequence), which strongly correlate at active medulloblastoma enhancer loci that activates transcription of an oncogene [15]. Atypical Teratoid Rhabdoid Tumor (ATRT) defined by the dearth of the chromatin remodeling protein SNF5 (SMARCB1) [16] results in prominent expression of the Polycomb gene EZH2. These Polycomb genes are responsible for neuronal stem cell self-renewal. EZH2 shows an increased level of expression in both adult and pediatric brain tumors, in ATRT the molecules targeted by polycomb are widely methylated at H3K27 and down regulated. The reversing effects of SNF5 is achieved in ATRT cells by inhibiting histone deacetylases (HDACs) through rebuilding acetylation of histone H3 and H4 in the promoter region [17].

In addition to histone acetylation, histone methylation also plays a critical role in brain tumor, there are some research finding that proves. IDH1 or IDH2 genes, which encodes for isocitrate dehydrogenase enzymes of the citric acid cycle, are reported to be mutated in lower grade diffuse gliomas and secondary glioblastoma of adults, but are lesser seen in young children [18]. A substitution mutation in IDH1R132H and IDH2R172H inhibits a wide range of histone demethylases through the production of oncometabolite 2-HG [18, 19 & 20]. These IDH1/2 mutations have an impact on histone methylation and are associated with DNA hypermethylation in cancer (21, 22). GSKJ4 was a known small molecule inhibitor of JMJD3 (a histone H3K27 demethylase) on admitting the tumor cells showed a decreased cell viability and increased the level of H3K27me3 in K27M glioma cell lines and also K27M mutant glioma xenografts prolonged the survival rate of mice [23]. Another importance of histone methylation in glioma is H3K36 trimethylation, which induces transcription of RRM2, a ribonucleotide reductase subunit responsible for the establishment of deoxyribonucleotide from ribonucleotide which is reduced on H3K36 methylation results in low level of dNTP for DNA replication and provokes apoptosis [24]. Regulation of gene

expression depends on histone lysine methylation, in which K27 is either methylated or acetylated [25]. Mutation in K27 may lead to increased trimethylation that downregulates the tumor suppressor gene transcription [26]. Redistribution of H3K36 methylation happens in glioma G34R/V mutation that alters the gene transcription, and noticeable upregulation of the MYCN (V-Myc avian myelocytomatosis viral oncogene neuroblastoma-Derived Homolog) oncogene [27]. MYCN expression also noticed to be high in diffuse gliomas when histone H3K36 trimethylation (H3K36me3) reduced within the nucleosomes that contain an H3.3 G34R/V mutated tail [28].

Polycomb repressive complexes PRC2 and PRC1 mainly perform gene repression through demethylating or trimethylating H3K27 [29]. The PRC2 complex accomplishes this histone methyltransferase activity with its enzymatic subunits [29]. In ependymoma, current research identified the impact of PRC2 on the poor prognosis of hindbrain ependymoma, where PRC2 activity was found to be at the peak and that is associated with increased trimethylation of H3K27 leading to tumor suppressor gene silencing [30]. The tumor formation in H3.3 K27M mutated High-Grade Gliomas is motivated by chromatin modifications and this modification resulting from loss and gain of H3K27 methylation at different gene loci [31]. An experiment was done in G34R/v Mutated Pediatric HGG by mutating the H3.3-ATRX- DAXX chromatin remodeling pathway and showed that H3F3A/ATRX-DAXX/TP53 mutations showed a change in the length of telomere without the aid of telomerase activity, suggesting that this responsible for the uncontrollable cellular division in pediatric glioblastoma [31]. Thus, this knowledge of histone acetylation and methylation helps to develop new therapies that target specific molecules responsible for epigenetic changes of various brain cancers.

3. Epigenetic changes during irradiation of brain tumor

The advanced stage of brain cancers treated with radiation therapy, where high energy

ionizing radiations are used to kill the cancer cells [32]. Apart from treating cancer cells, these radiations also cause brain injury such as cognitive impairment, leukoencephalopathy, inhibition of neurogenesis and dementia [33]. Radiation also disrupts the Blood-Brain Barrier (BBB), which set as detection of radiation-induced brain injury [34]. There was a change in the integrity of BBB on irradiating the rat brain with gamma rays at the dose of 20-40 Gy [34], this was also proved by Zhou et al., where they concluded that BBB of rats got disrupted at the 4th week on irradiation with 40Gy of gamma rays. These disruptions increased the permeability of BBB and also increases the water content of the BBB [35]. Reactive oxygen species (ROS) produced by radiation is said to increase the expression of pro-inflammatory factors TNF-alpha, IL-1beta and also increases the mRNA levels of several chemokine's mainly CCL2, Gro/KC, CXCL9, CXCL10 and CXCL11 [36, 37, 38, 39]. Radiofrequency electromagnetic radiation can also induce DNA double-strand breaks in rat brain cells after exposure of 2450 MHz for 2 hours [40]. Ionizing radiation affects the brain by causing cognitive decline, memory deficits, fatigue, and brain tumors [41]. Patients with hereditary disorders such as ataxia-telangiectasia or Nijmegen breakage syndrome showed an increased radiosensitivity, and research says that these genetic defects are key responsible for severe radiotherapy side effects such as ulcerative oropharyngeal mucositis, febrile neutropenia in some brain tumor patient [42].

On a molecular level, bystander effects of radiation clearly showed increased DNA damage, mutations, change in gene expression, and alters the levels of cellular proliferation and apoptosis, which are tightly regulated by epigenetic modification including DNA methylation, demethylation and histone acetylation [43]. Whitefield and coworkers found that DNA methylation amplified in the range of 15% in E.coli 15T-(555-7) upon radiation exposure [44]. Rakova et al., [45] conducted an experimented in Wistar and outbreed rats are irradiated in bone marrow and thymus regions with 6.5 Gy and 7 Gy by using 60Co γ

radiation for evaluating the 5-methylcytosine (5-mC) and concluded that radiation-induced global DNA methylation is tissue-dependent and species-dependent [46]. Kalinich group estimated the epigenetic response in the following four different cell lines that describing four different tissues, for neural they tested with mouse neuroblastoma cells (C-1300N1E-115), estimation in lungs and ovary was done using Chinese hamster lung fibroblasts (V79A03) and Chinese Hamster Ovary (CHO) respectively. In cervical cancer with human HeLa (S-3) cells, all these cells were subjected to radiation with dose ranging from 0.5 Gy to 10 Gy of γ radiation with 60 Co as the source. Later estimation at the end of 24h, 48h, and 72h after irradiation, they found a global loss of DNA methylation in all four cell lines with a prominent decrease in 5-mC in a dose-dependent manner [47]. Further, the estimation of global DNA methylation in *vivo* was done using X-ray and concluded that tissues exposed to radiation higher than 1 Gy would subsequently result in the loss of global DNA methylation in tissues such as the spleen, thymus and mammary gland [47]. Apart from global DNA methylation, ionizing radiation also causes gene-specific DNA-methylation. Studies by Su et al., verified a noteworthy DNA hypermethylation of cyclin-dependent kinase 2A, and O-6-methylguanine-DNA methyltransferase (MGMT) genes in the sputum of uranium miners [48]. The human-hamster hybrid cell line exhibits a loss of LINE-1 DNA methylation when subjected to 2 Gy of X-rays. A Similar loss in LINE-1 methylation was detected in the spleen tissue when seven months old rat irradiated with 20 Gy of X-rays in the cranial region, which was later found to be related to LINE-1 reactivation [49], leading to genomic instability and tumor progression [50]. Further the same was reported by Goetz and the group in human colorectal carcinoma cells where they exposed the RKO cells to 1 Gy of X-rays at the rate of 2.4 Gy/min, on the other hand when AG01522D primary human diploid skin fibroblasts on exposure to the same Gy of X-ray at the same rate exhibited hypermethylation of LINE-1 [51]. Though there are as many studies done on whole radiation exposure, clinical

radiotherapy imparts fractionated exposure. Only certain studies reported the effect of fractionated radiation exposure. Fractionated exposure of 0.5 Gy given for ten consecutive days also resulted in the loss of global DNA methylation in mice but the phenomenon was sex-specific, the loss was identified only in male mice, not in females. Fractionated doses also affected the levels of DNA methyltransferases Dnmt1, Dnmt3b and methyl-binding protein MeCP2, which sets as a proof for DNA hypomethylation [52]. The radiation-induced histone modification changes in brain tumor have not been known much and that is a hot spot of research for the future.

4. Radio sensitizers role in radiation-induced epigenetic changes in brain tumor

Radiation therapy plays an important role in the treatment of cancers, however, the major problem encountered is the recurrence of cancers as the cells become radioresistance. There are many mechanisms proposed for radioresistance, primary among was hypoxia. In hypoxia condition, the Linear-Energy-Transfer of mitochondria gets disturbed due to low concentration of oxygen, which decreases the formation of peroxide [53]. Hence to increase the efficiency of radiotherapy radiosensitizers are used. Some of the radiosensitizers used in brain cancer are Topotecan, Gemcitabine, Efaproxiral, Temozolomide (TMZ), Imatinib [54. Topotecan inhibiting topoisomerase I causing irreversible DNA damage and also it is capable of crossing the blood-brain barrier and improves radiotherapy effect on brain cancer cells. Research also proved that DNA lesions could be achieved along with radiation by directing the DNA alkylating agent, BO-1051, towards glioblastoma cells, which accumulates the G2/M population. Cells are more sensitive to radiation in the G2/M phase and the same mechanism is followed by paclitaxel, indomethacin, 2-methoxyestradiol and TMZ [55]. Other than these synthetic organic compounds gold nanoparticles are also used to increase the sensitivity of hela cells exposed to gold nanoparticles and irradiated with X-ray 220 kV and found an increased expression of γ-H2AX and 53BP1 proteins that are responsible for DNA

double-strand break [55]. Albendazole is another effective radiosensitizer that causes DNA damage through phosphorylation of H2AX [57]. Krista Van Nifterik et al., [58] tried to enhance the sensitivity to gamma-radiation of D384 (astrocytoma grade III) and T98 cells by pretreatment of valproic acid with TMZ. They contradicted the hypothesis that VPA acts as antagonist for TMZ by demethylating the promoter of MGMT gene and producing MGMT proteins (O6-methylguanine-DNA-methyltransferase) thus making resistance of cells to TMZ. The DNA methyltransferase inhibitors can also act as a radiosensitizer when compared to other small molecule inhibitors [59]. Giovanni L Gravina et al., suggested that the reason for choosing DNMT inhibitors is that these agents can induce radio sensitization, at lower concentrations compared to the concentrations available in plasma [60]. Another small inhibitor Zebularine enhances radiosensitivity in glioblastoma cells by inhibiting DNA methylation and also inhibits DNA repair, which was evident by the expression of γH2AX [61]. Following table 1 shows the list of radiosensitizers which are in various phases of clinical trial available to date.

5. ROS generation during radiation-induced epigenetic changes in brain tumor

In normal physiological conditions ROS produced as a byproduct during aerobic respiration that serves as an essential signaling molecules that regulate numerous cellar processes, for example regulating inflammation, aging and cancer cell proliferation [62]. These ROS causes biological damage when the balance between free radicals and the anti-oxidant system gets disrupted [63]. Ionizing radiation (IR) is one such cause for the over-production of ROS. The Major source of free radicals in cells is water, which constituents about 80% of cells. Water molecules absorb these radiation and get ionized to form free radicals. The products produced in the radiolysis of pure deaerated water are $^{\bullet}OH$, $H^{\bullet}$, H_2, H_2O_2 and other free radical species. Ionizing radiation is capable of producing reactive nitrogen species by stimulating nitric oxide synthase, thus producing nitric oxide (NO^{-}).

Nitric oxide reacts with superoxide anion ($O2•^{-}$) form the peroxynitrite anion ($ONOO^{-}$) is also highly reactive and capable of attacking cellular targets, including lipids, thiols, proteins, and DNA bases. But these free radicals generated from water has only a short life span of 10^{-9} s [64]. The late production of radiation-induced ROS brought about by mitochondria. Mitochondrial DNA (mtDNA) are directly affected by IR, leading to dysfunction of mitochondria by increasing the DNA copy number further increase in the production of protein. This over-production may affect the proper function of electron transport chain (ETC). This disruption gives pro-oxidants as by product. This oxidative stress causes oxidative damage to all biological components after a long duration of radiation exposure.

ROS plays an important role in epigenetic changes during aging and cancer [65]. ROS affects DNA demethylation by HIF-1alpha regulated lysine demethylase, DNA oxidation and TET-mediated hydroxymethylation (5hmc). ROS can indirectly modulate the activity of histone-modifying enzymes depend on intracellular levels of essential metabolites, such as Acetyl-CoA, Fe, ketoglutarate, NAD^{+}, and S-adenosylmethionine [1]. Some experiments proved that ROS could cause DNA hypermethylation, Lim So and the group [66] identified increased methylation at the promoter region of E-cadherin gene through the recruitment of DNMT1 and HDAC1 by Snail that brings about the down-regulation of the E-cadherin protein expression In human HCC cells [67]. Another group also proved hypoxia-induced hypermethylation in isolated fetal rat hearts and cardiomyocytes [68]. Interestingly, ROS also reduces the hydroxymethylation (5hmc) which directly increases methylation (5mc) by decreasing the availability of Fe (II) or ascorbate, that in turn decreases the activity of TET proteins and demethylase enzyme activity is disrupted [68]. In contrast, ROS also causes DNA hypomethylation by inhibiting the binding of methyl-CpG binding protein (MBP) 2, a critical epigenetic regulator that directly affects the DNMT and histone HDAC recruitment to the DNA [67]. ROS oxidizes guanosine to 8-oxo-2'-deoxyguanosine (8-oxodG) which

prevents the methylation of cytosine, leading to hypomethylation and transcriptional activation [69]. All tumors exhibit global DNA hypomethylation and is thought to induce genomic instability and activation of proto-oncogenes. ROS affects the histone methylation by affecting the histone methyltransferase enzyme, SET7 domain [70], which was evident in various diseases like diabetes, stroke, hypertension, cancer [71,72 & 73]. Similarly, ROS also modulates histone acetylation and deacetylation through interacting with HAT and HDCA, respectively [74, 75]. Thus, during brain tumor ROS acts via epigenetic mechanism creates a lot of complication for treating brain tumors.

6. Transcriptome heterogeneity and epigenetics in Glioblastoma

Diverse genetic and epigenetic mechanisms drive Glioblastoma. It is important to note that extensive intra and inter-tumor heterogeneity and associated tumor microenvironments also contribute to the progression of glioblastoma. Vascular niche, in addition to microenvironmental factors, also affects the distribution of tumor cells. Tumor Treatments mainly Chemo- and radiotherapy are known to enrich for GSCs due to the heterogeneity among patient tumors and within the same tumor, there is no unique marker that can distinguish the GSCs from non-GSCs was the major problem [78]. The distinct genetic alterations present in the individual tumors originating from the same organ contribute to inter-tumor heterogeneity. Various studies have demonstrated the intra-tumor heterogeneity and corresponding functional alterations in glioblastoma. Parker et al., reported intra-tumoral heterogeneity at the mutational, transcriptional and epigenetic levels based on multiple spatially distinct biopsies from different glioblastoma tumors [79]. A noticeable high frequency of extrachromosomal DNA amplification reported in different cancer types, including Glioblastoma which can increase oncogene copy number and intra-tumor heterogeneity [80, 81]. The recent developments in next-generation sequencing (NGS)-based applications and the availability of comprehensive databases such as TCGA (The Cancer

Genome Atlas) enabled the accumulation and integrative analysis possibility of large scale genomic, transcriptomic and epigenetic data from genome-wide experiments. An earlier gene expression-based molecular classification of TCGA data suggested four subtypes of GBM, which includes Proneural, Neural, Classical, and Mesenchymal [82]. In general Proneural subtypes includes *IDH1* mutations, *Tp53* mutations and *PDGFRA* abnormalities, which are mostly associated with secondary GBM [82, 83]. Classical subtypes associated with a cluster of most common genomic aberrations with 95% showing EGFR amplification and 95% with a homozygous deletion of ARF locus. This classical subtype in contrast to Proneural, lacks abnormalities in TP53, PDGFRA and others [82]. The mesenchymal subtype characterized by increased expression of *CH13L1* and *MET* high frequency of NF1 mutation/deletion and low levels of NF1 gene expression [83]. One more feature of GBM is its intratumoral genetic heterogeneity, it is challenging for our understanding of the pathology of the diseases and our ability to effect meaningful therapeutic responses to targeted agents. Cameron et al., reported EGFR was the most frequently mutated gene in GBM clinical samples and also RNA-seq detected a diversity of mutated transcripts [84]. The whole-exome and transcriptome sequencing data confirmed the major mutations in GBM are PI3K pathway (*EGFR, PDGFRA & NF1*), p53 pathway (*MDM2* & *TP53*) and the Rb pathway (*CDK4*, RB1and others) [82]. The cytokines mainly, IL-6 are induced by mutated ΔEGFR-expressing cells to act upon nearby cells by paracrine fashion expression well amplified wEGFR (wild). If the tumor cells are co-expressing both ΔEGFR and wEGFR genes autocrine mechanism may also operate in some tumor conditions and this might be because of regions of predominant wEGFR-only expression and also cells would be benefited thus maintains both paracrine and autocrine mechanisms coexist to maintain receptor heterogeneity in GBM cells. The cytokine IL-6 also increased to form neurosphere self-renewal in GBM [82]. IL-6-cytokines was a well-known potent activator of the JAK/STAT3 pathway and the inhibition of cytokines induces the

apoptosis in GBM cells [85]. The possible mechanism here is cytokines activate gp130, which was believed to activate wEGFR in surrounding cancer cells, leads to an increased overgrowth of tumor; thus minor tumor cell population can actively drive the accelerated growth of total tumor mass. The genome/transcriptome sequencing of glioblastoma samples by Reifenberger et al., report the important role of methyl guanine DNA methyltransferase (MGMT) promoter methylation and isocitrate dehydrogenase 1 or 2 (IDH1/2) mutation in glioblastoma long-term survival [86]. A recent effort to understand the heterogeneity of glioblastoma by an integrative approach involving single-cell RNA-sequencing of tumors, large scale genetic and expression analysis of TCGA specimens, functional approaches, and single-cell lineage tracing revealed the existence of four main cellular states that recapitulate distinct neural cell types. Those cell types were influenced by the tumor microenvironment, and exhibit plasticity [87]. The comprehensive cataloging of somatic alterations of glioblastoma was done by Brennam et al., through different omics-based profiling such as the genome, exome and RNA sequencing copy number, transcriptomic, epigenomic and targeted proteomic profiling [88]. Such large scale integrated approaches facilitate the discovery of biomarkers, understanding the mechanism of disease propagation, and generating novel hypotheses for further validation. A correlation between dysregulated DNA methylation with gene expression and clinical prognosis observed in genome-wide methylomic and transcriptomic analyses lead to the identification of subtype-specific epigenetic signatures in glioma stem cells and glioblastoma [89]. A multi-platform transcriptome-methylome-genome study by Binder et al., revealed large molecular heterogeneity in WHO grade II/III gliomas and split into eight expression and six methylation subtypes and the subtypes differ in overall promoter methylation, WHO grade and prognosis(9). Significant past work described above indicating that genetic alterations, gene expression changes, and associated epigenetic modifications clearly underlie glioblastoma heterogeneity and can impact the sensitivity to

radiation [90]. Ke. C et al., reported that the radiation-induced changes in the redox and metabolic state of subgroups of glioma can alter the radiation sensitivity and possible to impact the radio curability [91]. However, the proof of actively maintaining tumor cell heterogeneity during irradiation to brain cells is unclear and Investigation of such interactions provides a new avenue for therapeutics of Glioblastoma. Moreover, such intratumoral heterogeneity may be because of interactions between tumor cells, genomic imbalance at the transcriptional, translational and post-translational level needs to be investigated.

7 Epigenetics of the microenvironment of brain tumor:

Tumor microenvironment comprises mainly contains non-cancerous cell types in addition to tumor cells including ontogenetically distinct macrophages, tissue-resident microglia and also bone marrow originated macrophages. In the case of Glioblastoma treatment even after surgery, radiation therapy and Temozolomide therapy, the average life expectancy is only around 14 months [92]. Primary solid tumors with the simple score showing increased degree of intra-tumor transcriptional heterogeneity also give rise to microenvironmental factors which plays an important role in molecular plasticity. The integrating factors of single-cell analysis of new TCGA classification and microenvironmental factors classified the subtypes into three with mesenchymal subtype with the highest among the GBM with around ~34% of the samples [93]. The tumor cells with Proneural subtype may die for chemo/radiotherapy response, while the mesenchymal subtype develops resistance and dominate the recurrence tumor. This is because of tumor micro-environment of immune cells and vasculature components of the tumor. The GBM patients with classical and mesenchymal subtypes are more resistant to the chemo- and radiotherapy treatment than the Proneural which was sensitive due oligodendroglia characters. GBM is mostly driven by Tumor-initiating cells (TICs), which remain after surgical procedure and are resistant to radiation therapy and chemotherapy. The Tumor microenvironment plays a key role in controlling the spread of

solid tumors by TIC multipotency. Tumor microenvironment directly controls the pronuclear subtypes derived TIC acquires MES signature via NF-κB dependent pathway or TNF-α pathway with increased expression of CD44 and also shows increased radioresistance [88, 95]. The preclinical data on GBM patients shows that radioresistant GSCs expressing specifically $CD133^{+}$ cells activate the DNA damage checkpoints and develop radioresistance both *in vitro* and *in vivo*. It was demonstrated that the GSCs with mesenchymal subtype are more radio-resistant than Proneural subtype. After irradiation treatment of GBM samples shows PMT (Proneural-mesenchymal transition) within a short time of post-irradiation treatment with NF-κB, STAT3, P53 and snail has its mediators. Radiation treatment also induces mesenchymal markers like α-SMA, MMP2 and MMP9, while downregulating Proneural markers, mainly PDGFR-α. Radiation treatment upregulated CEBPB and mesenchymal markers (CD44, α-SMA/ACTA2, VIM, FN1, COL1A1 and COL1A2, MMP2, MMP9, and YKKL-40), while downregulating Proneural markers (SOX10 and PDGFR-α) [94, 92]. The PMT induced human GBM cells after post-irradiation treatment shows increased cell invasion, cell motility, chemotherapy resistance and radioresistance properties compared to non-irradiated glioma cells. In a mouse model of glioma, chemotherapy and radiotherapy cause the release of HMGB1 from the damaged tumor cells into the tumor microenvironment, which acts via TLR2 to increase the anti-tumor activity [96]. Tumor Microenvironment also decides the molecular fate of TICs by molecular class switching upon tumor rebound. It was expected that microenvironment significantly modifies the epigenetic landscape of glioblastoma cells with unknown mechanism. Hypoxia induces histone methyltransferase MLL in glioblastoma cells and its loss decreases the HIF expression and targets. This epigenetic mechanisms of HIF1α induced during hypoxia may promote the TIC self-renewal and also enhances tumor growth. Studies has shown that the perivascular niche (PVN) in Glioblastoma serves as a glioma stem cell reservoir, which acts as tumor-initiating

cell pool. The astrocytes in the PVN induces osteopontin, which enhances radiation resistance and also forms $CD44^+$ glioma stem cells leading to enhanced cancer growth. The epigenetic chromatin modifiers EZH2 and BMI1 show differential expression level in two GBM subtypes mesenchymal and Proneural. EZH2, which is well-known histone methyltransferase known to catalyze H3K27me3, shows a higher level of expression in Proneural subtypes than mesenchymal and classical subtypes [92, 97]. The survival data on GBM patients showed that patients with low EZH2 and *BMI1* expression have the best life expectancy, while those with increased expression of both EZH2 and *BMI1* have less survival rate. The epigenetic profile of mesenchymal and Proneural subtype GSCs were different from their parental cancer tissues. These reports indicate that patients with more heterogeneous tumors impact the survival of the patients along with epigenetics and microenvironmental factors.

8 Conclusion and future perspective

We conclude that brain tumor biology and pathophysiology is complicated and involves various epigenetic factors that can influence tumor development. From the studies over the past decade, it has become clear that brain tumor epigenetics along with heterogeneity and microenvironment factors are fundamental regulator of tumor progression and treatment efficacy. It will be very necessary to advance our current knowledge in understanding the tumor resistance developed during standard care, especially radioresistance has become big problem and to overcome that, our knowledge on the molecular aspects are limited. The complete understanding of epigenetics of various subtypes and their transitions during various stages of tumor gives idea to target therapeutically. The epigenetics knowledge of complex microenvironmental landscape and heterogeneity of Glioblastoma is very limited. Radiation therapy employed all over the world to treat the various cancer type. Most of the cancer patients need radiotherapy during course the course treatment. Here we reviewed

different epigenetic aspects that are affected upon radiation in relation to brain cancer. The small molecule inhibitors like HDAC inhibitors, HME inhibitors are already employed in most of the cancer treatments like lung cancer treatment and radiation-induced toxicity also reduced by the HDAC inhibitors like Trichostatin A has been shown and reported. In a general point of view, we need to improve active research on drug delivery, the standard of care response to therapy for all brain malignancies. The current field of brain tumor and radiation research is not much explored much and much research is needed to much unidentified stuff to the scientific community.

Methodology for review

Articles referenced in this review articles were identified by a literature search in Web of Science and PubMed using various terms including gliomas, brain tumor, epigenetics and others selected topic in relevance to narrow focus of this short review.

ABBREVIATIONS

BRD4: Bromodomain-containing protein 4

GBM: Glioblastoma

PRC; Polycomb repressive complexes

PMT: Proneural Mesenchymal transition.

ROS: Reactive oxygen species

HDAC: Histone Deacetylases,

HME: Histone methyltransferase enzyme

H3K927me3: Histone 3 lysine 27 trimethylation

G34R/V: Glycine 34 to Arginine or Valine Substitution

GSCs: Glioma stem cells

TICs: Tumor-initiating cells

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CONFLICT OF INTEREST:

No conflict of interest

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Table 1: Radio sensitizers used for various cancer

RADIOSENSITIZER	CANCER TYPE
Metformin	Lung Cancer(70)
Capecitabine	Rectal cancer (19)
Nedaplatin	Cervical cancer(28)
5-Fluorouracil	Pancreatic cancer(18)
Gemcitabine	non-small cell lung cancer (16)
panobinostat	Bladder cancer(31)
Cordycepin	Oral cancer(76)
Vicenin-2	non-small cell lung cancer (2)
Resveratrol	breast cancer(11)
Erlotinib	Brain cancer(77)

Figure captions:

Figure 1: Generation of ROS on radiation exposure: There are three main routes by which ROS is generated in a cell. ROS can be produced in the cytoplasm either by radiolysis of water resulting in H^+, OH^- and eq^- or it may produce peroxynitrite anion ($ONOO^-$) by reaction of NO^- with nitric oxide synthase. Another source of ROS generation is from the mitochondria where the electron transport chain gets disrupted producing pro-oxidants as by-product.

Figure 2: Role of ROS on epigenetics: ROS produced from ionizing radiation is capable of interacting with lipid molecule, DNA and proteins. ROS can affect the methylation status of DNA either by directly interacting with DNA or interacting with the necessary enzymes like TET and DMNT. ROS also influences the chromatin packing by regulating the certain enzymes like HAT, HDAC and HMT leading to acetylation, deacetylation and methylation of histone respectively.

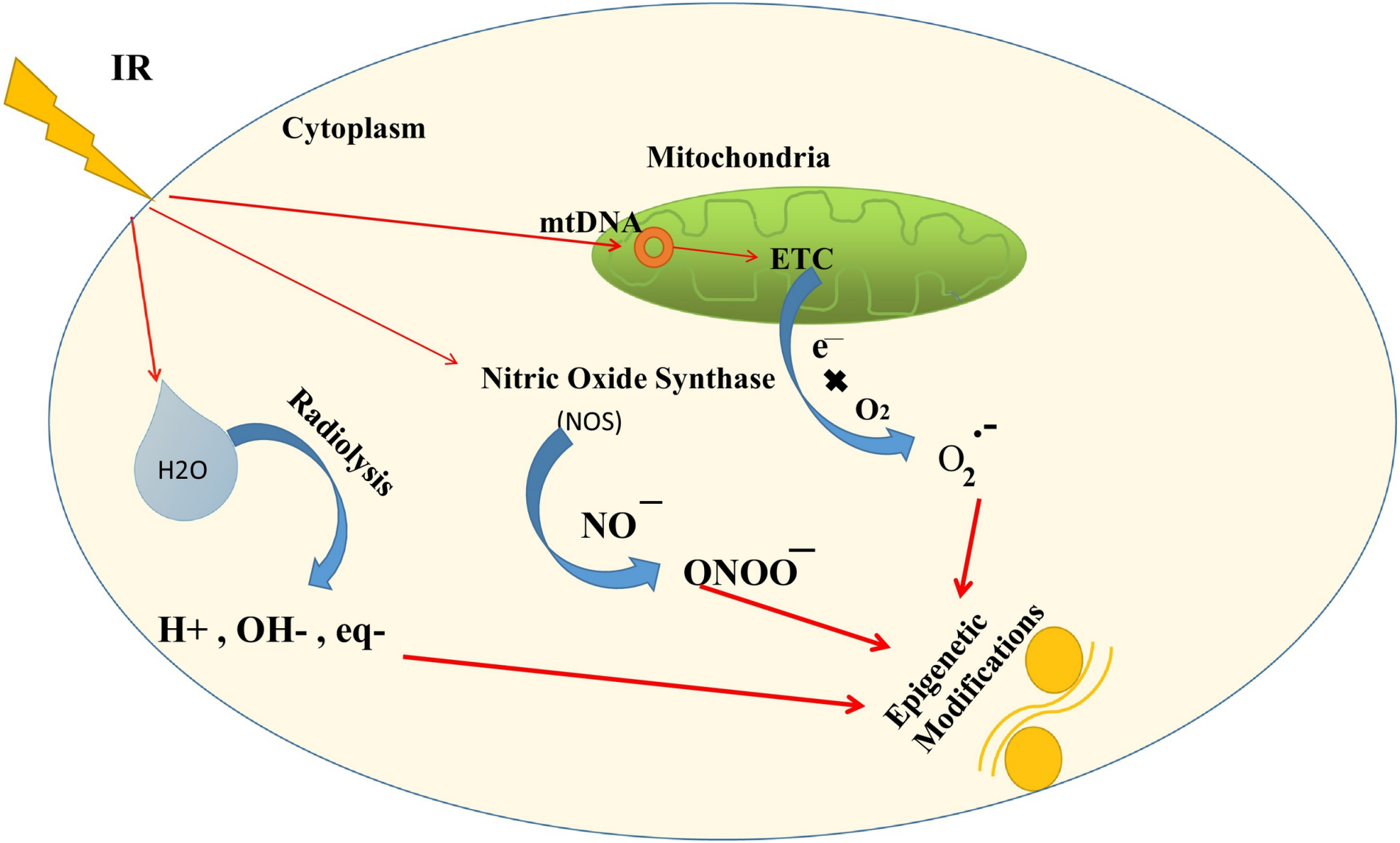
IR
Cytoplasm
Mitochondria
mtDNA
ETC
e⁻
O2
Nitric Oxide Synthase
(NOS)
H2O
Radiolysis
NO⁻
ONOO⁻
O2•-
H+ , OH- , eq-
Epigenetic
Modifications

Figure 1

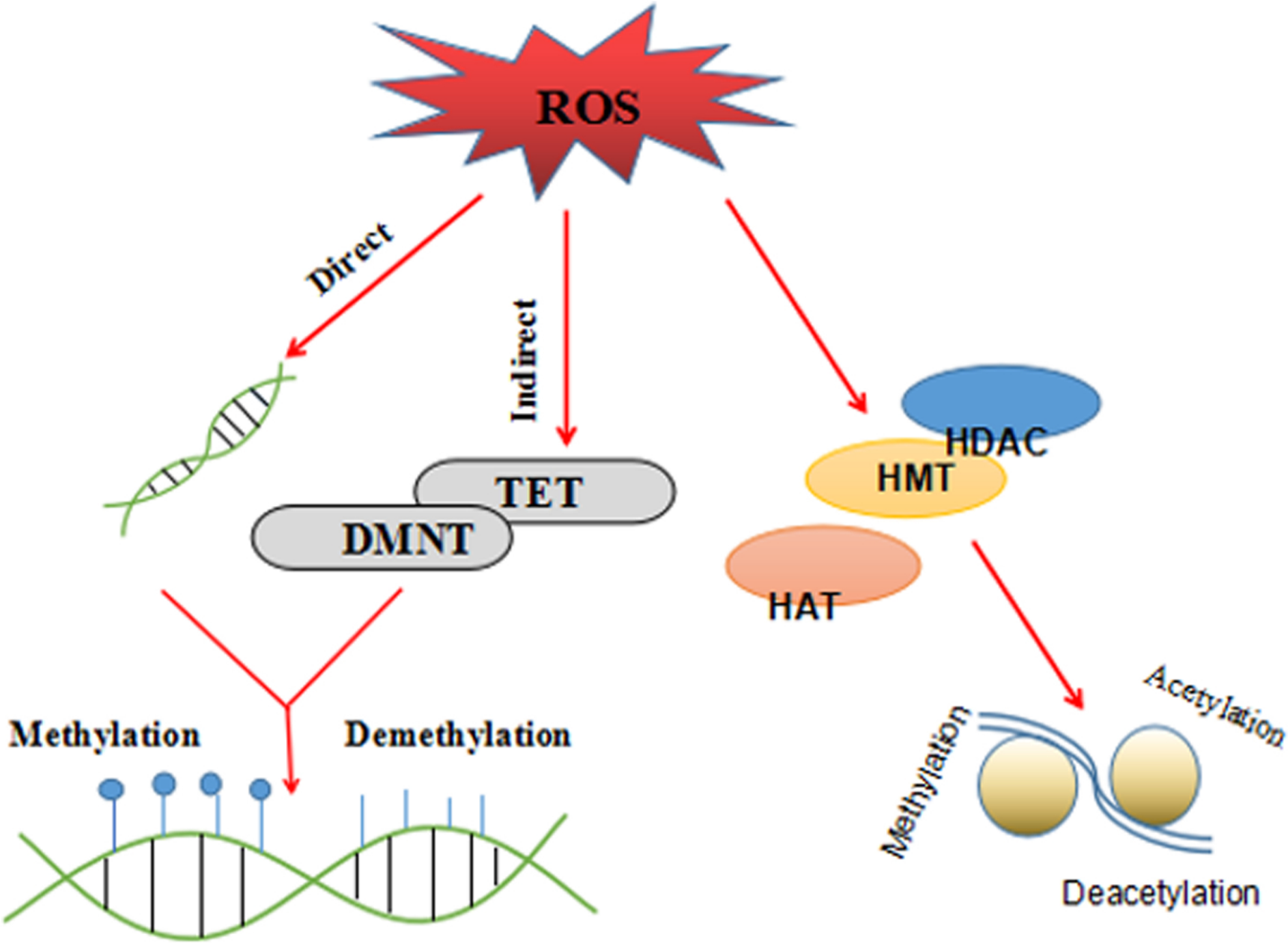
ROS
Direct
Indirect
TET
DMNT
HDAC
HMT
HAT
Methylation
Demethylation
Acetylation
Methylation
Deacetylation

Figure 2