Chapter

Molecular Classification of Diffuse Gliomas

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Abstract

In 2016 WHO classification of CNS tumors genotypic and phenotypic parameters were integrated to define a new nomenclature of diffuse gliomas on the basis of presence or absence of isocitrate dehydrogenase mutations. This resulted in more homogenous and narrowly defined categories with better accuracy of prognostic information, thus, playing a crucial role in patient management. Broadly, astrocytomas are now histologically and genetically distinct with IDH-mutant, ATRX-mutant, 1p/19q-intact and oligodendroglial tumors has IDH-mutant, ATRX-wildtype and 1p/19q-codeleted profile. Glioblastoma are now classified into primary and secondary on the basis of IDH mutations independent of clinical history.

Keywords: Diffuse glioma, astrocytoma, oligodendroglioma, IDH, 1p/19q codeletion, ATRX, TERT, EGFR, PTEN, CDKN2A, MGMT, H3F3K27M

1. Introduction

In 2016 World Health Organization (WHO) classification of tumors of central nervous system, there was a paradigm deviation from earlier morphology based classification of gliomas to a new classification and nomenclature by integrating the molecular and histomorphological parameters. This approach provided finely defined diagnostic categories resulting in better correlation with prognostic and treatment parameters. Now diffuse gliomas whether astrocytoma or oligodendroglioma are grouped together on the basis of their shared *IDH1* or *IDH2* mutation status. Oligodendrogliomas also show 1p/19q codeletion. So, in 2016 diffuse glioma category comprise of WHO grade II and III astrocyctic tumors, grade II and III oligodendroglioma and grade IV glioblastoma, IDH mutant and wildtype. Continuing evolving knowledge on pathology of glioma led to a pediatric midline glioma with mutations in histone H3 genes to be also included with these adult diffuse gliomas. This excludes astrocytoma with circumscribed growth pattern and lacking IDH mutations i.e. pilocycytic astrocytoma, pleomorphic xanthoastrocytoma, subependymal giant cell astrocytoma. So, diffuse astrocytoma and oligodendrogliomas are now nosologically more similar than are diffuse astrocytoma and pilocytic astrocytoma and family tree of tumors is being redrawn [1].

This new classification was testament to rapid advancement in the field of molecular biology and reducing cost, easy availability for masses in the present times. Now there is possibility of detecting some of these mutations on immunohistochemistry. This journey from discovery of isocitrate dehydrogenase mutations peculiar to gliomas leading to their radical reclassification and new taxonomy based on the presence or absence of mutations happened over a period of just 8 years [2].

2. Principle mutations

2.1 Isocitrate dehydrogenase mutations

Metabolism in cancer cells is rewired compared to normal cells since challenge is production more building blocks for proteins, nucleic acids rather than production of more ATP molecules as cell fuel. But very few tumors show mutations in genes directly involved in metabolic pathways. Isocitrate dehydrogenase (IDH) is an enzyme in the tricarboxylic acid (TCA) cycle of aerobic respiration and catalyzes the oxidative carboxylation converting isocitrate to α –ketoglutarate (α –KT). It exists in 3 isoforms i.e. IDH1, IDH2 and IDH3; isoenzymes are multiple forms of an enzyme catalyzing the same reaction but differ in amino acid sequence and kinetic properties (**Table 1**).

Only IDH 3 is a part of TCA cycle and dependent on nicotinamide adenine dinucleotide (NAD) as co factor [3–5].

Isocitrate + $NAD^+ \rightarrow$ α-ketoglutarate + CO_2 + NADH+ H^+.

IDH 1 is found in cytosol and peroxisomes while IDH 2 in mitochondria,both using nicotinamide adenine dinucleotide phosphate (NADP) as cofactor. They both catalyze reversible reaction and prevent oxidative damage by generating NADPH [2].

Isocitrate + $NADP^+ \rightleftarrows$ α -ketoglutarate + CO_2 + NADPH+ H^+.

In diffuse gliomas, heterozygous mutations seen in cytosolic IDH1 or mitochondrial IDH2 are considered as driver mutations [1–6]. Active site of both enzymes is formed by many arginine residues which is a polar amino acid. It forms hydrophilic bonds with isocitrate which is negatively charged. Most common mutation in diffuse gliomas is heterozygous point mutation at nucleotide position 395 of the *IDH1* replaces guanine by adenine G395A resulting in replacement of arginine by histidine (less polar amino acid) at amino acid residue 132 of the protein (R132H). However, new enzyme IDH1-R132H homodimer is not completely inactive despite losing a critical substrate-binding amino acid residue. It gets a neomorphic activity resulting in reducing α-KG to D-2-hydroxyglutarate (D-2-HG) and oxidizing NADPH to NADP+ [3–5].

This mutation was first discovered in 2008 when next generation sequencing was used to study 22,661 protein coding genes in 22 glioblastomas and 5 of them

	IDH 1	**IDH2**	**IDH3**
LOCATION	Cytosol Peroxisomes	Mitochondria	Mitochondria
COFACTOR	NADP+	NADP+	NAD+
STRUCTURE	Homodimer	Homodimer	Hetrooctamer (2 alpha, 1 beta, 1 gamma subunit)
REVERIBILITY OF REACTION	Reversible	reversible	irreversible
MAJOR ROLE	Prevent oxidative damage by generating NADPH	Prevent oxidative damage by generating NADPH	Catalytic role in TCA cycle (citric acid cycle)
GENES	IDH 1 gene: 2q33	IDH 2 gene: 15q26.1	IDH3A: 15q25.1–25.2 IDH3B: 20p13 IDH3G: Xq28

Table 1.
Properties of three isoforms of IDH.

showed expressed IDH mutations all at this same codon [2]. Subsequently, Yan and colleagues (26) analyzed IDH1 and IDH2 loci of nearly 1,000 central nervous system (CNS) tumors and found mutually exclusive mutations of IDH1 or IDH2 in more than 70% of WHO grade II and III astrocytomas and oligodendrogliomas and in secondary GBMs that developed from these lower-grade lesions [6]. Since then, numerous studies throughout the world substantiated the similar findings with heterozygous mutations in IDH 1 or less commonly in IDH2 been identified in nearly 74% diffuse astrocytoma WHO grade II, 59% anaplastic astrocytoma WHO grade III, all secondary glioblastoma, 76% oligodendroglioma WHO grade II and 67% anaplastic oligodendroglioma WHO grade III. Most common hot spot mutations identified are R132C, R132S, R132G, R132L in IDH1 and R172K, R172M, R172W and R172G in IDH2 [1, 5].

Mechanism of oncogenesis of IDH is still under research. These IDH mutations result in metabolic dysregulation in tumor glial cells affecting glucose sensing, glutamine metabolism, lipogenesis, and regulation of cellular redox status. In normal cells, IDH 1and 2 wild type (wt) are an important source of NADPH. They are responsible for nearly 65% of total NADPH production in the cytoplasm of IDH wildtype (IDHwt) glioblastoma [3–5]. Glioma cells overexpressing IDH1-R132H or other mutations in IDH 1 or 2 had decreased NADPH levels so consequently, levels of reactive oxygen species (ROS) increased and GSH decreased. Apart from role of ROS, increased production of D-2-HG also has its implications on the tumor cell. D-2-HG and α-KG are structurally similar; only differ for the C2-linked oxygen atom in α-KG, which is replaced by a hydroxyl group in D-2-HG. So D-2-HG causes competitive inhibition of α-KG–dependent dioxygenases thus exerting its direct oncogenic effects. Approximately 60 dioxygenases regulate diverse and important cellular processes by hydroxylating target acceptor proteins by using α-KG as the donor substrate. Important among these are prolyl hydroxylases that regulate hypoxia-inducible factor (HIF) 1α, chromatin-modifying enzymes like histone N-methyl-lysine demethylases and ten-eleven translocations (TET) 5-methylcytosine hydroxylases. The global histone demethylation by D-2-HG causes hypermethylation at a number of gene loci forming glioma-CpG island methylation phenotype and affect chromatin modification. D-2-HG also inhibits TET-mediated 5-methylcytosine hydroxylases levels which affect the expression of many regulatory proteins and possibly tumor suppressors that also contribute to tumorigenesis [5].

IDH mt gliomas can occur anywhere in the central nervous system but are preferentially located supratentorially in the frontal lobes [1, 7, 8]. Hence it is hypothesized IDH mt gliomas arise from a neural precursor population that is spatially and temporally restricted in the brain.

Since IDH mutations play a key role in tumourogenesis. Their diagnostic and prognostic role is well incorporated into routine neuropathology. IDH 1/2 mutations can be detected by Direct Sanger sequencing, pyrosequencing, allele-specific hybridization polymerase chain reaction (PCR), Real-time PCR, digital droplet PCR and high-throughput next-generation sequencing. IDH1 R132H accounts for nearly 90% of all IDH associated mutations in gliomas, so a monoclonal antibody has been developed against the mutant protein, allowing its use in paraffin-embedded specimens [1]. IHC IDH R132H mutation (clone H09) has shown sensitivity of 94% and a specificity of 100%. Positive staining is strong cytoplasmic and weak nuclear in tumor cells. Endothelial cells, perivascular lymphocytes, residual glial cells should be negative. Weak background staining and staining of macrophages is negative. Normal brain does not show staining for mIDH1 R132H IHC but granular staining in the neurons can be seen due to non specific binding to lipofuscin. One caveat to kept in mind while interpreting IHC is that macrophages can show strong cytoplasmic

granular staining even in IDH wt tumors. IHC is very useful for small samples where quantity of DNA extracted is too low for definite results by sequencing since it can highlight single infiltrating tumor cells [9]. IDH helps to separate gliosis (IDH negative) from low grade astrocytoma (IDH mt), if there is doubt in grade I and grade II glioma, IDH presence indicates we are dealing with grade II astrocytoma and also primary glioblastoma (IDH wt) from secondary glioblastoma (IDH mt). If IHC is negative, DNA sequencing is a must before calling a glioma IDH wt.

In all studies IDH mt gliomas have shown better improved progression free survival, longer time for treatment failure and extended overall survival in each of three treatment arms: radiotherapy, radiotherapy with PCV (procarbazine, lomustine and vincristine) or radiotherapy plus temozolomide [5]. So in addition to traditional good prognostic factors i.e. age < 40 years, lower tumor grade, tumor not crossing midline, absence of neurologic deficit before resection and tumor <6 cm, IDH mutation status has emerged as most important favorable prognostic factor in current times. Some studies have reported median survival of 10.9 years in IDH mt diffuse astrocytomas [1, 2, 6, 10].

Due to role of key role IDH mutations in glioma tumorogenesis, many isocitrate dehydrogenase inhibitors like hydroxypyridin-2-one, bis-imidazole phenol, tetrahydropyrazolopyridine are some of the drug under trails [5].

2.2 1p/19q codeletion

Nearly 60–80% of oligodendroglial neoplasms show co-deletion of 1p/19q: unbalanced translocation t(1;19)(q10;p10) after which only one copy of the short arm of chromosome 1 and one copy of the long arm of chromosome 19 remain and der (1;19) (q10;p10) is produced. It is hypothesized that translocation creates two derivative chromosomes, der(1;19)(p10;q10) and der(1;19)(q10;p10), and is followed by loss of the derivative chromosome containing 1p and 19q [11–16]. IDH wild type gliomas do not have 1p/19q codeletion.

Polysomy of 1p, 19q or both is also noted in a subset of oligodendrogliomas and has been associated with a poor prognosis, independent of deletion status [12, 13]. Oligodendrogliomas of grades II and III that have 1p/19q co-deletion also have a high frequency of TERT promoter mutations, CIC mutations on the remaining chromosome 1p allele and FUBP1 mutation on the remaining 19q allele (**Figure 1**) [14].

1p/19q codeletion are an essential part of molecular diagnostics of oligodendroglioma. Fluorescence in situ hybridisation (FISH), Cytogenomic microarray (CMA), Loss of heterozygosity and next generation sequencing are used to detect 1p/19q co-deletion. FISH is a reliable and validated most commonly used laboratory technique among these. Normal cells show a 2O2G signal (two test and two control probes, test: control ratio = 1.0). Loss of a test signal yields a 1O2G signal pattern (ratio = 0.5) and represents absolute deletion of a chromosome. Presence of aneuploidy, polyploidy and polysomy affects the interpretation since it become unclear what percentage of nuclei are displaying genuine co-deleted signals, so a ratio is calculated dividing total number of test signals by total number of control signals. Atleast 60 non-overlapping nuclei are counted and ratio < 0.8 should be both chromosomes for 1p/19co-deletion. Ratio of 0.75–0.90 is considered borderline [11].

PCR-LOH analysis can be used for borderline cases, it has better specificity than FISH because it tests for multiple loci in a single assay. But PCR is more labour intensive, requires more tissue, a higher proportion of neoplastic cells (at least about 70%) [11].

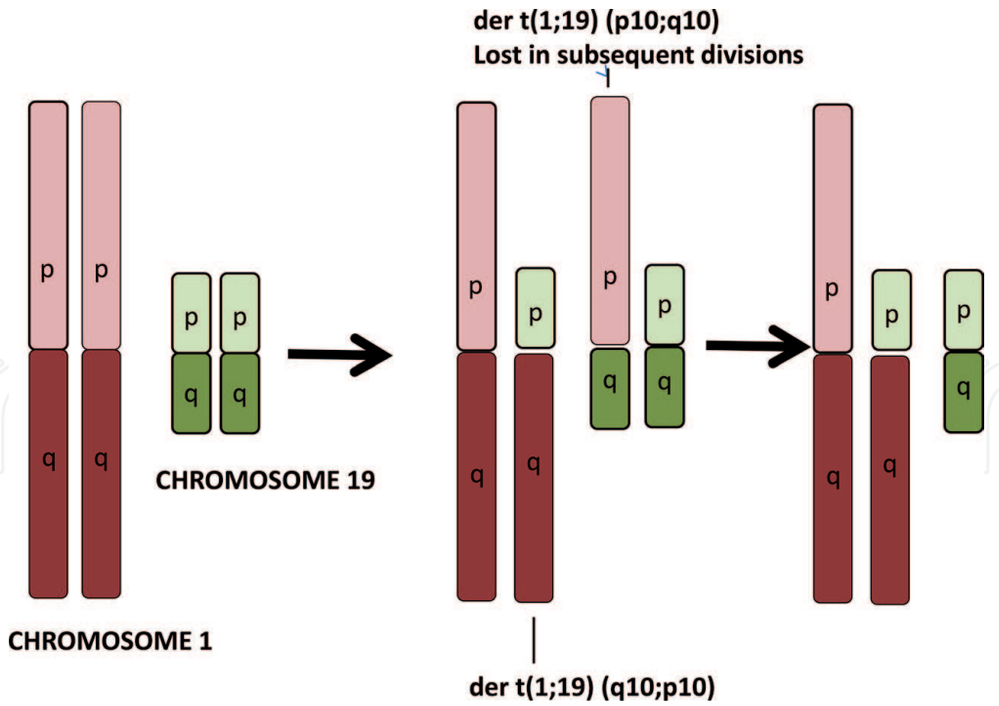


Figure 1.
Unbalanced whole-arm translocation between chromosomes 1 and 19 [t(1,19)(q10;p10)] resulting in 1p/19q codeletion in glioma(11,15,16).

The 1p/19q codeletion confers a favorable prognosis and is predictive of responses to alkylating chemotherapy and combination of radiotherapy and chemotherapy [1, 11–16].

2.3 Alpha Thalassemia/Mental Retardation Syndrome X-linked (ATRX)

Another critical marker that defined molecular classification of gliomas is ATRX gene present on Xq21.1. It is named so since it was first discovered through a study of assessing patients with the X-linked mental retardation syndrome presenting with α-thalassemia, severe psychomotor impairments, urogenital abnormalities, and patterns of characteristic facial dysmorphism. This gene encodes a protein which belongs to a chromatin-remodeling pathway (ATRX-DAXX) and is required for genomic stability by the incorporation of H3.3 at telomeres [17]. ATRX inactivation within gliomas can be due to mutations, deletions, gene fusions, or any combination of these. These mutations induce abnormal telomeres that are characteristic of a telomerase-independent telomere maintenance mechanism termed ALT (alternative lengthening of telomeres) [18].

Numerous studies have shown that ATRX mutations have a strong association with IDH mutations but never with 1p/19q codeletion. This property is exploited as diagnostic marker since ATRX inactivation indicates astrocytic lineage and rules out oligodendroglioma. ATRX mutations can be detected by direct Sanger sequencing, pyrosequencing, allele-specific hybridization polymerase chain reaction (PCR), Real-time PCR and high-throughput next-generation sequencing. ATRX IHC: clone CL0537 when show loss of nuclear expression >90% tumor nuclei is indicative of mutated ATRX. Nuclei of non-neoplastic cells such as endothelia, microglia, lymphocytes and reactive astrocytes are strongly positive and serve as positive internal control. When tumor cells show retained nuclear expression of ATRX IHC it indicates wild type ATRX [1, 17–20].

Low-grade glioma patients with ATRX retention and IDH mutations have lower progression-free survival and overall survival (OS) than tumors with 1p/19q codeletion and IDH mutations and longer time to treatment failure than those patients with IDH mutation and wild-type ATRX (55.6 vs. 31.8 months, respectively). Thus ATRX mutation infer a favorable prognosis to tumor [1, 19, 20].

2.4 TP53

Mutations of *TP53* are found in over 60–80% of infiltrative astrocytomas, anaplastic astrocytomas and secondary GBMs, yet are rare in oligodendrogliomas. There is a strong association between IDH1 mutation and *TP53* mutation in diffuse astrocytomas, and this combination of mutations is helpful in distinguishing astrocytomas from oligodendroglimas [1].

TP53 mutations can be analyzed by direct Sanger sequencing, pyrosequencing, PCR, allele-specific hybridization, real-time PCR and high-throughput next-generation sequencing. P53 IHC is also easily available and widely used. Immunostain reacts with both the normal and mutant forms of p53. Wild type P53 is rapidly degraded and has short half-life, hence is not detected by p53 IHC. Mutant p53 degrade more slowly, accumulate within nucleus of tumor cells creating a stable target for IHC. IHC detection of overexpressed protein is thus used as a surrogate method for mutation analysis, But it is not sensitive or specific. Over the last 25 years, studies have shown concordance rates between p53 IHC and TP53 mutation status ranging from 55 to 89% in grade I–IV gliomas [1, 21, 22].

TP53 alterations are usually missense producing stable full-length protein. Nonsense, frameshift, or deletion mutations results in incomplete translation of p53 gene producing a truncated protein product or loss of protein expression. This anomalous p53 structure may not be recognized during p53 IHC analysis resulting in false negativity. Some studies considered >10% tumor nuclei staining as positive for TP53 mutation status while others consider >50% as positive [21, 22].

2.5 *TERT* promotor mutations

One of the hallmarks of cancer is its ability to proliferate indefinitely. In normal somatic cells, the number of cell division is limited by the telomere length of chromosomes as it decreases with each replicative cycle. Cancer cells often overcome this limit by activating their telomerase. Telomerase consists of an RNA subunit and a reverse transcriptase catalytic subunit (TERT), which adds telomeric repeats to chromosome ends, therefore, maintaining telomere length. *TERT* gene on 5p15.33 encodes catalytic active site of telomerase and one of the mechanisms of telomerase activation in gliomas is somatic mutations in the promoter region of *TERT*. Most common mutations are C228T and C250T. The frequency of mutation was nearly 72% of IDH wt glioblastomas and in 95% of IDH mt oligodendrogiomas while relatively low in diffuse astrocytomas and anaplastic astrocytomas (19 and 25%, respectively) [23]. ATRX mutations are mutually exclusive of TERT gene mutations [1].

TERT mutations are detected by sequencing. IDH mutation, with 1p/19q codeletion and TERT mutation is characteristic of oligodendroglioma. TERT mutation in absence of IDH mutation indicates astrocytoma. In IDH wt gliomas, one with TERT mutation is associated with reduced overall survival compared to those lacking it. TERT mutation in IDH mt gliomas carries good prognosis [24].

2.6 EGFR

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase, whose ligands include EGF and TGF-α. Most frequently amplified oncogene in astrocytic tumors. EGFR amplification is seen in nearly 40% of primary/ IDH wt glioblastoma and rarely in secondary/ IDH mt glioblastoma [1]. There are also specific EGFR mutations (the vIII mutant), which produce a truncated transmembrane receptor with constitutive activity. Both EGFR amplification and the EGFRvIII mutant are mutually exclusive with IDH mutations [25].

2.7 PTEN

Loss of large regions at 10p, 10q23 and 10q25–26 loci, or loss of an entire copy of chromosome 10 is the most frequent genetic alterations in primary glioblastoma. It is specific for astrocytic differentiation and are rare in oligodendrogliomas [25].

2.8 *CDKN2A:*

Cyclin-dependent kinase inhibitor 2A (CDKN2A) is a gene located at chromosome 9, band p21.3. CDKN2A homozygous deletion is associated with poor prognosis among IDH-mutant gliomas [26, 27].

2.9 MGMT methylation

MGMT (O6-methylguanine-DNA methyltransferase) is a DNA repair enzyme and reverses the damage caused by alkylating agent temozolomide(TMZ) which adds methyl group at O6 position of guanine and this alkylation forms cross-links between adjacent strands of DNA. The MGMT protein rapidly reverses alkylation at the O6 position of guanine thereby averting the formation of lethal cross-links resulting in TMZ resistance. Promotor methylation of MGMT inactivates the gene so patients with MGMT promotor methylation are more benefitted with TMZ than patients without it [28].

MGMT promotor methylation is an essential part of molecular workup of all grade III and IV gliomas. Promoter methylation of O6-methylguanine–DNA methyltransferase (MGMT) is detected by methylation specific PCR, pyrosequencing or array based studies. MGMT determination by immunohistochemistry lacks standardization, reproducibility and, most importantly, correlation with clinical outcome so it is no longer recommended.

MGMT promotor methylation is commonly associated with IDH mutations and genome wide epigenetic changes (G-CIMP).

3. Diffuse gliomas

Difuse astrocytoma, IDH mutant, and WHO grade II: Diffuse astrocytoma composed of well differentiated fibrillary astrocytes in loose microcystic matrix. They show nuclear atypia in the form of variation in nuclear shape or size with accompanying hyperchromasia. All show mutation in *IDH 1* or *IDH2* supported by presence of *ATRX* characterized by gemistocytes forming nearly 20% of the tumor cells is a variant of IDH mt diffuse astrocytoma [1, 29, 30].

Difuse astrocytoma, IDH wild type, and WHO grade II: diffusely infiltrating astrocytoma without mutations in the IDH genes. It is extremely rare.

Anaplastic astrocytoma, IDH mutant: Diffusely infiltrating astrocytoma with focal or dispersed anaplasia, significant mitotic activity and mutation in *IDH 1* or *IDH*. TP53 or ATRX mutations are found in majority of tumors.

Glioblastoma, IDH wild type/ primary glioblastoma: They are high grade astrocytoma with nuclear atypia, cellular pleomorphism, mitosis, microvascular proliferation and necrosis. They lack IDH mutations but show TERT promotor mutations (80% cases), homozygyous deletion of CDKN2A/CDKN2B (60% cases), loss of chromosome 10p (50% cases), 10q (70%), EGFR alterations (55% cases) and PTEN (40% cases) [14, 15]. They account for nearly 90% of all glioblastoma [1].

Glioblastoma, IDH mutant/ secondary glioblastoma: IDH mutations in glioblastomas are considered as a marker for glioblastoma that arise by transformation from lower-grade gliomas, regardless of clinical history. IDH mt/secondary glioblastomas differ from IDH wt/primary glioblastoma in preferential frontal location and lesser extent of necrosis. Radiologically IDHmt glioblastoma exhibited more frequent non-enhancing tumor component, larger size at diagnosis, lesser extent of edema, and increased prevalence of cystic and diffuse components [31, 32]. Median age of IDHmt glioblastoma at diagnosis is 43 years while that of primary IDH wt glioblastoma is nearly 60 years [1].

Hence in routine histopathology practice, for older patients >55 years old, glioblastoma not in midline location and no prior history of lower grade glioma, IDH wt type designation can be given solely on the basis of negative IDH R132H immunohistochemistry. Sequencing is not required as the probability of an alternate IDH mutation is <1% [1, 31, 32].

IDH mt glioblastoma manifest longer overall survival and showed more frequent promoter methylation of MGMT [6].

Oligodendroglioma: Diffusely infiltrating slow growing glioma composed of monomorphic cells with uniform round nuclei and variable perinuclear haloes with IDH1 or IDH2 mutation and codeletion of chromosomes arms 1p and 19q.

IDH mutant gliomas which do not show ATRX loss on IHC should be considered for 1p/19qcodeletion studies even in absence of clear cut oligodendroglial histology [33].

Rarely tumors with oligodendroglial morphology but lacking IDH mutations or 1p/19q codeletion are noted. This group belongs to pediatric type oligodendroglioma. It is important to rule out histological mimics like dysembryoplastic neuroectodermal tumor, extraventricular neurocytoma, clear cell ependymoma and pilocyctic astrocytoma before rendering diagnosis of pediatric type oligodendroglioma [34, 35]. They show FGFR1 duplications or rearrangements of MYB related MYBL1 translocation [33].

Tumors with 1p/19 q codeletion without IDH mutations are usually IDH wt high grade astrocytomas and must be evaluated for possibility of incomplete deletion of on 1p and 19q [1].

Diffuse midline glioma: They are Infiltrative midline high grade glioma with astrocytic differentiation and mutations in histone proteins. Pons, thalamus, spinal cord are the common locations. Median age is 5–11 years. They are always IDH wild type and are considered grade IV tumors. In humans, there are main three histone H3 proteins: H3.1 encoded by HIST1H3B and HIST1H3C, H3.2 encoded by HIST2H3C and H3.3 encoded by H3F3A and H3F3B. Most common histone mutation is H3K27M (lysine to methionine substitution in H3F3A gene) which inhibits trimethylation of H3.3 histone resulting in decrease in H3K27me3. Other less frequent mutations occur in *HIST1H3B* or *HIST1H3C*. This can be detected by Sequencing for H3F3A and HIST1H3B. However, monoclonal H3F3A K27M

antibody is also available and intense nuclear staining in more than 80% of cells is taken as positive. Concordance between immunohistochemistry and sequencing is nearly 95%. H3 K27M mutated tumors show loss of H3K27me3 staining which can also be detected by IHC but it is not specific [1, 36].

Not otherwise specified (NOS) designation: It is used in tumors when either molecular testing is not available (e.g., in low-resource settings), or was performed but did not yield adequate results (assay failure), or was deliberately not done (e.g., not testing IDH status in an elderly patient with glioblastoma because of lack of implications for therapeutic management. But when molecular tests have been performed but results do not lead to a precise categorization of the tumor within the framework of the WHO 2016 classification, then term not elsewhere classified (NEC) is used [1, 26].

Final histopathological report:

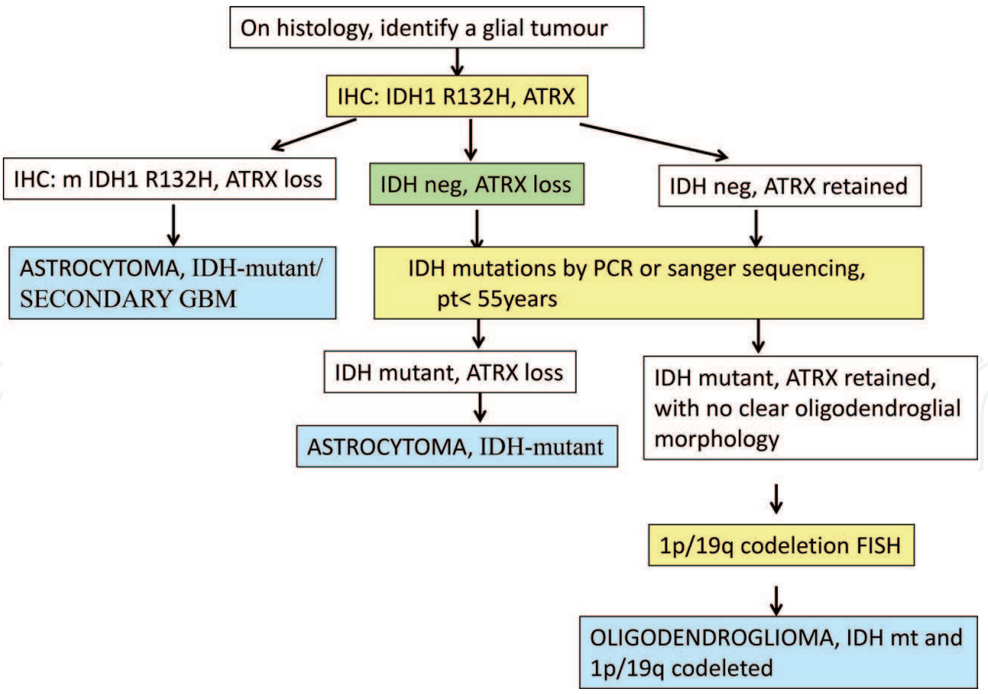
- Layer 1: Integrated diagnosis
- Layer 2: Histological classification (i.e. cellularity, mitosis, necrosis, vascular endothelial proliferations, variants)
- Layer 3: Histologic (WHO) grade (based on morphology)
- Layer 4: Biomarker studies (IDH, ATRX, p53, 1p/19q codel, EGFR)

Example:

- **Integrated diagnosis**: Diffuse Astrocytoma IDH mt, WHO grade II
- **Histological diagnosis**: Diffuse astrocytoma, WHO (histological) grade II
- **Molecular information**:
- **IDH**: positive (R132H immunohistochemistry; consistent with mutant type)
- **ATRX**: nuclear expression loss (immunohistochemistry; consistent with mutant type)
- **p53**: positive, >60% (immunohistochemistry; consistent with mutant type)

Key points

- Astrocytoma= *IDH*+/*ATRX* loss / mutated *TP53*
- Oligodendroglioma = *IDH* mutant/*ATRX* retained/1p/19q co-deleted
- Primary GBM: no IDH mutation/ EGFR/ TERT/PTEN/ TP53
- Secondary GBM: *IDH*+/*ATRX* loss / mutated *TP53*
- ATRX mutations are strongly associated with IDH mutations and are mutually exclusive with 1p/19q codeletion.



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