

# Overview of The Current Histopathologic and Molecular Techniques Used in Diagnosis of Diffuse Glioma

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**Abstract-** Diffuse gliomas are brain tumors that develop from glial cells and are notable for being very invasive and difficult to eradicate. Getting the right diagnosis of diffuse gliomas is critical for proper therapy and prognosis. Significant progress has been made in histopathologic and molecular approaches for identifying diffuse gliomas in recent years. In this study, it provides an overview of the most recent histopathologic and molecular methods used to diagnose diffuse gliomas, including immunohistochemistry, next-generation sequencing, fluorescence in situ hybridization, and DNA methylation profiling. The advantages and limitations of each technique are to be discussed to provide recommendations for the optimal approach to diagnosis in different clinical scenarios.

**Index Terms-** Diffuse glioma, Brain tumor, Histopathology, Molecular techniques, Immunohistochemistry, Next-generation sequencing, DNA methylation profiling.

## I. INTRODUCTION

Diffuse gliomas are a specific kind of brain tumor that arises from cells called glial cells, which are non-neuronal cells that support and nourish neurons in the brain. Diffuse gliomas are extremely invasive and difficult to treat due to their tendency to permeate normal brain tissue. These tumors are categorized based on their cell type, location, and molecular features, and may differ from low-grade to high-grade carcinoma. Some of the most frequent kinds of diffuse glioma include oligodendrogliomas, astrocytomas, and glioblastomas.

A proper diagnosis of diffuse glioma is of the utmost importance for deciding most effective therapy and prognosis. Histopathologic examination, which includes studying tissue samples from the tumour under a microscope to determine the tumour type and grade, is the present gold-standard method for diagnosis. However, this method has drawbacks since gliomas can be highly heterogeneous, making it difficult to get a representative tissue sample. Furthermore, histopathologic examination is subjective and may not adequately reflect the tumor's molecular features.

In recent years, there has been a substantial trend towards incorporating molecular approaches to help in the diagnosis of diffuse gliomas. These approaches can give more accurate and objective information about the tumor's molecular properties, such as genetic alterations, expression of genes patterns, and methylation of DNA profiles. This data may serve as the basis to guide treatment decisions and predict patient outcomes.

The primary objective of this review article is to provide an overview of the most recent cutting-edge histopathologic and molecular approaches for diagnosing diffuse gliomas. The benefits and drawbacks of each strategy will be investigated and subsequently provide recommendations for an effective way to diagnosis in various clinical settings. Hence, this article is to offer a thorough overview of the present diagnostic tools for diffuse glioma and to emphasize the promise of molecular approaches to enhance diagnosis, prognosis, and treatment of this difficult illness.

## II. HISTOPATHOLOGIC TECHNIQUES USED IN DIAGNOSIS OF DIFFUSE GLIOMA

Histopathology is defined simply the study of tissue changes or abnormalities using a microscope. It is an important tool in the diagnosis of cancer and has been used for many years in the diagnosis of diffuse glioma. Glioma histopathology is the analysis of tumor tissue acquired by biopsy or surgical excision. The analysis of the tissue aids in establishing the diagnosis, evaluating the kind and grade of the tumor, and estimating the patient's prognosis.

The WHO categorization of tumors of the brain and spinal cord is the gold standard for diagnosing brain tumors, including diffuse gliomas. The categorization system is based on the tumor's histopathologic characteristics and has been modified multiple times throughout the years. The most recent update occurred in 2016, when major revisions in the categorization of diffuse gliomas were made. Diffuse gliomas are classified into three types: astrocytic tumors, oligodendroglial tumors, and mixed gliomas.

The histologic grading of diffuse gliomas is a critical component of their diagnosis. The system for grading is based on histopathologic characteristics of the tumor, such as mitotic activity, nuclear atypia, and the presence of necrosis. Presently, the WHO grading system for diffuse gliomas is as follows:

Grade I: Pilocytic astrocytoma

Grade II: Diffuse astrocytoma, oligodendroglioma, oligoastrocytoma

Grade III: Anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic oligoastrocytoma

Grade IV: Glioblastoma

The tumor's histologic grade is a significant indicator of the patient's medical prognosis, with higher-grade tumors having a poorer prognosis.

Histopathologic approaches, although being an important tool in the diagnosis of diffuse gliomas, have significant limitations. Inter-observer variability, sample error, and difficulties discriminating between different forms of gliomas, particularly those that are morphologically identical, are among them. Even among expert neuropathologists, inter-observer variability can be high, resulting in discrepancies in tumor grading. Sampling error can also be a serious issue, especially in heterogeneous tumors, leading to an incorrect diagnosis. Furthermore, histopathologic approaches may not always be able to differentiate between different forms of gliomas, particularly those with comparable morphology, such as oligodendroglioma and astrocytoma. This emphasizes the need of using molecular approaches to supplement histopathologic investigation in the identification of diffuse gliomas.

### III. Molecular Techniques Used in Diagnosis of Diffuse Glioma

#### *Overview of Molecular Techniques*

Diffuse gliomas are a kind of primary brain tumor that is characterized by infiltrative development and diverse histologic characteristics. Histopathologic analysis of tissue taken from a surgical resection or biopsy has typically been used to make the diagnosis of diffuse glioma. However, molecular methods have emerged as significant tools for improving tumor detection and prognosis. This discussion will go through the molecular approaches that are currently employed in the diagnosis of diffuse glioma.

- Immunohistochemistry (IHC)

IHC is a procedure that detects proteins that are relevant in tissue samples by using particular antibodies. IHC may be used to detect the presence of different markers that are helpful in determining diagnosis and prognosis in diffuse gliomas. IHC, for example, could determine the expression of the transcription factor IDH1, which is utilised to discriminate between IDH-mutant and IDH-wildtype gliomas. Similarly, ATRX protein expression can be utilised to discriminate between ATRX-mutant and ATRX-wildtype gliomas.

- Fluorescence in situ hybridization (FISH)

FISH is a method that utilizes fluorescently tagged probes to identify specific sequences of DNA in tissue samples. FISH can identify genetic abnormalities such as amplifying mutations or deletions of certain genes or chromosomal parts in diffuse gliomas. FISH, for example, can be performed to identify EGFR gene amplification in glioblastoma or the deletion of the CDKN2A/B locus in oligodendroglioma.

- Polymerase chain reaction (PCR)

PCR is a technique that works by amplifying particular DNA sequences in a sample, permitting them to be detected and quantified. PCR is frequently employed to detect mutations in particular genes in diffuse gliomas, such as the IDH1 or TP53 genes. PCR may additionally be used to identify the presence of particular viral DNA, such as human cytomegalovirus (HCMV) or Epstein-Barr virus (EBV), both of which have been linked to the development of certain gliomas.

- Next-generation sequencing (NGS)

NGS is a high-throughput technique that can sequence millions of DNA fragments in a sample simultaneously. In diffuse gliomas, NGS can be used to detect mutations in multiple genes simultaneously, providing a comprehensive view of the genetic alterations present in a tumor. This can help to refine the diagnosis of diffuse glioma, as well as provide prognostic information. For example, NGS can be used to detect mutations in the TERT promoter, which are associated with poor prognosis in gliomas.

- DNA methylation profiling

DNA methylation profiling is a technique that analyzes the pattern of DNA methylation in a sample. In diffuse gliomas, DNA methylation profiling can be used to classify tumors into specific subtypes based on their methylation pattern. This can help to refine the diagnosis of diffuse glioma, as well as provide prognostic information. For example, DNA methylation profiling can be used to distinguish between IDH-mutant astrocytomas and oligodendrogliomas.

### IDH1/2 Mutations

IDH1 and IDH2 are two genes that encode isocitrate dehydrogenase enzymes, which play an important role in cellular metabolism. Mutations in these genes have been identified in a large proportion of diffuse gliomas and are now recognized as key molecular markers in the diagnosis and classification of these tumors. In this overview, we will discuss the role of IDH1/2 mutations as molecular techniques used in the diagnosis of diffuse glioma.

Mutations in the IDH1 and IDH2 genes were first identified in gliomas in 2008 and have since been found to be present in up to 80% of diffuse gliomas. These mutations are highly specific to diffuse gliomas and are rare in other types of brain tumors. The most common IDH1 mutation is a single amino acid substitution at codon 132 (IDH1 R132H), while the most common IDH2 mutation is a single amino acid substitution at codon 172 (IDH2 R172K).

IDH1/2 mutations are hypothesized to be early events in gliomagenesis and to play a role in tumour development and maintenance. Mutated IDH enzymes generate a metabolite called 2-hydroxyglutarate (2-HG), which is not found in normal brain tissue. 2-HG has been demonstrated to decrease the action of a variety of enzymes involved in cellular metabolism, as well as to induce epigenetic alterations that lead to tumor growth.

IDH1/2 mutations are now required for the diagnosis and categorization of diffuse glioma. The presence or absence of IDH1/2 mutations is now utilized as a crucial diagnostic criterion for differentiating between distinct subtypes of diffuse glioma in the 2016 revision of the World Health Organisation (WHO) classification of tumors of the central nervous system.

IDH1/2 mutations are utilized to differentiate between two primary subgroups of diffuse glioma: IDH-mutant and IDH-wildtype. Based on other histologic and molecular characteristics, IDH-mutant gliomas are further categorized as astrocytoma, oligodendroglioma, and oligoastrocytoma. Glioblastoma and anaplastic astrocytoma are the two types of IDH-wildtype gliomas.

Immunohistochemistry (IHC), a method routinely used in pathology laboratories, may identify the presence of an IDH1 R132H mutation. IHC for IDH1 R132H has a good sensitivity and specificity for identifying this mutation in gliomas, making it an essential diagnostic tool in the assessment of these tumors.

In diffuse glioma, IDH1/2 mutations have a significant prognostic relevance. IDH-mutant gliomas outlive IDH-wildtype gliomas in terms of overall survival and progression-free survival. This link between IDH mutations and increased survival is likely to be connected to the tumors' different molecular and histologic properties, as well as their sensitivity to therapy.

IDH1/2 mutations have been discovered as possible therapeutic targets in diffuse glioma, in addition to their prognostic importance. Several preclinical investigations have indicated that inhibiting mutant IDH enzymes can lower 2-HG levels and slow tumour development. Clinical studies to assess the effectiveness of IDH inhibitors in the treatment of IDH-mutant gliomas are presently underway.

### 1p/19q Co-Deletion

The molecular marker 1p/19q co-deletion has become an essential diagnostic and prognostic tool in the treatment of diffuse glioma. The importance of 1p/19q co-deletion as a molecular approach utilized in the diagnosis of diffuse glioma is of high interest area. In oligodendroglial tumors, both the shorter arm of chromosome 1 (1p) and the longer arm of chromosome 19 (19q) are usually removed. This chromosomal defect causes the loss of genetic material, which is considered to be involved in tumor growth and progression.

1p/19q co-deletion is a specific genetic alteration that is present in a subset of diffuse gliomas, particularly oligodendrogliomas and oligoastrocytomas. The frequency of 1p/19q co-deletion is approximately 50-70% in oligodendrogliomas and 20-40% in oligoastrocytomas.

The presence of 1p/19q co-deletion is now recognized as a key diagnostic criterion for distinguishing between different subtypes of diffuse glioma. In the 2016 revision of the World Health Organization (WHO) classification of tumors of the central nervous system, the presence or absence of 1p/19q co-deletion is used to classify tumors into different subtypes.

Oligodendrogliomas and oligoastrocytomas with 1p/19q co-deletion are classified as IDH-mutant and 1p/19q-codeleted. These tumors have distinct histologic and molecular features, and are associated with a better prognosis compared to other subtypes of diffuse glioma. Also, the diagnosis of 1p/19q co-deletion can be made using a variety of techniques, including fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR). FISH is a commonly used technique in pathology laboratories, and involves the use of fluorescent probes to detect the presence of the 1p/19q co-deletion in tumor cells.

1p/19q co-deletion has important prognostic significance in diffuse glioma. Oligodendrogliomas and oligoastrocytomas with 1p/19q co-deletion are associated with a better prognosis compared to those without this genetic alteration. Additionally, the improved prognosis associated with 1p/19q co-deleted tumors is thought to be related to their distinct molecular and histologic features, as well as their response to treatment. These tumors are more likely to respond to chemotherapy, particularly with the use of temozolomide and procarbazine, lomustine, and vincristine (PCV) regimen, which has become a standard treatment for these tumors.

### MGMT Promoter Methylation

MGMT (O-6-methylguanine-DNA methyltransferase) promoter methylation is a molecular marker that has become an important diagnostic and prognostic tool in the management of diffuse glioma. In this overview, we will discuss the role of MGMT promoter methylation as a molecular technique used in the diagnosis of diffuse glioma. Hence, the MGMT gene encodes for a DNA repair enzyme that removes alkyl adducts from the O6 position of guanine. In gliomas, the MGMT gene is frequently silenced by promoter methylation, resulting in decreased MGMT protein expression and increased sensitivity to alkylating chemotherapy, such as temozolomide.

MGMT promoter methylation is present in approximately 40-60% of diffuse gliomas, with higher frequencies observed in glioblastomas compared to lower-grade gliomas. Otherwise, the presence of MGMT promoter methylation is now recognized as an important diagnostic and prognostic marker in the management of patients with diffuse glioma. In the 2016 revision of the World Health Organization (WHO) classification of tumors of the central nervous system, MGMT promoter methylation status is used to classify glioblastomas into two subtypes: MGMT methylated and MGMT unmethylated.

Glioblastomas with MGMT promoter methylation are classified as the MGMT methylated subtype, and are associated with a better prognosis compared to those without MGMT promoter methylation. MGMT promoter methylation is also associated with increased response to alkylating chemotherapy, particularly temozolomide.

The diagnosis of MGMT promoter methylation can be made using a variety of techniques, including methylation-specific PCR (MSP), pyrosequencing, and methylation-specific microarray analysis. MSP is a commonly used technique in pathology laboratories, and involves the use of PCR primers that are specific to methylated or unmethylated DNA. The PCR products are then analyzed by gel electrophoresis to determine the presence or absence of MGMT promoter methylation.

MGMT promoter methylation has important prognostic significance in diffuse glioma, particularly in glioblastomas. Patients with glioblastomas with MGMT promoter methylation have a better prognosis compared to those without MGMT promoter methylation, with increased overall survival and progression-free survival. The improved prognosis associated with MGMT promoter methylation is thought to be related to the increased sensitivity of these tumors to alkylating chemotherapy, as well as other factors such as decreased invasiveness and increased immune response.

### Limitations of Molecular Techniques

Molecular techniques have revolutionized the diagnosis and classification of diffuse gliomas. However, these techniques are not without limitations. In this section, we will provide an overview of the limitations of molecular techniques used in the diagnosis of diffuse gliomas.

- **Tumor heterogeneity:** Tumor heterogeneity is a major limitation of molecular techniques. Different areas of the same tumor may have different molecular profiles, which can lead to inconsistencies in diagnosis and classification. Additionally, the biopsy may not capture the most aggressive or representative part of the tumor.
- **Sampling errors:** Sampling errors are a common limitation of molecular techniques. The accuracy of molecular diagnosis depends on the quality and quantity of the tissue sample obtained. Biopsy samples can be affected by necrosis, inflammation, or inadequate tumor cellularity, which can lead to false-negative results.
- **Technical limitations:** Technical limitations of molecular techniques can also limit their accuracy. For example, the sensitivity and specificity of PCR-based assays depend on the primer design, PCR conditions, and quality of the template DNA. In addition, some molecular assays may not be able to detect certain genetic alterations or may be affected by inter-laboratory variability.
- **Lack of standardization:** There is a lack of standardization of molecular techniques for the diagnosis of diffuse gliomas. Different laboratories may use different assays, which can lead to inconsistent results. The lack of standardization also makes it difficult to compare results between different studies.
- **Limited clinical utility:** The clinical utility of molecular techniques in the diagnosis of diffuse gliomas is limited. While molecular profiling can aid in tumor classification and prognosis, the clinical significance of certain genetic alterations is unclear. In addition, the use of molecular techniques in clinical decision-making is limited by the lack of targeted therapies for most genetic alterations.
- **Cost and availability:** Molecular techniques can be costly and may not be available in all settings. This limits their widespread use, particularly in resource-limited settings.

#### **IV. Integration of Histopathologic and Molecular Techniques in Diagnosis of Diffuse Glioma**

Diffuse glioma is a challenging tumor to diagnose and manage. The diagnosis of diffuse gliomas requires the integration of histopathologic and molecular techniques. Integrating these techniques offers several advantages, including improved diagnostic accuracy, better understanding of tumor biology, and personalized treatment planning. However, there are several challenges that need to be addressed to realize the full potential of integrated diagnosis in the management of diffuse gliomas.

The integration of histopathologic and molecular techniques can improve the diagnostic accuracy of diffuse gliomas. The combination of morphologic features and molecular alterations can provide a more complete picture of the tumor and aid in accurate classification and grading. Additionally, integrating these techniques can provide a better understanding of the biological characteristics of the tumor, which can aid in predicting the tumor behavior, determining the prognosis, and identifying potential therapeutic targets.

Integrated diagnosis plays a crucial role in treatment planning for diffuse gliomas. The integration of histopathologic and molecular techniques can aid in accurate classification and grading of the tumor. This information can guide treatment decisions and help predict the response to therapy. The integration of histopathologic and molecular techniques can also aid in prognostic assessment of the tumor. This information can guide treatment decisions and help predict the outcome of the disease.

Knowledge of the molecular alterations in the tumor can guide the selection of targeted therapies and assist in determining the optimal treatment approach. Additionally, integrating histopathologic and molecular techniques can aid in monitoring the response to therapy. Knowledge of the molecular alterations in the tumor can assist in determining the response to therapy and guide treatment modifications.

Despite the advantages of integrating histopathologic and molecular techniques, there are several challenges that need to be addressed. These challenges include technical challenges, standardization, cost and accessibility, and interpretation. Integrating histopathologic and molecular techniques requires specialized expertise and technology. The availability of these resources can vary across different centers, which can affect the accuracy and reproducibility of the results.

There is a lack of standardization of histopathologic and molecular techniques for the diagnosis of diffuse gliomas. This can lead to inconsistent results and make it difficult to compare results between different studies. The cost and accessibility of histopathologic and molecular techniques can be a challenge. These techniques can be expensive and may not be available in all settings, particularly in resource-limited areas. Integrating histopathologic and molecular techniques also requires careful interpretation of the results. The interpretation of complex molecular data requires specialized expertise, which may not be available in all centers.

## V. CONCLUSION

In conclusion, the integration of histopathologic and molecular techniques in the diagnosis of diffuse glioma represents a major advancement in the field. The use of these techniques offers improved diagnostic accuracy, better understanding of tumor biology, and personalized treatment planning. While histopathologic techniques remain the gold standard for diagnosis, the integration of molecular techniques has significantly improved the accuracy of diagnosis and classification, allowing for better prognostic assessment and treatment planning. However, there are still challenges that need to be addressed, including technical difficulties, standardization, cost and accessibility, and interpretation of results. Further research and improvements in technology and standardization will be necessary to optimize the benefits of integrated diagnosis in the management of diffuse glioma.

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