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Molecular Neuropathology versus Histopathology in the Diagnosis of Central Nervous System Tumors: Review Article about Novel Diagnostic and Investigative Technologies

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Abstract

Background: While the diagnostic roles of histopathology and cytopathology remain highly significant nowadays, the roles and applications of molecular techniques in neuropathology are expanding and becoming noteworthy. In the presence of high-throughput techniques such as next-generation sequencing, an exciting application for molecular techniques is genetic or epigenetic profiling, which is known as a rapid, cost-effective, and sensitive technique to elucidate and identify novel genetic or epigenetic alterations. **Purpose and Method:** The current report is a review article to discuss the significant roles of molecular pathology and advanced molecular technologies, including DNA methylation arrays and spatial transcriptomics, in the neuropathology of central nervous system tumors. **Results:** The DNA Methylation array is considered a diagnostic support and investigative tool, while spatial transcriptomics is only an investigative tool so far. However, spatial transcriptomics enables visualizing the cells spatially according to their messenger ribonucleic acid and genetic expression. Both of the techniques help in the discovery of different and novel genetic or epigenetic alterations, which may provide opportunities to develop a clinically relevant classification of tumors, elucidate diagnostic and prognostic markers, and ascertain therapeutic checkpoints. There is tremendous growth in the role of these novel technologies, and they are becoming of major importance gradually. **Conclusion:** Histopathology and cytopathology, as conventional diagnostic disciplines of pathology may have a minor role in the future with further development and advancement of these technologies, especially when they are verified totally

and their quality is ensured to serve patients with high healthcare standards. Therefore, different calls from experts in the field of pathology have asked to prepare and train pathologists not only in histopathology but in molecular pathology and its advanced technologies under what they have termed next-generation pathologists.

Keywords

CNS, Tumors, Methylation, Spatial Transcriptomics, Histopathology, Molecular

1. Introduction

1.1. Historical Context

For decades, histopathology has been considered the gold standard for diagnosing and classifying tumors in general and central nervous system (CNS) tumors in particular [1]. At the end of the seventies of the past century, the World Health Organization (WHO) issued the first book for CNS tumor classification. In 1993, the second edition was released and was followed by two editions in 2000 [2] and in 2007. All these previous editions considered histology as the main feature for classification. In WHO 2007, however, there were genetic alterations included as prognostic factors after the histological diagnosis. The WHO classification of CNS tumors in 2007 included the following groups of tumors according to histological features: tumors of neuro-epithelial tissues; tumors of meninges; neuronal and mixed neuroglial tumors; tumors of the pineal region; embryonal tumors; tumors of cranial and para-spinal tumors; lymphoma and hematopoietic tumors; germ cell tumors; tumors of the sellar region; and metastatic tumors [3].

In 2016, the WHO issue of CNS tumor classification came out with a substantial change, which was the integration of molecular and histological entities for the first time. However, the significant roles of histopathology and immunohistochemistry (IHC) remain highly valuable. The 2016 classification of CNS tumors involved the following entities: diffuse astrocytic and oligodendroglial tumors, other astrocytic tumors, ependymal tumors, other gliomas, choroid plexus tumors, neuronal and mixed neuroglial tumors, tumors of the pineal region, embryonal tumors, tumors of cranial and para-spinal regions, meningioma, mesenchymal and non-meningoepithelial tumors, melanocytic tumors, lymphoma, histiocytic tumors, germ cell tumors, tumors of the sellar region, and metastatic tumors [1]. In this edition, it was reported that if the diffuse glioma histologically appears astrocytic but shows an isocitrate dehydrogenase (IDH) mutation with the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) co-deletion, the diagnosis depends on the genotype regardless of the histological phenotype. Therefore, the diagnosis would be oligodendroglioma, IDH-mutant and 1p/19q-codeleted. This is an example of the substantial changes that

have been introduced to 2016 WHO CNS tumor classification, whereas the genotype of the tumor trumps the histopathological features.

In 2021, a new version of the WHO classification of CNS tumors was published and introduced new changes that advance the role of molecular pathology, genetics, and epigenetics in CNS tumor diagnosis and classification [4]. For instance, some of the new tumor types and/or subtypes are included now based on novel diagnostic techniques such as deoxyribonucleic acid (DNA) methylation array profiling. Using DNA methylation profiling, the paraganglioma of the cauda is now classified as a distinct entity from the other common paragangliomas diagnosed at other anatomical positions. Another example of DNA methylation array-based diagnosis, two studies [5] [6] reported that pineoblastomas could be classified into four molecular subtypes, and this subtyping was included in the new version of the WHO classification of CNS tumors in 2021. The WHO-2021 classification of CNS tumors involved the following groups: gliomas, glioneuronaltumors & neuronal tumors; embryonal tumors; pineal tumors; cranial and para-spinal tumors; meningioma; melanocytic tumors; hematolymphoid tumors; germ cell tumors; tumors of the sellar region; and metastatic tumors [4]. Finally, it might be noted that the number of main groups in the 2021 classification of CNS tumors is less than what was reported in the WHO-2016 classification. This may be owed to the more organized grouping and classification of the tumors in the newest version of the WHO classification of CNS tumors. For instance, the diffuse gliomas were classified in WHO-2016 under one main group and named diffuse astrocytic and oligodandroglioma according to integrated histological features as well as molecular and cytogenetic biomarkers such as IDH and 1p19q Co-deletion, respectively [1]. On the other hand, the WHO-2021 classified glioma, glioneuronal and neuronal tumors into one large group, with the exception of choroid plexus tumors. This group includes six broad categories as follows: adult-type diffuse gliomas; pediatric-type diffuse low-grade gliomas; pediatric-type diffuse high-grade gliomas; circumscribed astrocytic gliomas; glioneuronal and neuronal tumors; and ependymomas [4].

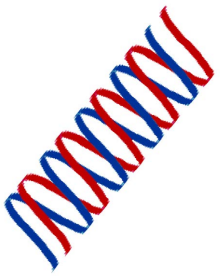
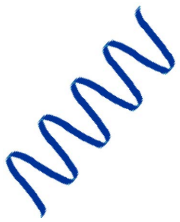
Conclusively, from 2007 all the way to 2016 and then to 2021, the roles of molecular pathology and molecular techniques are growing gradually and provide a more organized classification of CNS tumors with high clinical significance regarding diagnosis, prognosis, and treatment. However, due to the unavailability of molecular techniques in all health centers or unclassified CNS tumors, the last two WHO books for CNS tumor classification included two suffixes, NOS and NEC. The suffix NOS stands for not otherwise specified, while NEC stands for not elsewhere classified. The suffix NOS indicates that the diagnosis which is given to a tumor was based on histological features due to unavailability or technical failure of laboratory techniques that are necessary to provide a specific WHO diagnosis, which mostly integrates histological and molecular findings. While the suffix NEC refers to a descriptive diagnosis of the tumor when there is no conclusive result or no relation between the findings of IHC, genetic, epige-

netic, histology, or the clinical scenario. Therefore, the suffix NEC would alert the clinicians that the diagnosis does not meet a specific WHO diagnosis, although all histological and molecular investigations have been performed successfully [1] [4].

1.2. Molecular Pathology

In general, molecular pathology is defined as the assessment of nucleic acids and proteins to diagnose diseases, select treatment, and predict the prognosis [7]. There are a variety of molecular techniques that are used in research and diagnostic contexts (**Table 1**) [8] [9]. While histopathology still forms the cornerstone in reporting the stage and grade of tumors, molecular pathology has been expanding and its role has become critical. In the practice of histopathology and cytopathology, an important list of ancillary tests, including IHC, conventional & molecular cytogenetics, and molecular techniques, has been incorporated into the practice of histopathology and cytopathology [10]. The application of molecular

Table 1. Techniques used in molecular diagnostics and molecular pathology.

Molecule	Molecular test	Diagram
DNA	• Manual sequencing • Southern hybridization • Genomic Polymerase Chain Reaction (PCR) (gene deletion, mutation) • Restriction Fragment Length Polymorphism (RFLP) • Gene dosage study by GeneScan analysis • Genotyping by microsatellite analysis • Single Nucleotide Polymorphism (SNP) detection by Sequenom/ABI PRISM Sequencer detector • Automatic DNA sequencing • Fluorescent in situ hybridization • DNA profiling array	
RNA	• Northern hybridization • Reverse Transcription (RT)-PCR • Quantitative-PCR • RNA profiling • RNA Seq. • Transcriptome Seq.	
Protein	• Western blotting • IHC • Mass Spectrometry	

diagnostics in molecular pathology has been focused on disease-associated mutations [11] [12] [13], which aid pathologists in developing classification systems for diseases and providing correct and exact diagnoses. For instance, the implementation of PCR and FISH in routine testing has become essential for a long list of genetic alterations that are associated with tumors such as hematopoietic tumors, CNS tumors, and sarcomas [7] [14] [15]. For instance, it was reported that many oligodendroglial tumors present with loss of heterozygosity and co-deletion of 1p19q [16] [17], and this alteration becomes important in the diagnosis of this type of diffuse gliomas. Additionally, after the discovery of mutations in IDH 1 and IDH 2, they became essential in diagnosing and classifying glioblastoma and other diffuse gliomas [15].

Another advance in the application of molecular diagnostics has been the identification of useful molecular markers to predict responsiveness or resistance to certain therapies, such as target therapy. Treatment with selective inhibitors such as anti-epidermal growth factor receptor (EGFR) and anti-*ALK*/*ROS*1 therapies has been reported to shrink tumors and prolong survival in patients diagnosed with lung cancer [18]. Deficient mismatch repair, microsatellite instability-high and programmed death-ligand-1 is well-known markers to select immunotherapy for patients diagnosed with colon cancer [19]. Another example is the status of O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, which has been reported to guide treatment for glioblastoma and some astrocytic tumors [20]. In addition to that, the 1p/19q co-deletion is associated with a higher sensitivity to Procarbazine–Lomustine–Vincristine (PCV) chemotherapy [21] [22]. Moreover, besides the clear role of molecular pathology in the selection of target therapy, personalized medicine, also known as precision medicine, significantly depends on molecular techniques. Precision medicine relies on the fact that each human has a unique pathogenesis due to heterogeneous genetic and epigenetic alterations that may affect the interaction between the internal environment of the human body and the external environment [23].

Another exciting application for molecular pathology in the presence of high-throughput techniques such as next generation sequencing; it becomes rapid, cost-effective, and sensitive to elucidate and identify new genetic or epigenetic alterations for diagnostic, prognostic, and therapeutic purposes [24] [25] [26] [27]. Finally, there is a tremendous growth in the role of molecular pathology and its different technologies, and they are becoming of major importance gradually. Accordingly, histopathology and cytopathology as conventional diagnostic disciplines of pathology may have a minor role in the future with further development and advancement of these technologies, especially when they are verified totally and their quality is ensured to serve patients with high healthcare standards. Therefore, different calls from experts in the field of pathology have asked to prepare and train pathologists not only in histopathology but also in molecular pathology and its advanced technologies under what they have termed next-generation pathologists [10] [28].

2. Novel Diagnostic and Investigative Technologies

2.1. CNS Tumor Classification Using DNA Methylation Arrays

DNA methylation is an epigenetic marker and is defined as the addition of a methyl group to DNA at the cytosine of cytosine-guanine (CpG) islands. DNA methylation is important in both normal development and disease [29] [30]. DNA methylation may affect gene expression without affecting DNA sequencing and consequently alter various normal biological functions. Moreover, DNA methylation has been linked to the etiology of many diseases, including cancer [31], kidney disease [32], diabetes type 2 [33], psychiatric disorders [34], and sepsis [35]. Additionally, it has been reported that DNA methylation could be affected by estrogen treatment and is considered an explanation for gene expression changes by treatment in experimental models [36]. In cancer, DNA methylation patterns show both the origin of the cell type and acquired changes resulting in the formation of tumors [29] [30].

DNA methylation profiling of CNS tumors has resulted in entity-based signatures leading to the identification of tumor types and subtypes with a high clinical relevancy [37] [38] [39]. Accordingly, a CNS tumor classifier has been developed by the German Cancer Research Center and Heidelberg University, Germany. A classifier is a free tool that is accessible on a website named molecular neuropathology (https://www.molecularneuropathology.org/) [29] [40]. This website is a platform entitled "next generation neuropathology". However, it is documented on the classifier website that the classifier and the platform are still research tools. This is in the presence of different studies that have discussed the usefulness of DNA methylation array implementation in routine diagnostic neuropathology [41] [42] [43] [44] [45]. In addition to that, the new WHO classification of CNS tumors in 2021 incorporates tumor entities that are based on DNA methylation profiling [4]. As well, the power of DNA methylation in diagnosing brain tumors as a support tool was reported [46] [47]. However, there are some issues, including the optimization of the DNA methylation approach and the availability of the technique itself [48].

There were different suggestions to implement the classifier in routine diagnostic neuropathology [29] [41]. Currently, it is suggested to keep routine diagnostic procedures in neuropathology in parallel with the DNA methylation array-based diagnosis, and to provide a final integrated report that includes both the conventional diagnosis and the classifier-based one [41] [44] [45]. It was recommended during handling of the DNA methylation profiling results by pathologists or specialists in molecular diagnostics to pay careful attention to the reporting DNA methylation based-diagnoses with scores below 0.84 and better to ignore the suggested diagnosis with a score of less than 0.50.

The detailed procedure of the DNA methylation profiling of CNS tumors has been published in different reports [29] [43] [45]. The procedures start with the selection of the enrichment area of tumor cells and extraction of DNA, followed by assessment of the quantity and quality of the extracted DNA. Next, the 450 K

or EPIC (Illumina) DNA methylation array is used to profile the methylation of uploaded DNA. This is followed by generating data as idat files, which later are uploaded into the classifier to obtain the automated reports as the final step. The automated report that is generated by the classifier has three important parts (http://www.molecularneuropathology.org/), including 1) the diagnosis as a histological entity with a molecular subtype of the tumor; 2) the copy number variation showing the whole 46 chromosomes and the cytogenetic abnormalities; and 3) the DNA methylation status of the MGMT promoter. All of these details are clinically important regarding diagnosis, prognosis, and treatment. For instance, copy number variation can show important diagnostic cytogenetic information such as 1p/19q co-deletion, the signature of +7/−10, amplifications, deletions, and fusion abnormalities. The DNA methylation status of the MGMT promotor is an essential biomarker for prognosis and treatment with Temozolomide [20].

The implementation of DNA methylation array in the diagnostic field has been regarded for different reasons [41] [43], including but not limited to 1) unusual or vague microscopic morphology; 2) contradictory results of molecular tests; 3) small or inadequate samples; and 4) the need for additional molecular sub-classification such as ependymoma and medulloblastoma [49] [50]. As well, there are recommendations to apply a DNA methylation array if the case belongs to the pediatric age group, even if the histology of the CNS tumor is clear and conclusive. This is because methylation profiling offers relevant diagnostic information for the vast majority of pediatric CNS tumors [41] [45]. These diagnostic details include a) the tumor class, which is the main diagnosis and has been determined through histopathology and cytopathology for years; b) the tumor subclass, which sometimes needs a number of molecular techniques and/or histological features to be concluded; c) Copy number analysis, which is commonly ordered in oncology practice during the diagnosis and treatment of pediatric tumors and is performed using conventional or emerging molecular cytogenetics; and d) Guidance for additional molecular testing and important information regarding the epidemiology, treatment, or prognosis of the tumor classification and sub-classification [45]. The last point regarding the guidance for further molecular testing and relevant information is found mainly in the description of the CNS tumor classification on the automated report. For example, the report of methylation class diffuse midline glioma H3 K27M mutant includes a detailed description with different aspects, such as: i) the definition of the tumor histologically; ii) the frequent anatomical position of the tumor, which in the case of diffuse midline glioma H3 K27M mutant is located in midline structures thalamus, cerebellum, brainstem and spine; iii) the median age, which in this case is about 13 years and ranging from 3 to 54 years; iv) additional mutations according to the anatomical position e.g. *ATRX* and *TP*53/*PPM*1*D* are common in most of the midline structures tumors but in brainstem tumors an additional mutation affecting the gene *ACVR*1 is recurrently reported and observed; and v) prognosis of the tumor and its group or classification which is

in the case of methylation class diffuse midline glioma H3 K27M mutant is generally very poor despite the presence of lower grade tumors with K27M mutation have also been reported rarely [4] [29] [44] [45] [46].

In fact, DNA methylation array profiling has been used to study other tumors such as lung cancer [51] [52] [53], soft tissue & bone tumors [54] [55] [56] [57] [58], and ovarian epithelial tumors [59]. In a paper published in 2021, there was a project to develop a DNA methylation profiling-based tool for the classification of soft tissue and bone sarcomas [30]. Finally, there are many studies and the literature is growing regarding DNA methylation profiling and its huge role as a diagnostic or investigative tool in the advancement of diagnostic accuracy and healthcare quality [43]-[48].

2.2. Spatial Transcriptomics

Spatial transcriptomics analysis is unlike DNA methylation profiling since it cannot be used as a diagnostic tool directly, at least nowadays. However, it is considered an investigative tool that provides many possibilities to elucidate biomarkers that can be used in the diagnosis, treatment, and follow-up of cancer patients. Spatial transcriptomics enables quantitative gene expression data and visualization of messenger ribonucleic acid (mRNA) distribution within histological sections [60]. Single cell-based analysis of transcriptomics has revealed heterogeneity within different cancer types, resulting in identifying the cells that cause drug resistance and predict metastasis [61]-[65]. Spatial transcriptomics advances the understanding of the various steps of molecular pathogenesis in cancer, inflammatory, and degenerative disorders, which may lead to the identification of new diagnostic, prognostic, and/or therapeutic markers [66] [67] [68].

There are basic procedures for spatial transcriptomics and they are summarized in **Figure 1**. The protocol for performing spatial transcriptomics on a fresh frozen section has been approved without technical or procedural obstacles. However, formalin-fixed and paraffin-embedded (FFPE) tissues were hard to apply spatial transcriptomics to. This is because of affected nucleic acid integrity due to formalin-induced strand cleavage and the formation of cross-linking between RNA and other molecules [69]. During the past year 2021, a suggested protocol to apply and optimize spatial transcriptomics on FFPE samples was published [70]. Because FFPE sections are the most preferred method for preserving histological sections in research and routine diagnostic pathology [71], this protocol provides useful technical steps and procedures for resolving formalin-related issues. The protocol starts with the recovery of fixed mRNA through deparaffinization and removing the cross-links within the section on a special slide with barcodes. Next, tissue sections are stained with routine histological stains, hematoxylin and eosin, and subjected to pre-permeabilization with collagenase to disrupt the extracellular matrix. Cross-link treatment is heat-induced with alkaline Tris-EDTA buffer to stop degradation of RNA. After this, the free mRNA is used to prepare cDNA libraries employing a protocol for optimization,

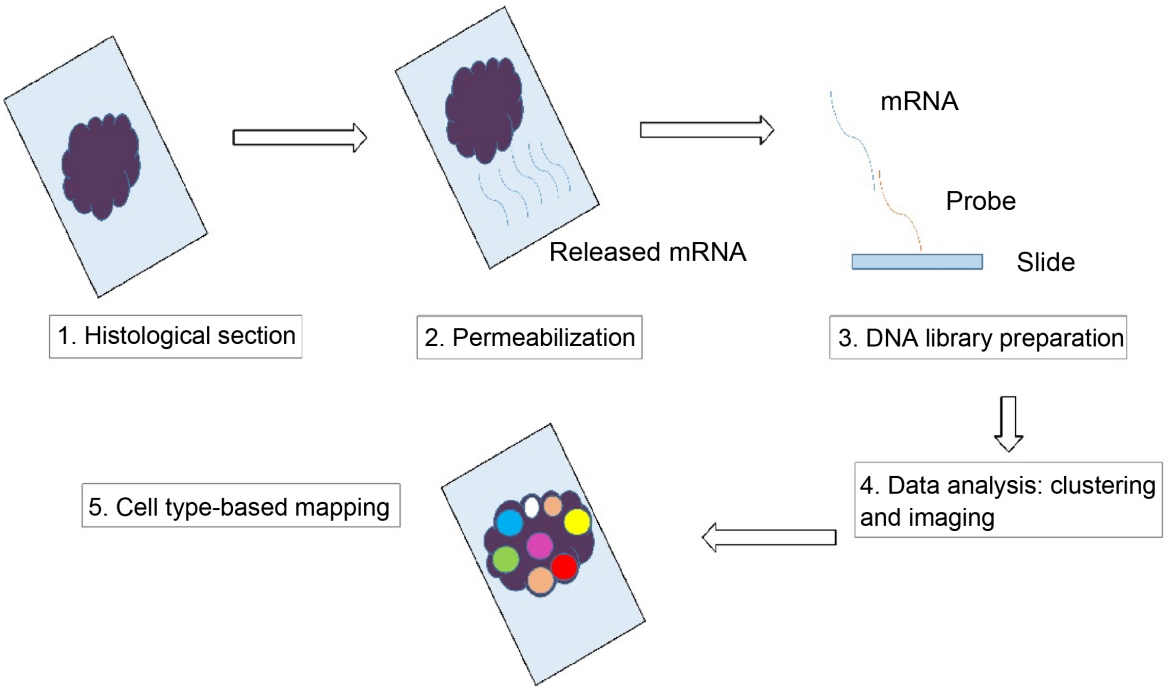


Figure 1. The basic procedures of spatial transcriptomics.

and then the sequencing of libraries is executed and data are collected for further analysis [70].

Using spatial transcriptomics, a tumor-specific keratinocyte population, immune cells, and heterogeneity of the tumors in cutaneous squamous cell carcinoma were identified. In this study, it was concluded preliminarily that patients with a high expression of the markers *ITGB*1 and *PLAU* are associated with low progression-free survival after treatment with *PD*-1 inhibitors [61]. This demonstrates how spatial transcriptomics can provide information about treatment and its effectiveness. In another study, spatial transcriptomics was applied to prostate cancer samples, and it was reported that spatial analysis ascertains the area of tumor cell enrichment although histology showed normal appearance [66]. The latter observation reveals how it is important to detect tumor progression at molecular levels before it becomes histologically notable.

Regarding inflammation and the application of spatial transcriptomics, a report studying the profiles of periodontal tissues affected by inflammation due to periodontitis showed distinct inflammatory areas of chronic inflammatory process as a part of the molecular pathogenesis of disease progression [72]. Additionally, spatial transcriptomics has a valuable and significant role in analyzing the molecular pathogenesis of inflammation due to infectious diseases. In a published report, a study integrated spatial transcriptomics and single-cell based RNA sequencing to analyze cardiac histological sections isolated from mice as an experimental model infected with a virus. This study reported that spatial transcriptomics and single-cell based RNA sequencing provided detailed processes of myocarditis at different time points by exploring the molecular changes in-

duced by the virus [73]. In addition, spatial transcriptomics was used to describe molecular immune cell mechanisms and their variations according to tissue type during chronic rheumatoid arthritis inflammation [74]. Collectively, these different studies provide a better understanding of the significant role of spatial transcriptomics in studying and investigating inflammatory disorders.

Another study has applied spatial transcriptomics to obtain gene expression of mouse spinal cords over the course of amyotrophic lateral sclerosis (ALS), as well as of postmortem histological sections from ALS patients. It was reported that there were dynamic changes in the area of microglia and astrocyte populations at early stages, which were accompanied by different transcriptional pathways in both mouse models of ALS and human postmortem nervous tissue [68]. This study is an example of how the progressive changes of molecules and the molecular pathogenesis could be studied at different stages of disease, which may help in a better understanding of disease and consequently find diagnostic and therapeutic novel markers at different stages of disease progression.

In distinguishing ductal carcinoma from invasive ductal carcinoma of breast cancer, a combination of spatial transcriptomics and a machine learning program was reported to have a prediction accuracy of 90 - 95 percent [75]. Integration of spatial transcriptomics and single-cell based RNA sequencing was used to study pancreatic ductal adenocarcinoma and revealed significant outcomes. An example of these outcomes is that the integration of the two techniques determines the degree of overlap between genes spatially, which reveals different levels of functional interactions between tumor cells, inflammatory immune cells, and normal cells [76]. In a study that involved multiple analyses of transcriptomes of more than 2000 histological sections of cases diagnosed with stage III melanoma, the transcription was visualized spatially within the tissue according to specific gene expression profiles [77]. The latter conclusion was a result of another significant application of spatial transcriptomics, which is the quick identification of the clones of cells that are responsible for either drug resistance or sensitivity to target therapy [78]. All in all, these different reports show the important role of spatial transcriptomics as an investigative tool in studying tumors.

Regarding the application of spatial transcriptomics in the CNS and its tumors, there was a report regarding the analysis of the brain and how highly important it is to explore the complexity of its functions in relation to macroscopic and microscopic morphology and molecular features [79]. Another study applied RNA sequencing after laser microdissection of tumor cells from cases diagnosed histologically as cases of glioblastoma, and this study has concluded a protocol that allows pure isolation of glioblastoma cells from other histological compartments. This step will be followed by further studies, as the authors mentioned, which may provide a deep understanding of the molecular and transcriptional changes in glioblastoma. This study is another example to show the future and potential significant roles of RNA sequencing and spatial transcrip-

tomics in diagnosing and classifying CNS tumors [80]. Finally, the microenvironment of brain tumors and its details are highly significant and are related to treatment resistance and even failure. However, in the presence of spatial transcriptomics and its application as an investigative tool, there was promising preliminary data to understand and overcome such challenges [81].

2.3. Differences and Similarities between DNA Methylation Array and Spatial Transcriptomics

There are a number of aspects regarding differences and similarities between DNA methylation arrays and spatial transcriptomics (Table 2). DNA methylation and spatial transcriptomics belong to different molecular fields; DNA methylation is considered as an epigenetic marker, while transcriptomics is known as the analysis of mRNA and gene expression profiles. However, both techniques have been reported to reveal spatial heterogeneity of tumors [60] [82]. A DNA methylation array is considered as a diagnostic support and investigative tool, while spatial transcriptomics is an investigative tool, not a diagnostic tool so far.

Table 2. Differences and similarities between DNA methylation array and spatial transcriptomics.

Aspect	Methylation array	Spatial transcriptomics
Field	Epigenetics	Genetic expression
Technique	Optimized for FFPE and Frozen tissue	Optimized for FFPE and Frozen tissue
Diagnostic or ancillary technique	Yes	Not yet
Revealing molecular heterogeneity of tissues in health and disease	Yes	Yes
Under development and verification for absolute diagnostic and clinical applications	Yes	Yes
Discovery of different and novel genetic or epigenetic alterations	Yes	Yes
Classification of tumors	Yes	Under development
Elucidate diagnostic and prognostic markers	Yes	Yes
Detection of therapeutic checkpoints.	Yes	Yes
Detect the resistance of response for certain type of treatment	Yes	Yes
Applications in neuropathology	Research and support diagnostic tool	Research tool

However, spatial transcriptomics enables a deep understanding by visualizing the cells according to their mRNA and gene expression. Both of the techniques are still under development and verification for absolute diagnostic and clinical applications. Both of these techniques could be applied to detect the resistance or response to certain types of treatment. Finally, both of the techniques help in the discovery of different and novel genetic or epigenetic alterations, which may provide opportunities to develop a clinically relevant classification of tumors, elucidate diagnostic and prognostic markers, and ascertain therapeutic checkpoints.

3. Conclusion

While the diagnostic roles of histopathology and cytopathology remain highly significant nowadays, routine diagnostic neuropathology depends on different complementary molecular and cytogenetic techniques such as IHC, pyrosequencing, FISH, and real-time PCR. The application of molecular diagnostics has focused on pathogenic mutations to aid in disease classification and provide accurate and precise diagnoses. This is seen clearly in the assessment of IDH1 and IDH2 to classify and diagnose diffuse gliomas. Another advance in the application of molecular diagnostics has been the identification of useful molecular markers to predict responsiveness or resistance to certain therapies. The status of MGMT promoter methylation has been reported to guide treatment for glioblastoma and some diffuse astrocytic tumors. Another exciting application for molecular pathology in the presence of high-throughput techniques such as next-generation sequencing is the identification of new genetic or epigenetic alterations. During the last few years, the role of molecular pathology has expanded after introducing novel diagnostic and investigative technologies, mainly in the classification of CNS tumors. There is tremendous growth in the role of these novel technologies, including DNA methylation profiling and spatial transcriptomics, and they are gaining major importance gradually. Accordingly, histopathology and cytopathology as conventional diagnostic disciplines of pathology may have a minor role in the future with further development and advancement of these technologies, especially when they are verified totally and their quality is ensured to serve patients with high healthcare standards. Therefore, different calls from experts in the field of pathology have asked to prepare and train pathologists not only in histopathology but in molecular pathology and its advanced technologies under what they have termed next-generation pathologists.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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