Molecular diagnostics

Molecular diagnostics is a branch of medical diagnostics that uses molecular biology techniques to identify and analyze genetic material, proteins, and other biomolecules to diagnose and monitor diseases. This field has revolutionized the way healthcare professionals detect and manage various diseases, providing faster, more accurate, and precise diagnostic information.

Key components and techniques of molecular diagnostics include:

Nucleic Acid Testing (NAT): Nucleic acid testing involves the detection and analysis of DNA or RNA sequences specific to a particular pathogen or genetic marker. Polymerase Chain Reaction (PCR) and its variants, such as quantitative PCR (qPCR) and reverse transcription PCR (RT-PCR), are commonly used techniques for amplifying and detecting target nucleic acids.

Next-Generation Sequencing (NGS): NGS technologies enable the high-throughput sequencing of DNA or RNA, allowing the simultaneous analysis of multiple genes or even entire genomes. NGS is particularly valuable for identifying genetic mutations, genomic variations, and rare genetic disorders.

Immunodiagnostics: This technique involves the detection of specific proteins or antibodies using immunoassays like enzyme-linked immunosorbent assays (ELISA) or Western blotting. It is widely used in diagnosing infectious diseases, autoimmune disorders, and cancer biomarkers.

Microarrays: Microarrays are solid supports (e.g., slides or chips) with thousands of DNA or RNA probes immobilized on them. They allow simultaneous screening of multiple genes or genetic variations and are commonly used in gene expression profiling and genotyping.

Fluorescence In Situ Hybridization (FISH): FISH is a cytogenetic technique that uses fluorescent-labeled DNA or RNA probes to visualize specific DNA sequences or chromosomal abnormalities in cells or tissues.

Mass Spectrometry: Mass spectrometry is used to identify and quantify proteins and other biomolecules based on their mass-to-charge ratio. It is commonly applied in clinical laboratories for protein profiling and identification of disease-specific biomarkers.

Applications of Molecular Diagnostics:

Infectious Diseases: Molecular diagnostics play a crucial role in detecting and identifying various pathogens, including bacteria, viruses, fungi, and parasites. It enables rapid and accurate diagnosis of infectious diseases, such as COVID-19, HIV, hepatitis, and tuberculosis.

Genetic Disorders: Molecular diagnostics is used to detect genetic mutations and variations associated with inherited diseases, such as cystic fibrosis, sickle cell anemia, and Huntington's disease.

Oncology: In cancer diagnostics, molecular techniques help identify genetic mutations and biomarkers that guide targeted therapies and predict disease prognosis.

Pharmacogenomics: Molecular diagnostics can identify genetic variations that influence an individual's response to specific drugs, enabling personalized medicine approaches.

Prenatal Testing: Molecular diagnostics is used for prenatal screening and diagnosis of genetic disorders in unborn babies.

Molecular diagnostics has significantly improved disease detection, treatment decisions, and patient outcomes. It continues to advance rapidly, driven by innovations in technology and our understanding of the molecular basis of diseases.

Since the introduction of integrated histological and molecular diagnostics by the 2016 World Health Organization (WHO) Classification of Tumors of the Nervous System, an increasing number of biomarkers have been found to have prognostic significance in infiltrating gliomas, many of which have now become incorporated as diagnostic criteria in the 2021 WHO Classification. This has increased the applicability of targeted-next generation sequencing in the diagnostic work-up of neuropathology specimens and in addition, raises the question of whether targeted sequencing can, in practice, reliably replace older, more traditional diagnostic methods such as immunohistochemistry and Fluorescence in situ hybridization. Slocum et al. demonstrated that the Oncomine Cancer Gene Mutation Panel v2 assay targeted-next generation sequencing panel for solid tumors is not only superior to IHC in detecting mutation in IDH1/2 and TP53 but can also predict 1p/19q co-deletion with high sensitivity and specificity relative to Fluorescence in situ hybridization by looking at average copy number of genes sequenced on 1p, 1q, 19p, and 19q. Along with detecting the same molecular data obtained from older methods, targeted-next generation sequencing with an RNA sequencing component provides additional information regarding the presence of RNA based alterations that have diagnostic significance and possible therapeutic implications. They advocate for expanded use of targeted-next generation sequencing over more traditional methods for the detection of important molecular alterations as a part of the standard diagnostic work up for Central nervous system tumors [1)]

Bächli et al., report a single-institutional collection of pediatric brain tumor cases that underwent a refinement or a change of diagnosis after completion of molecular diagnostics that affected clinical decision-making including the application of molecularly informed targeted therapies. 13 pediatric CNS tumors were analyzed by conventional histology, immunohistochemistry, and molecular diagnostics including DNA methylation profiling in 12 cases, DNA sequencing in 8 cases and RNA sequencing in 3 cases. 3 tumors had a refinement of diagnosis upon molecular testing, and 6 tumors underwent a change of diagnosis. Targeted therapy was initiated in 5 cases. An underlying cancer predisposition syndrome was detected in 5 cases. Although this case series, retrospective and not population based, has its limitations, insight can be gained regarding precision of diagnosis and clinical management of the patients in selected cases. Accuracy of diagnosis was improved in the cases presented here by the addition of molecular diagnostics, impacting clinical management of affected patients, both in the first-line as well as in the follow-up setting. This additional information may support the clinical decision making in the treatment of challenging pediatric CNS tumors. Prospective testing of the clinical value of molecular diagnostics is currently underway [2)].

It has been reiterated many times that molecular typing of (brain) tumors is more reliable and precise than histological classification, but data confirming this belief are largely missing. While it appears intuitive that searching for absence versus presence of a mutation is more straightforward and afflicted with less inter-rater variability than a diagnosis based on the bewildering variety of histological pictures, it still remains a hypothesis that needs to be tested in systematic inter-rater reliability studies. Preliminary endeavors have revealed surprisingly high inter-rater variability of molecular neuropathology. In an unpublished German study involving 22 neuropathology institutions,

20 gliomas were examined for MGMT promoter methylation. Uniform results of methylation versus non-methylation among all institutions were obtained in only four of 20 cases (20%), which is most probably lower than reliability expected for microscopical diagnosis. MGMT analysis may predispose to relatively high variability due to heterogeneous techniques and molecular targets, while assays for hotspot point mutations (IDH) or deletion (1p/19q) are expected to be more reliable, but this remains to be demonstrated and urgently calls for inter-laboratory studies and consensus protocols to guarantee reliable molecular and integrated diagnoses.

Are there valid and convenient surrogate markers? In general, molecular classification is performed using appropriate molecular methods, such as sequencing or methylome analysis. These techniques tend to be expensive and need to be well controlled. Existence of reliable and valid surrogate markers using more convenient and standard methods such as immunohistochemistry would be advantageous. While current agreement indicates that surrogate markers for 1p/19q co-deletion do not exist, a few neuropathologists (including Banan and Hartmann, who are authors of the corresponding review article in this issue) believe that molecular classification of medulloblastoma (WNT, SHH, non-WNT/SHH) and ependymoma, RELA fusion-positive, can be performed using appropriate immunohistochemical markers. The problem is that the spectrum of markers as suggested by different experts is variable, and sensitivity and specificity of these markers is less than ideal. Furthermore, in any institution, immunohistochemical markers need to be validated against molecular methods in a large series of tumors before diagnostic application, because immunohistochemical methods and their evaluation may vary widely among institutions.

For example, immunohistochemistry for L1CAM has been suggested as a potential surrogate marker for the diagnosis of ependymoma, RELA fusion-positive. Unfortunately, L1CAM may also be expressed by other ependymoma subtypes and other brain tumors, and only 82% of RELA fusion-positive ependymomas have been shown to be positive for L1CAM in a systematic, well-controlled study [3)].

It appears reasonable to assume that sensitivity and specificity will be even lower in a routine setting when immunostaining is performed in a single case every few weeks or months. In a similar vein, a variety of immunohistochemical markers have been recommended for the molecular classification of medulloblastoma, but their sensitivity and specificity are currently less clear than neuropathologists occasionally believe, who otherwise would be unable to classify medulloblastoma according to WHO 2016 or make only a NOS (“not otherwise specified”) diagnosis. Since molecularly defined entities of the WHO Classification are clinically, prognostically and potentially therapeutically relevant, the exclusive use of surrogate markers with 50, 80, or even 98% sensitivity does not appear diagnostically, scientifically, and ethically appropriate, if exact molecular methods are available elsewhere and neuropathology should still be considered the gold standard of diagnosis. Much more work remains to be done.

Economics or ethics?

Undoubtedly exact neuropathological diagnosis of brain tumors has become more expensive with WHO 2016. In an ideal world without financial constraints, every brain tumor would be comprehensively genetically characterized, including whole genomic sequencing and methylome analysis. In general, a classification system does not include statements about what is affordable, in part because there are huge differences between and within nations. However, the Blue Book makes an interesting point with respect to the molecular diagnosis of glioblastoma. The 2016 Classification includes glioblastoma, IDH wild type (also referred to as primary glioblastoma, about 90%) and glioblastoma, IDH mutant (also referred to as secondary glioblastoma, about 10%). The two tumor types differ with respect to age, length of clinical history, and prognosis, making a correct diagnosis clinically relevant. About 90% of IDH mutations are represented by IDH1 R132H, which can be reliably detected using an antibody specific for the mutant protein, whereas the other 10% mutations (IDH1

non-R132H, IDH2) can only be revealed by sequencing IDH1 and IDH2 genes. The proportion of glioblastomas with IDH mutation substantially decreases with age. Accordingly, the Blue Book states that it may be sufficient or “safe” in older patients to rely solely on negative immunohistochemistry for making the diagnosis of glioblastoma, IDH wild type, because in an immunohistochemically negative glioblastoma from a patient without prior lower-grade glioma, the probability of an alternative IDH mutation is <6% in a 50-year-old patient and decreases to <1% in patients aged >54 years. It is debatable whether saving cost and workload by refraining from sequencing IDH1/IDH2 genes in all glioblastomas justifies molecular mis-classification in <5% of patients.

How long does it take to make the final diagnosis? As molecular diagnostics is performed following histological and immunohistochemical analysis, time to final diagnosis inevitably increases for brain tumors with integrated diagnosis. For example, since oligodendroglioma requires molecular pathology, and criteria of anaplasia differ for astrocytic versus oligodendroglial neoplasms, it is not unusual that in a glioma with ambiguous histology and a few mitoses a preliminary diagnosis of diffuse glioma (without type and grade) has to be made for a week or so. Diagnostic turnaround times mainly depend on types of methods and frequency of assays in individual labs.

How can we move forward between WHO classification updates? Most probably, the number of molecularly defined brain tumor types will soon increase. Examples may include meningioma, atypical teratoid/rhabdoid tumor, diffuse astrocytoma IDH wild type, and pilocytic astrocytoma. Other molecular tumor types have not yet been introduced into the WHO Classification system, although they have been already included in consensus suggestions on clinical management, such as ependymoma with YAP fusion or infratentorial ependymoma types A and B [4].

Furthermore, new molecular or surrogate markers that are important for classification and diagnosis will be developed. The current intervals of 7–9 years between WHO Classification updates are certainly too long in this era of rapid progress. In order to provide prompt suggestions for the neuro-oncology community, members of the WHO Working Group and an associated Clinical Advisory Panel have recently constituted cIMPACT-NOW (Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) [5].

As the suffix NOW indicates, this is Not Official WHO. Suggestions will be solicited from the neuro-oncology community, evaluated in working groups, and guidelines for diagnostics and suggestions for possible WHO updates will be regularly published [6].

1)
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