Clinical Neuropathology practice news: ATRX, a new candidate biomarker in gliomas

By: Qinwen Mao, MD, PhD, Assistant Professor in Pathology

Genome-wide molecular approaches have substantially elucidated molecular alterations and pathways involved in the oncogenesis of brain tumors. In gliomas, several molecular biomarkers including IDH mutation, 1p/19q co-deletion, and MGMT promoter methylation status have been introduced into neuropathological practice. Recently, mutations in the ATRX (α-thalassemia/mental retardation syndrome X-linked) gene have been detected in gliomas of various subtypes and grades. In independent studies, a prognostic impact of ATRX mutations in the context of other molecular markers could be demonstrated, thus turning ATRX into a new candidate biomarker for routine clinical practice.

ATRX

The ATRX gene is located on chromosome Xq21.1 and encodes a protein that belongs to the H3.3–ATRX–DAXX chromatinremodeling pathway. Mutations in ATRX give rise to characteristic developmental abnormalities including severe mental retardation, facial dysmorphism, urogenital abnormalities and α-thalassemia. ATRX is required for the incorporation of the histone variant H3.3 at pericentric heterochromatin and at telomeres, as well as at several transcription factor binding sites. Perturbation of ATRX has been associated with a wide range of effects: altered patterns of DNA methylation (at subtelomeres, heterochromatic repeats, and ribosomal DNA), aberrant chromosome congression in mitosis, and segregation in meiosis, as well as telomere dysfunction.

ATRX expression in gliomas

Loss of nuclear ATRX seems to be a good surrogate marker for ATRX mutations and ATRX expression can – similarly to mutant IDH1 protein – be easily assessed in the routine neuropathological setting with a commercially available antibody (HPA001906, Sigma–Aldrich, St. Louis, MO, USA). Physiologically, ATRX protein is ubiquitously expressed in cell nuclei. Mutations in the ATRX gene result in a loss of nuclear protein expression in tumor cells, but retained expression in non–tumor cells (e.g., endothelial cells, pre–existing glial cells), which serve as a positive internal control, analogously to...
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analogously to the expression of INI1/SMARCB1 protein in atypical teratoid/rhabdoid tumors. Yet, interpretation of the immunohistochemical staining results might be difficult in diffusely infiltrating tumors with very low tumor cell content and areas with squeezing artifacts which are usually not well stained.

Diagnostic value of ATRX
As ATRX mutations have been to date not detected in pilocytic astrocytomas, ATRX expression analysis might be helpful in diagnostically challenging small biopsies, especially with regard to the differential diagnosis of pilocytic versus diffuse astrocytoma.

Prognostic value of ATRX
ATRX is a prognostic candidate biomarker in adult patients with malignant gliomas, where it might help to define a group of anaplastic astrocytomas with a better prognosis. The currently available literature suggests that anaplastic astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas can be divided based on IDH and ATRX mutations and 1p/19q status in three prognostically different groups:
-- Tumors with IDH mutations, no ATRX mutation and 1p/19q co-deletion. These tumors are most frequently oligodendrogliomas and oligoastrocytomas and have the best prognosis.
-- Tumors with IDH mutations and ATRX mutation and without 1p/19q co-deletion. These tumors are most frequently astrocytomas or oligoastrocytomas and show an intermediate but significantly better prognosis than group 3 tumors.
-- Tumors without IDH mutation. These tumors have the poorest prognosis and seem to behave clinically like glioblastomas.

In conclusion, ATRX seems to be a promising candidate biomarker in gliomas, which could help to refine, in combination with IDH and 1p/19q status, the prognosis of patients with malignant gliomas.

Errors Associated with Clinical Laboratory Testing
By: Andrew Bandy, MD, Resident in Pathology

There are three kinds of errors associated with clinical laboratory testing: pre-analytical, analytical and post-analytical. Their descriptions follow.

Pre-analytical Errors
A pre-analytical error is one that occurs before the related sample is tested. The vast majority occur even before the sample arrives in the laboratory. Indeed, most do not involve lab personnel. Pre-analytical errors include, among other things, patient misidentification, inappropriate patient preparation, collection of an in
Errors Associated with Clinical Laboratory Testing (Contd.)

appropriate sample, collection of an appropriate sample into an inappropriate receptacle, and sample degradation due to mistreatment, e.g., overheating or freezing, or delayed transport. Pre-analytical errors are by far the most common errors associated with clinical laboratory testing. The primary reason for this is that the steps involved in sample collection have not been standardized or uniformly accepted by all those involved in the process, e.g., physicians, nurses, healthcare trainees, patient care technicians, clerical staff, phlebotomists, etc. Fortunately, many pre-analytical errors are recognized or ‘caught’ by laboratory personnel before the related sample is tested. Examples include discrepancies between lab requisitions and tube labels, inappropriate samples (for a given test), inappropriate sample volumes, etc. Sometimes, however, pre-analytical errors are not recognized before sample analysis occurs. In such instances, the consequences of wrongful testing can range from minimal, e.g., assignment of normal results to an unrelated normal individual, to disastrous, e.g., administration of incompatible blood. So as to minimize untoward outcomes attributable to pre-analytical errors, persons involved in the process should undergo regular task-specific education and training, including competency assessment.

Analytical Errors

Analytical errors are those that occur after receipt of the sample by the laboratory, sometime during the analytical phase of testing. They constitute a relatively small percentage of the errors, in large part because much of the testing in today’s clinical laboratory is automated. Errors in the analytical phase – almost all of which are attributable to laboratory personnel – involve, among other things, missteps in specimen processing and storage, unrecognized quality control failures, instrument miscalibration, instrument misuse, procedural deviations and errors, interfering substances, and use of inappropriate or expired reagents. Laboratories are best able to control the analytical phase of testing by strict adherence to standard operating procedures, attentiveness to quality control outcomes, regular preventative maintenance of instrumentation, and scheduled proficiency testing. Although relatively few in number, analytical errors can be vexing, often coming to light only after the lab has been notified that a finalized result is inconsistent with a patient’s condition, status or treatment. Rigorous inspection of laboratories by accrediting organizations, e.g., College of American Pathologists, recurring education and training of laboratory personnel, continuous improvement in quality control measures and careful adherence to defined operating procedures all help limit the frequency of analytical errors in the clinical laboratory.

Post-analytical Errors

Post-analytical errors are those made during the reporting and/or interpretation of test results. Some are made by laboratory personnel, others by those who order the tests. Post-analytical errors occur more frequently than analytical errors, but much less frequently than pre-analytical ones. Failure to post results, result entry errors (especially for tests recorded manually), result misassignment, inappropriate test utilization, failure to relay critical values in timely fashion, and slow turnaround time are all examples of post-analytical errors. Importantly, many recurring post-analytical errors are best addressed by means of one or another IT solution. Moving forward, such solutions will be used increasingly to reduce error rate.

In closing, errors associated with clinical laboratory testing have gone down dramatically over time, due both to advances in instrumentation and automation and to development and integration of laboratory information
Errors Associated with Clinical Laboratory Testing (contd.)

systems. The three phases of the analytical cycle provide convenient means by which to parse errors associated with clinical laboratory testing and deal with them accordingly. Inasmuch as all three types of errors have potential to cause patient harm and waste resources, it behooves all those involved in any aspect of lab testing to think carefully about their role in the global process, working especially to optimize and standardize practice.

Hemoglobin Electrophoresis

By: Kim Bolek MT (ASCP) and Alicia Franken M.D.

Hemoglobin electrophoresis is a method used to detect abnormal hemoglobins. They are identified and separated based on their variation in net charge. Hemoglobin electrophoresis was traditionally performed on both a cellulose acetate gel at an alkaline pH of 8.4 and on a citrate agar gel at an acidic pH at 6.2. The abnormal hemoglobin is then determined by the migration pattern. Our laboratory currently uses a newer method called capillary hemoglobin electrophoresis which allows us to detected hemoglobins more accurately, and to analyze a larger number of cases in a shorter period of time.

In our laboratory, approximately 15,000 to 20,000 hemoglobin capillary electrophoresis assays are performed each year. At least 4 mL of whole blood or 2 lavender microtainers are required for this assay. The tube should be completely filled and inverted 8–10 times to prevent clotting and refrigerated upon collection. The specimen may be rejected if it is clotted or if the sample volume is inadequate. The red blood cells are hemolyzed and then subjected to a method of alkaline electrophoresis through a capillary electrophoresis tube at a pH of 8.6. The capillary membranes containing the migrated hemoglobins are then evaluated for peaks at various wavelengths. These peaks are measured by fractionation and reported as a percentage. The abnormal hemoglobin variants are then confirmed on a traditional cellulose acetate gel at an acidic pH of 6.2. Hemoglobin electrophoresis can detect a variety of abnormal hemoglobins including hemoglobin S, C, E, D and β-thalassemia. An example of hemoglobin S trait is shown in Figure 1.

Hemoglobin electrophoresis can detect β-thalassemia based on elevated Hb A2 levels and red cell indices; however, α-thalassemia cannot be detected by this method. Molecular testing by polymerase chain-reaction (PCR) methods or sequencing of the α-globin gene is necessary for identification of the various mutations that cause α-thalassemia.
Hemoglobin Electrophoresis. (contd.)

Figure 1. A. Normal Patient with 96.9% Hb A and 3.1% Hb A2. B. Patient with HbS Trait with 57.2% Hb A, 40.1% HbS, and 2.7% HbA2.

References:
Middle East Respiratory Syndrome (MERS)

By: Chao Qi, PhD, Associate Professor of Pathology, Director of Clinical Microbiology Laboratory

Middle East Respiratory Syndrome (MERS) is a viral respiratory illness first reported in Saudi Arabia in 2012. It is caused by a coronavirus identified as Middle East Respiratory Syndrome Coronavirus (MERS–CoV). Most people with MERS–CoV infection developed severe acute respiratory illness. About 30% of these people died. The first confirmed case of MERS–CoV infection in United States was a traveler from Saudi Arabia to Indiana. The second case was identified in Florida and he also traveled from Saudi Arabia. Hospitals have been informed by the Public Health Department to look out for MERS patients in order to prevent the spread of MERS–CoV in the U.S.

MERS–CoV is different from other coronaviruses that have been found to infect people. It’s not the same virus that caused SARS in 2003. However, like SARS it has caused severe acute respiratory illness and pneumonia in many reported cases. Patients with MERS–CoV infection have been reported in Egypt, Greece, Jordan, Kuwait, Lebanon, Malaysia, Oman, Philippines, Qatar, Saudi Arabia, United Arab Emirates, United States of America, and Yemen. The incubation period of MERS–CoV infection is from 2 to 14 days (average of approximately 5 days), and it is believed that patients are not contagious during the incubation period. The infectivity begins in the prodromal phase characterized by fever and non-specific symptoms. Early systemic symptoms are followed by dry cough and/or shortness of breath. Infectivity increases when respiratory symptoms develop. Droplet and direct contact appear to be the predominant mode of transmission, although airborne and indirect contact through fomites remains a possibility. The virus has been shown to spread among family members and to care takers in hospital setting, where there has been close contact but there is no definitive evidence of sustained spread in community. Several lines of evidence indicated the virus may come from camels.

Healthcare professional should immediately report to their local health department any person being evaluated for MERS–CoV infection as a patient. As soon as MERS–CoV infection is suspect, a mask should be placed on the patient and the evaluation should continue after the patient has been placed on airborne and contact precautions to prevent additional exposures.

Little is known about pathogenic potential and transmission of MERS–CoV. To increase the likelihood of detecting the virus, CDC recommends collecting multiple specimens from different sites at different times after symptom onset. Lower respiratory specimens are preferred, but collecting nasopharyngeal and oropharyngeal (NP/OP) specimens as well as stool and serum are strongly recommended. Respiratory specimens should be collected as soon as possible after symptoms begin – ideally with 7 days and before antiviral medication is administrated. However, if more than a week has passed since symptom onset and the patient is still symptomatic, respiratory samples should still be collected, especially lower respiratory tract specimens since respiratory virus can still be detected by RT–PCR. Currently MERS–CoV can’t be detected by any of the commercial available respiratory viral detection assays. Tests for MERS–CoV are performed by the CDC laboratory. Specimens from suspected MERS cases must be packaged and transported according to the current edition of International Air Transport Association (IATA) Dangerous Goods Regulations. Specimens should be stored and shipped at 2–8°C in less than 72 hours of collection. If samples can’t be shipped within 72 hours of collection, they should be stored on dry ice.
Proficiency Testing in the Clinical Laboratory

By: Audrey Deeken, MD, Resident in Pathology

In the United States, Clinical Laboratory Improvement Amendments of 1988 (CLIA) are federal regulatory standards that apply to all clinical laboratories testing human specimens, either for health assessment or for disease diagnosis, prevention or treatment. CLIA sets the standards that ensure accuracy of a test no matter the location of the lab. A component of CLIA is the requirement for proficiency testing. Proficiency testing, or PT, uses blinded samples, i.e., ‘unknowns,’ to assess a lab’s analytical accuracy. Such testing, also referred to as inter-laboratory comparison, is applicable to every analyte assessed by a lab. It is used specifically to monitor and grade lab performance.

PT is performed multiple times throughout the year. Labs enrolled in accredited PT programs receive and analyze unknown samples, treating them as they do patient samples. PT results are then reported to the accrediting agency, e.g., The College of American Pathologists (CAP), for comparison to the mean value derived from measurement of the same unknown by all PT program participants (generally hundreds of labs). To monitor time-dependent variance in analyte measurement, multiple unknowns are distributed over an extended period. After receiving results from all participating labs, the PT-accrediting agency grades the analyte-specific performance of each lab, referencing a lab’s result to the sample mean. Participating labs are then notified of their performance with respect to the global performance of all participants. If any PT performed by a lab ‘fails,’ i.e., deviates significantly from the sample mean, then the lab is required to investigate and document the root cause of the failure and implement corrective action. Serial PT failures are reported to governmental regulatory agencies, limiting a lab’s ability to perform further testing until it documents reproducible improvement in testing outcome.

Proficiency testing by clinical laboratories is essential because it compels the labs to verify the accuracy and reliability of their work. That, in turn, enables the labs to regularly evaluate and, as necessary, improve their performance. Being CLIA-certified, the NMH Clinical Laboratory actively participates in the proficiency testing programs of its accrediting agency, CAP. By strict adherence to CAP’s standards of best lab practice, including proficiency testing, the NMH Clinical Laboratory ensures the tests it performs are both accurate and timely, result attributes essential to quality patient care.
Clinical and Anatomical Pathology Services at NMH

Northwestern Memorial Hospital's medical staff in the Department of Pathology, are led by William Muller, MD, PhD (Chair) and Gregory Retzinger, MD, PhD (Associate chair). The department offers full-service clinical and anatomic pathology services to patients and physician offices. With 42 pathologists and 300 staff members conducting over 9 million reportable tests every year, our department is structured to offer the very highest level of customer service.

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