Detection of Isocitrate Dehydrogenase 1 Gene (IDH1) and Isocitrate Dehydrogenase 2 Gene (IDH2) Mutations: Role in Diagnosis and Characterization of Infiltrating Gliomas

Background

Infiltrating gliomas are uncommon, lethal tumors of the brain and spinal cord that are not currently curable. Infiltrating gliomas are broadly classified as astrocytoma, oligodendroglioma or mixed oligoastrocytoma according to their resemblance to normal central nervous system cells. Microscopic appearance is the current gold standard for diagnosis of these lesions, but is limited by poor interobserver agreement (particularly in grade II and III neoplasms), tissue artifacts, sampling error, and the presence of histologic mimics including benign tumors. Hence, a tool that would allow the distinction between infiltrating gliomas and other disorders would be highly desirable.

IDH1/2 Testing Background, Rationale and Clinical Significance:

Genomewide mutation screening of glioblastoma revealed somatic mutations of isocitrate dehydrogenase 1 gene (IDH1) (1), most frequently within tumors that were known to have arisen from lower grade gliomas. Subsequent work showed a high frequency of mutation of IDH1 codon 132 and a smaller number of mutations within analogous codon 172 of related gene isocitrate dehydrogenase 2 (IDH2) (2). Mutations within infiltrating gliomas appear to be limited to these two codons.

The majority of grade II and III infiltrative gliomas and higher grade gliomas that arise from them contain these mutations. Testing of other neoplasm types has shown mutations of IDH1/2 only very rarely (3). The mutations appear to be absent from the vast majority of non-CNS tumors, circumscribed brain tumors (including pilocytic astrocytoma, ependymoma, and ganglioglioma) and reactive astrogliosis. Hence, IDH1/2 testing may help distinguish between infiltrating grade II and III gliomas and other tumor types, both neoplastic and non-neoplastic. IDH1/2 mutations also have prognostic significance, since gliomas with mutations of these loci tend to survive longer than grade-matched gliomas without mutations (1-2,4-5).

Given the very limited distribution of mutations within the genes (codon 132 within IDH1 and codon 172 within IDH2), testing for IDH1/2 mutations is straightforward. IDH1/2 sequencing has been performed on formalin-fixed paraffin-embedded material (6); furthermore, fluorescence melting curve analysis has also been validated to more conveniently detect IDH1 and IDH2 mutations (7). Finally, an antibody to the most common mutant form of IDH1 protein has been developed, and can be used in conjunction with molecular testing (8).

IDH1/2 testing should be considered in the following circumstances:

1. All grade II and III infiltrating gliomas
2. Circumscribed neuroepithelial tumors (pilocytic astrocytoma, pleomorphic xanthoastrocytoma, ganglioglioma, ependymoma) where the differential diagnosis includes infiltrating glioma
3. Selected glioblastomas to add prognostic information
4. Selected paucicellular samples when the differential diagnosis includes astrogliosis
**Method:**

DNA is extracted from FFPE tissue using the Roche DNA Preparation from FFPE kit. Segments of the IDH1 and IDH2 alleles encompassing the codons R132 and R172, respectively, of the patient's DNA are amplified by PCR. Amplification is followed by the hybridization of target-specific fluorescent probes to the sequences containing the codons and subjected to melting curve analysis. The probes melt off the target DNA at temperatures dependant on the underlying DNA sequence. If there is a mismatch (mutation), the probes melt off at a lower temperature, resulting in two peaks (heterozygous) corresponding to the different alleles. This assay is performed on a LightCycler® instrument and has been validated to detect mutations in tissue samples containing approximately 25% or greater neoplastic cells by subjective estimate.

**References:**


Questions regarding IDH1/2 testing should be referred to William McDonald, M.D. at (612) 863-6320