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Mutant IDH1-specific immunohistochemistry distinguishes diffuse astrocytoma from astrocytosis

Sandra Camelo-Piragua, Michael Jansen, Aniruddha Ganguly, J. ChulMin Kim, David N. Louis, and Catherine L. Nutt

S. Camelo-Piragua · M. Jansen · A. Ganguly · J. C. Kim · D. N. Louis · C. L. Nutt, James Homer Wright Pathology Laboratories, Massachusetts General Hospital, Boston, MA, USA

D. N. Louis, Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA

S. Camelo-Piragua · D. N. Louis · C. L. Nutt, Department of Pathology, Harvard Medical School, Boston, MA, USA

C. L. Nutt, Molecular Pathology Unit, Massachusetts General Hospital, CNY 6014, Building 149, Thirteenth Street, Charlestown, MA 02129, USA, cnutt@partners.org

One of the most vexing issues in diagnostic neuropathology relates to the distinction of diffuse astrocytomas (and other diffuse gliomas) from astrocytosis (gliosis) on biopsies, particularly small biopsies. This challenging differential diagnosis arises in two general situations: (1) low cellularity edges of infiltrating astrocytomas versus mild astrocytosis from a nearby reactive condition; and (2) florid astrocytosis (e.g., near a vascular malformation) versus more cellular astrocytomas.

The “holy grail” sought in such diagnostic dilemmas is a tumor-specific marker. To date, the most widely used marker for this purpose has been p53 detection by immunohistochemistry; since mutant p53 has a longer half-life than wild-type p53, it can be more readily detected immunohistochemically than wild-type protein [3,9]. However, p53 immunohistochemistry is not an entirely accurate marker since: (1) it may show light labeling of non-neoplastic cells; (2) not all TP53 gene mutations result in immunohistochemically detectable p53; and (3) some reactive conditions (notably progressive multifocal leukoencephalopathy) may be strongly positive [8]. Another immunohistochemical marker of tumor cells is the vIII mutant of the epidermal growth factor receptor (EGFR) protein. However, this marker is not of diagnostic utility in the above differential diagnosis, since EGFRvIII is primarily expressed in glioblastomas rather than lower-grade astrocytomas. Moreover, antibodies are not widely available or readily optimized for standard immunohistochemistry, again limiting its differential diagnostic utility.

Recently, isocitrate dehydrogenase 1 (IDH1) and IDH2 mutations have been demonstrated in a variety of diffuse gliomas, with IDH1 mutations occurring commonly in lower-grade gliomas [1,2,10,12]. Notably, nearly all IDH1 mutations are the same, with CGT→CAT transition causing a specific amino acid change from arginine to histidine at codon 132 (R132H). As a result, the detection of IDH1 mutations may be a specific means to aid in differentiating between glioma and gliosis. Indeed, one recent paper utilized a PCR-based assay to show that IDH mutations are found in astrocytomas but not in reactive conditions [6]. Of 57 non-neoplastic conditions, none showed IDH1/2 mutations. In contrast, 67.3% of grade II and grade

Correspondence to: Catherine L. Nutt.

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III diffuse gliomas did; in addition, in a small subset of gliomas, IDH mutations were demonstrated in the infiltrative edge of the tumor, an area represented in the “near miss” scenario in stereotactic biopsy.

Despite the promise of IDH mutations as a tumor-specific marker, not all institutions have ready access to mutation detection methods, and DNA extraction followed by sequencing may be problematic in very small biopsies. Furthermore, IDH1 immunohistochemistry, using an antibody specific to the common R132H mutant form of IDH1, may be more sensitive than sequencing to detect tumors with mutations [4]. Using R132H mutant IDH1 and p53 immunohistochemistry, we therefore studied 21 samples of WHO grade II diffuse astrocytoma and 20 samples of reactive conditions (10 resections for epilepsy, 7 infarcts, 2 evacuated hematomas and 1 traumatic brain injury), all surgical biopsy specimens. The mean patient age for low-grade astrocytomas and reactive cases was 33.4 and 32.5 years, respectively.

Immunohistochemical staining for IDH1 was done on BenchMark XT automated tissue staining systems (Ventana Medical Systems, Inc., Tucson, AZ) using validated protocols. Endogenous peroxidase activity was blocked by H₂O₂ and antigen retrieved using CC1 reagent (Ventana Medical Systems). After washing, tissue sections were incubated with mouse monoclonal anti-R132H-IDH1 antibody culture supernatant, followed by incubation with UltraView HRP-conjugated multimer antibody reagent (Ventana Medical Systems). Antigen detection was performed using UltraView diaminobenzidine chromogen step (Ventana Medical Systems). Tissues were counterstained with hematoxylin and scored independently by two investigators (SC-P, MJ). Immunohistochemical staining for p53 was performed using a mouse monoclonal antibody (Santa Cruz, CA; # SC47698) using standard protocol.

Positive granular cytoplasmic staining of tumor cells for mutant IDH1 was found in 9 out of 21 (42.9%) WHO grade II astrocytomas, but was entirely absent in all 20 reactive samples (Fig. 1). Positive nuclear staining of tumor cells with p53 was found in 10 out of 21 (47.6%) astrocytomas; of 20 reactive cases, none showed nuclear staining in astrocytes, but one showed positive nuclear signal in macrophages (CD68-positive). Five tumors showed co-expression of mutant IDH1 and p53. When used together, mutant IDH1 and p53 demonstrated the presence of tumor in 14 out of 21 (66.7%) cases.

To date, detection of IDH mutations in low-grade astrocytomas (WHO grade II) ranges from 59 to 88% [1,5,7,11,12]; as expected, the use of an antibody specific only for the R132H mutant IDH1 resulted in a slightly lower detection rate. However, in positive cases, tumor cells demonstrated staining both in the densely cellular areas of the tumor, as well as in the less cellular infiltrating tumor edges. This latter finding is important since the less cellular areas of tumors can be the most difficult to differentiate from non-neoplastic conditions in a stereotactic biopsy and may not yield sufficient tumor DNA after extraction to allow mutant IDH1 detection by sequencing.

We therefore demonstrate, for the first time, that use of immunohistochemistry with an antibody specific for the common mutant form of IDH1 is a powerful and easy adjunct to practical neuropathological diagnosis. The antibody is likely to find its place quickly alongside that of p53 in such cases. Indeed, our data further suggests that when p53 is used concomitantly with mutant IDH1, the ability of immunohistochemistry to confirm the morphologic impression of glioma is enhanced.

These findings also illustrate the increasing rapidity with which molecular assays are being converted to immunohistochemical stains. In the diagnosis of atypical teratoid/rhabdoid tumor, the transition from fluorescence in situ hybridization for chromosome 22q loss, to INI1 gene sequencing, to INI1 immunohistochemistry took well over 10 years; today, INI1 immunohistochemistry represents the commonly and widely used method. In the case of IDH1,

scarcely more than one year has passed between discovery of mutations in diffuse gliomas and the implementation of diagnostic IDH1 immunohistochemistry—attesting to the quickening pace of diagnostic change.

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Fig. 1.

R132H mutant IDH1 immunohistochemistry in WHO grade II astrocytoma and astrocytosis. Strong granular cytoplasmic mutant IDH1 staining in cellular area of astrocytoma (**a** H&E; **b** mutant IDH1) and in infiltrating tumor cells (**c** H&E; **d** mutant IDH1); example of two cases, individual mutant IDH1-positive infiltrating tumor cells in cortex (**d** *upper panels*, case 18) and at the edge of the tumor (**d** *bottom panel*, case 4). Marked astrocytosis adjacent to an infarct positive for GFAP (**e**) and negative for mutant IDH1 (**f**). Magnification **a–f** = 400×; **d**, *upper panels* = 1,000×