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SHORT REPORT

Monoclonal antibody specific for *IDH1* R132H mutation

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Abstract *IDH1* R132H mutations occur in approximately 70% of astrocytomas and oligodendroglial tumors. We developed a mouse monoclonal antibody targeting the *IDH1* R132H mutation. Here, we show the high specificity and sensitivity of this antibody on Western blots and tissue sections from formalin fixed paraffin embedded tumor specimens. This antibody is highly useful for tumor classification, in detecting single infiltrating tumor cells and for the characterization of the cellular role of mutant IDH1 protein.

Keywords IDH1 · Monoclonal antibody · Immunohistochemistry · Astrocytoma · Oligodendroglioma · Oligoastrocytoma

Somatic mutations in *IDH1* have recently been shown to be the most frequent structural alteration in astrocytomas, oligodendrogliomas and mixed oligoastrocytomas [1, 5, 9, 11, 14]. Approximately 70% of these tumor entities carry heterozygous point mutations in codon 132. Of these, more than 90% are mutations of the R132H type [4]. Because of the high frequency and the occurrence in WHO grade II tumors, *IDH1* mutations are believed to constitute early steps in tumorigenesis. Cancer of other organ systems exhibits rarely *IDH1* mutations; however, up to 10% of patients with acute myeloid leukemia (AML) exhibit such mutations [2, 6, 8]. The mutations have been shown to abrogate enzymatic activity in respect to NADPH generation [5, 14]. The mechanism of *IDH1* mutations in tumor formation is not known, however actual findings implicate activation of HIF-1 [15]. The detection of *IDH1* mutations is of major diagnostic and prognostic importance. The presence of mutations is restricted to astrocytomas and oligodendrogliomas and among glioblastomas mainly to so-called secondary glioblastomas proven to have arisen from a previously diagnosed lower grade astrocytoma. Presence of *IDH1* mutations in anaplastic astrocytomas and oligodendroglial tumors was shown to be associated with a significantly better outcome [10]. Further, in a phase III clinical trial, *IDH1* mutations emerged as the single most powerful predictor in these patients [13]. Interestingly, those few primary glioblastomas with *IDH1* mutations also have a significantly better prognosis, with *IDH1* mutation again being the most powerful predictor of prognosis in a prospective study [12].

Clinical relevance and the highly homogeneous pattern of *IDH1* mutations make this alteration a very attractive target for developing a mutation-specific antibody. We therefore immunized C57BL/6 mice with synthetic peptides of CKPIIIGHHAYGD sequence coupled to keyhole limpet hemocyanin matching the IDH1 amino acid sequence from codon 125 to 137 containing the R132H mutation. In order to obtain an antibody from another species directed against wild-type IDH1, Sprague–Dawley rats were immunized with recombinant protein fused to a hexahistidine tag spanning the region of codon 244–594.

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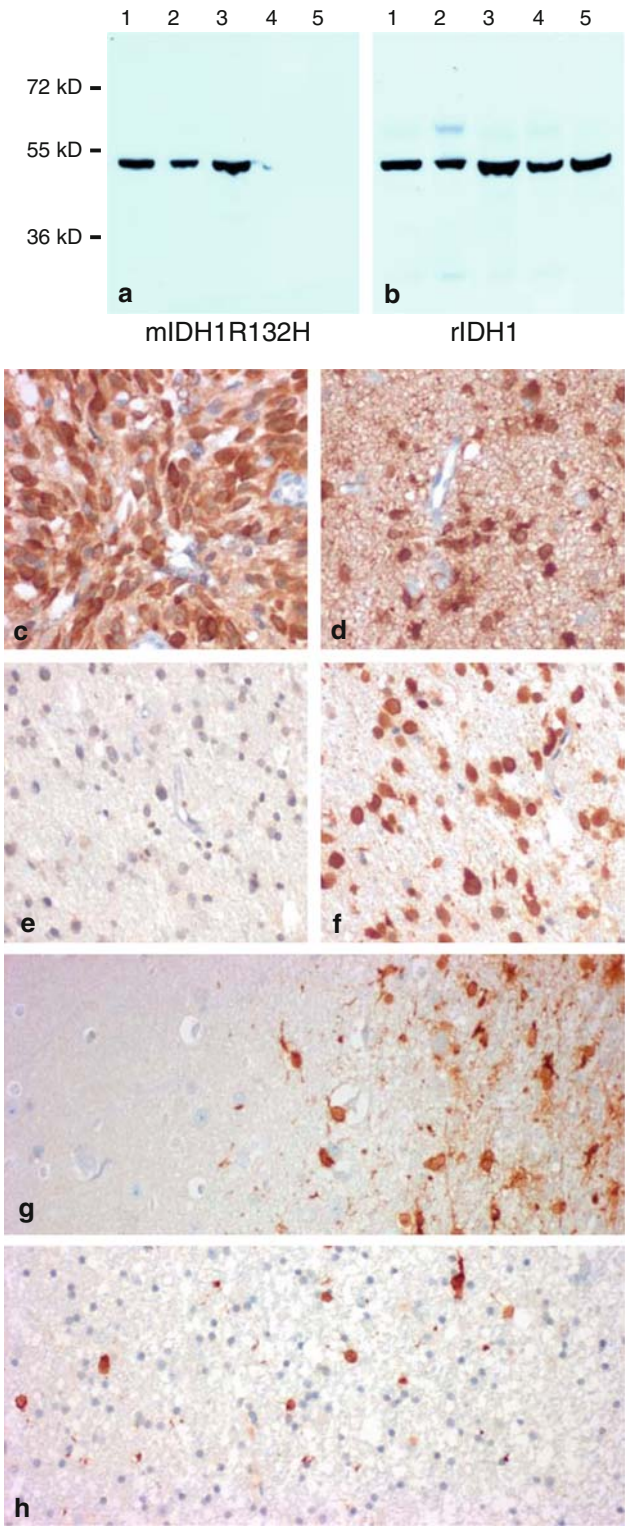

Fig. 1 **a** Western Blot showing binding of R132H mutation-specific antibody mIDH1R132H. Lysates in lanes *1* (ID 40954), *2* (ID 41560) and *3* (ID 41402) are from 3 different gliomas carrying the *IDH1* R132H mutation, lysate in lane *4* (ID 41654) is from a glioma with wild-type *IDH1* sequence and lysate in lane 5 (ID 41522) is from a glioma with R132C mutation. mIDH1R132H detects antigen exclusively in gliomas carrying the *IDH1* R132H mutation. **b** On the same blot both, wild-type and mutant IDH1 are detected by antibody rIDH1 in the same lysates 1–5. **c** Binding of mIDH1R132H to formalin fixed paraffin embedded tissue from ID 40954 (corresponding to lane *1* in Fig. 1a, b). **d** Binding of mIDH1R132H to ID 41402 (lane *3*). **e** No binding of mIDH1R132H to ID 41654 (lane *4*). **f** Binding of rIDH1 to ID 41654 (lane *4*). **g** Binding of mIDH1R132H to ID 43906 at the infiltration edge to cortex. **h** Binding of mIDH1R132H to ID 43906 at the infiltration edge to white matter

The monoclonal antibodies were raised according to the method described by Köhler and Milstein [7]. Consecutive subcloning, isotyping and purification were performed following published protocols [3]. Availability of this second antibody from another species recognizing both wild-type and mutant IDH1 protein allows simultaneous monitoring of the distribution of wild-type and mutant protein for example by double immunofluorescence. Analysis by Western blotting employing protein extracts from tumors with predetermined sequence status of *IDH1* codon 132 demonstrates binding of R132H-specific mouse clone mIDH1R132H (internal clone H14) only in the extracts from three tumors carrying this mutation but not in those with other mutations or with wild-type sequence. In contrast, rat clone rIDH1 (internal clone r41) detected IDH1 protein in wild-type tumors but also in tumors carrying different mutations due to its binding site distant from the motive including codon 132. Representative data is shown in Fig. 1a, b. Immunohistochemistry with mIDH1R132H on formalin fixed and paraffin embedded tumor tissues detected mutant protein only in tumors that previously tested positive for the R132H mutation but not in tumors with other mutations in codon 132 or with wild-type sequence. Antibody binding was restricted to tumor cells and did not occur in endothelial or lymphocytic cells. In contrast, rIDH 1 bound to all, tumor and non-tumor cells. Representative data is shown in Fig. 1c–f. The high specificity of mIDH1R132H to detect single tumor cells is demonstrated in Fig. 1g, h: individual tumor cells bind antibody in the infiltrating edge towards cortex (Fig. 1g) and the white matter (Fig. 1h). We find the ability of the mutation-specific antibody mIDH1R132H to detect single tumor cells within otherwise inconspicuous tissue of special interest. So far we did not detect antibody binding of mIDH1R132H to any cell other than tumor cells and, therefore, we regard single positive cells as unequivocal evidence for tumor. This feature is of major diagnostic interest for surgical specimens from small low-grade astrocytomas or oligodendrogliomas frequently not containing solid tumor but rather tumor infiltrated brain tissue.

The discrepancy between the mutation type observed in *IDH1*, namely, heterozygous mutations restricted to a single codon in absence of other truncating mutations which is supportive for an activating mutation and the proven inactivation of dehydrogenase activity upon mutation,

suggests additional functions for wild-type or mutant IDH1. The present set of antibodies will allow testing for binding partners and detection of potential differences between wild-type and mutant enzyme.

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References

1. Balss J, Meyer J, Mueller W et al (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597–602
2. Bleeker FE, Lamba S, Leenstra S et al (2009) IDH1 mutations at residue p.R132 (IDH1(R132)) occur frequently in high-grade gliomas but not in other solid tumors. Hum Mutat 30:7–11
3. Harlow E, Lane D (1988) Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbour
4. Hartmann C, Meyer J, Balss J et al (2009) Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1010 diffuse gliomas. Acta Neuropathol 118:469–474
5. Ichimura K, Pearson DM, Kocialkowski S et al (2009) IDH1 mutations are present in the majority of common adult gliomas but are rare in primary glioblastomas. Neuro Oncol 11:341–347
6. Kang MR, Kim MS, Oh JE et al (2009) Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. Int J Cancer 125:353–355
7. Köhler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497
8. Mardis ER, Ding L, Dooling DJ et al (2009) Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med 361:1058–1066
9. Parsons DW, Jones S, Zhang X et al (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812
10. Sanson M, Marie Y, Paris S et al (2009) Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol 27:4150–4154
11. Watanabe T, Nobusawa S, Kleihues P et al (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 174:653–656
12. Weller M, Felsberg J, Hartmann C et al (2009) Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma. A prospective translational study of the German Glioma Network. J Clin Oncol (in press)
13. Wick W, Stoffels M, Engel C et al (2009) NOA-04 Randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with PCV or temozolomide. J Clin Oncol (in press)
14. Yan H, Parsons DW, Jin G et al (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773
15. Zhao S, Lin Y, Xu W et al (2009) Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science 324:261–265