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Letter to the editor

Sequencing *IDH1/2* glioma mutation hotspots in gliomas and malignant peripheral nerve sheath tumors

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The isocitrate dehydrogenase 1 gene (*IDH1*) was in 2008 identified as a new glioblastoma pathogenesis candidate gene by Parsons et al.[1] Following this discovery, IDH1/2 mutated tumors have been studied more carefully, and in 2010 the *IDH1* mutation was found to be associated with a glioma cytosine–phosphate–guanine island methylator phenotype (G-CIMP).[2] Today, it is clear that the common mutations in the *IDH* genes result in an IDH enzyme with *de novo* activity producing 2-hydroxyglutarate.[3] This metabolite is an inhibitor of α-ketoglutarate–dependent dioxygenases, leading to genome-wide methylation alterations subsequently changing gene expression.[4,5]

Although large series of different tumor types have been analyzed, *IDH1/2* mutations have been reported at significant frequencies in only a few other neoplastic diseases, such as central and periosteal cartilaginous tumors, intrahepatic cholangiocarcinoma, acute myeloid leukemia, blast-phase myeloproliferative neoplasms, and angioimmunoblastic T-cell lymphoma.[6–15] The *IDH* mutation frequency is relatively high in oligodendrogliomas. Hence, we hypothesized that *IDH* mutations could also be frequent in malignant peripheral nerve sheath tumors (MPNSTs), tumors composed of Schwann cells, which are the peripheral nervous system's functional equivalent to oligodendrocytes. Previously, no *IDH1* R132 mutations were detected in a small series of 17 schwannomas[7] or in a series of 24 MPNSTs.[16] We sequenced the glioma mutation hotspots R132 and R172 in *IDH1* and *IDH2*, respectively, in gliomas and one of the world's largest series of MPNSTs.

Tumor samples ($n = 161$) from 157 glioma patients who underwent surgery at Oslo University Hospital–Rikshospitalet, Oslo, Norway from 2005 to 2010 were included in this study. High-grade gliomas from patients with known prior low-grade glioma were regarded as secondary, while other high-grade gliomas were regarded as primary. Histological diagnoses were reviewed by a neuropathologist (D.S.). MPNST tissue samples ($n = 93$, from 91 patients) diagnosed in the period 1973–2008 were collected from Oslo University Hospital; Skåne University Hospital, Lund, Sweden; University Medical Center Groningen, Groningen, Netherlands; and Istituto Ortopedico Rizzoli, Bologna, Italy. Histological diagnoses were determined by sarcoma reference pathologists. The biobanks and projects were approved according to national legislations.

DNA was extracted from fresh frozen tissue. From gliomas where fresh frozen tissue was not available we used tissue stored in RNA*later*. Regions of *IDH1* and *IDH2* including the mutation hotspots were amplified with primers in separate PCR reactions. Primer sequences for the *IDH1* reaction were 5′-GCA-CGG-TCT-TCA-GAG-AAG-CCA-3′ (forward, our design) and 5′-AGG-GGA-TCC-TAT-TGT-GCA-GCC-AG-3′ (reverse, our design), and for the *IDH2* reaction 5′-AGC-CCA-TCA-TCT-GCA-AAA-AC-3′ (forward[17]) and 5′-CTA-GGC-GAG-GAG-CTC-CAG-T-3′ (reverse[17]). Results were reviewed independently by 2 of the authors (A.B.H., H.H.). All mutations were technically validated in a separate run.

The detected *IDH1* mutation frequencies in the 161 gliomas (Table 1) are in line with earlier reports[18] and confirm that *IDH1* mutations are frequent in World Health Organization (WHO) grades II and III astrocytomas, oligoastrocytomas, oligodendrogliomas, and secondary glioblastomas. From 4 glioma patients included in the study, 2 samples of different grades were examined. The same mutation status was found in both samples. No mutation was detected in the *IDH2* hotspot R172, which, based on earlier reported *IDH2* mutation frequencies and our number of examined gliomas, is not surprising. This is particularly so because we found a relatively high frequency of *IDH1* mutations, and *IDH1* and *IDH2* mutations do not appear to occur together.[17]

The WHO grade III gliomas were divided into primary and secondary tumors depending on absence or presence of preexisting lower-grade lesions. All anaplastic oligodendrogliomas ($n = 8$) were *IDH1* mutated, regardless of primary/secondary status. In the subgroups anaplastic oligoastrocytoma and anaplastic astrocytoma, all primary tumors but one were *IDH1* nonmutated, whereas all secondary tumors were mutated. Interestingly, the only primary anaplastic astrocytoma with *IDH1* mutation had

Table 1. Frequency of *IDH1* mutations in codon R132

Diagnosis	Total Mutations	Base Change in Codon			
	% ($n_{mutated}/n_{total}$)	G > A	C > A	C > T	C > G
Commonly IDH mutated gliomas					
Diffuse astrocytoma	**93** (13/14)	10/13	2/13	1/13	–
Oligoastrocytoma	**100** (19/19)	17/19	1/19	1/19	–
Oligodendroglioma	**88** (7/8)	6/7	–	–	1/7
Anaplastic astrocytoma	**50** (2/4)[a]	1/2	–	1/2	–
Anaplastic oligoastrocytoma	**50** (2/4)[b]	1/2	1/2	–	–
Anaplastic oligodendroglioma	**100** (8/8)[c]	8/8	–	–	–
Secondary glioblastoma	**80** (8/10)	8/8	–	–	–
Total	**88** (59/67)	51/59	4/59	3/59	1/59
Other gliomas					
Low-grade neuroepithelial tumors (NOS)	**0** (0/2)	–	–	–	
Primary glioblastoma	**2** (2/92)	2/2	–	–	
Schwann cell tumors					
MPNST	**0** (0/93)	–	–	–	

Abbreviation: NOS, not otherwise specified.
[a]1/3 primary and 1/1 secondary.
[b]0/2 primary and 2/2 secondary.
[c]6/6 primary and 2/2 secondary.

areas with low-grade histology, suggesting that this may in fact have been a secondary tumor.

We found that 2 primary glioblastomas harbored the *IDH1* mutation. Both patients had a short clinical history and therefore no evidence of a preexisting low-grade glioma. Our neuropathologist found one of these primary glioblastomas to be diagnostically intermediate between an anaplastic oligoastrocytoma and a glioblastoma with a large oligodendroglial component. Nonetheless, the patient received standard glioblastoma treatment and was therefore included as a primary glioblastoma patient in our analyses.

No mutations were detected in the samples from the 91 MPNST patients, either in the hereditary cases ($n = 44$) or in the sporadic cases ($n = 47$). Our results suggest that although principally composed of functionally equivalent cells, gliomas and MPNSTs pursue different pathogenetic pathways. We cannot rule out the possibility of mutations occurring in other *IDH1/2* codons. However, few other *IDH1/2* mutations have so far been reported, and only a few of these have resulted in the same *de novo* activity as the glioma hotspot mutations.[19]

In conclusion, when analyzing 161 glioma samples, we detected the expected mutations in the analyzed *IDH1/2* glioma mutation hotspots at frequencies on a par with previous studies.[18] Furthermore, it would be interesting to analyze a larger series of WHO grade III gliomas to confirm our observations indicating that *IDH1* mutations are more frequent in secondary than primary anaplastic oligoastrocytomas and anaplastic astrocytomas. No *IDH1/2* glioma hotspot mutations were detected in MPNSTs, indicating that these pathologically similar but peripherally occurring tumors are pathogenetically different from CNS gliomas.

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