Published in final edited form as:

Acta Neuropathol. 2011 February ; 121(2): 279–281. doi:10.1007/s00401-010-0790-y.

R132H-mutation of Isocitrate Dehydrogenase-1 is not Sufficient for HIF-1 α Upregulation in Adult Glioma

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Mutations affecting codon 132 of the enzyme cytosolic isocitrate dehydrogenase 1 (IDH-1) have been found in 68%–86% of World Health Organization (WHO) grade II and III astrocytic and oligodendroglial tumors in adults, as well as in the majority of WHO grade IV secondary glioblastomas that evolved from these lower-grade tumors [3,4,8]. IDH-1 is localized in the cytoplasm and peroxisomes and serves as a major source for cytosolic nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is necessary for the regeneration of reduced glutathione, which functions as the main antioxidant in mammalian cells. Glioma-specific mutations in IDH-1 usually affect the amino acid arginine at position 132 of the amino acid sequence [3,5,8], resulting in a substitution of histidine for arginine. This mutation only affects one allele and results in a decrease in IDH-1 activity. Inactivation of IDH-1 has been linked to elevated oxidative stress, which has been hypothesized to confer an increased sensitivity to chemotherapy and radiotherapy [5].

A recent report suggests that the R132H mutation in IDH-1 may lead to accumulation of hypoxia-inducible factor 1 alpha (HIF-1 α) in glioma cells [10]. Given that the activation of the HIF-1 α pathway is associated with malignant progression and unfavorable outcomes in various tumor types [6], overexpression of HIF-1 α as a result of the R132H IDH-1 mutation has been hypothesized to act as a driver for malignant transformation in gliomas. To test the hypothesis that expression of R132H-mutated IDH-1 specifically leads to overexpression of HIF-1 α in human gliomas *in vivo*, we examined a large panel of glial tumors for association between R132H-mutated IDH-1 and HIF-1 α expression using immunohistochemistry.

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We analyzed a total of 120 archived formalin-fixed paraffin embedded glioma specimens from 115 patients. Immunohistochemistry for R132H-mutated IDH-1 was performed using mouse anti-human isocitrate dehydrogenase-1 mutation (R132H) antibody, clone H09, as previously described [1]. To determine the spatial localization of HIF-1 α in relation to R132H-mutated IDH-1 tumor cells, immunohistochemistry for HIF-1 α was performed in adjacent sections as previously described [9]. As a control, we also performed HIF-1 α immunostaining on five (including one pediatric) of the 37 IDH-1 negative primary GBMs.

The patients' clinical demographics and histology for the 115 tumor samples are shown in Table 1. Patient age at first surgery ranged from 1 to 80 years, with a median age of 40 years. There were 58 males and 57 females. Immunohistochemical evidence of tumor cells with R132H-mutated IDH-1 was present in 25/138 (18.1%) of patients. In patients positive for R132H-mutated IDH-1, ages ranged from 18 to 64 years of age with a mean age of 46 years. The large number of pediatric patients studied, i.e. 41/115 (35.7%) resulted in a younger median age (40 years) in our overall patient cohort compared to the adult series. However, all of the patients positive for R132H-mutated IDH-1 were adult patients (age 18 years) and therefore, the median age of the positive patients was higher (46 years) than the overall median age. The lower rate of IDH-1 mutations in secondary glioblastomas in our series is likely a result of the small number of patients with secondary glioblastomas in our cohort.

All tumors positive for R132H-mutated IDH-1 (n=25) were carefully evaluated for HIF-1 α expression in serial sections. We found that a subset of cells expressing R132H-mutant IDH-1 also expressed HIF-1 α . In specimens that showed necrosis, HIF-1 α up-regulation was evident in cells adjacent to areas of necrosis (Figure 1), as previously reported [9] although in several tumors HIF-1 α expression was not associated with necrotic zones. HIF-1 α , however, was not overexpressed in other areas of the tumors, including those with strong immunoreactivity for R132H-mutated IDH-1. HIF-1 α was seen in perinecrotic tumor cells in 8 cases, in groups of cells in 5 cases, and in both perinecrotic and groups of cells in 1 case. Therefore, no relation between the expression of R132H-mutated IDH-1 and HIF-1 α was evident. The five IDH-1 negative GBMs that were also stained for HIF-1 α showed a pattern of HIF-1 α immunoreactivity similar to the R132H-mutated GBMs.

Our findings indicate that although HIF-1 α is upregulated in a subset of gliomas *in vivo*, activation of the HIF-1 α pathway is not primarily regulated by R132H-mutated IDH-1, but more likely via other established mechanisms such as hypoxia and/or growth-factor dependent transcriptional regulation [7]. We feel that the picture of glioma tumor progression in regards to the R132H IDH-1 mutation is more complex than the current literature suggests, and is not entirely explained by the previously reported reduced levels of alpha-ketoglutarate that regulate HIF-1 α . Rather than HIF-1 α activation, our findings support alternate mechanisms that drive the progression of IDH-1 mutant gliomas, such as the recently reported excess accumulation of 2HG [2].

Acknowledgments

This work was supported by grants to D. Z. from the National Institutes of Health (R01 CA100426 and R21 NS065380-01) and the Musella Foundation.

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Figure 1. Expression of R132H-mutated IDH-1 and HIF-1α in a secondary glioblastoma (WHO Grade IV/IV) (a,b,c) and in anaplastic mixed glioma (oligodendroglioma/astrocytoma) (WHO grade III/III) (d,e,f)

(a) **H&E.** A necrotic focus with adjacent pseudopalisading cells is seen. Hyperplastic blood vessels are also present. (b) **R132H-mutated IDH-1.** Cytoplasmic immunostaining for R132H-mutated IDH-1 is seen as diffuse immunoreactivity in tumor cells. (c) **HIF-1α.** Nuclear staining for HIF-1α is restricted in the tumor cells adjacent to the necrosis. (d) **H&E.** The section shows abundant perivascular accumulation of tumor cells. (e) **R132H-mutated IDH-1.** The perivascular cells are immunoreactive for R132H-mutated IDH-1. (f) **HIF-1α.** No staining for HIF-1α is observed in the R132H-mutated IDH-1 immunoreactive cells.

Clinical characteristics of all study patients and summary of immunohistochemistry results for R132H-mutated IDH-1 and HIF-1 α

Table 1

Columns 2 and 3 summarize patient numbers and demographics of all study patients. Columns 4, 5 and 6 show R132H mutant IDH-1 staining results of pediatric (<18 years), adult (18 years) and all study patients, respectively. Column 7 summarizes HIF-1 α staining results for all tumors with R132H mutant IDH-1.

Histology	Number of Patients	Median Age (Range)	R132H- mutated IDH-1 Pediatric Patients (%)	R132H- mutated IDH-1 Adult Patients (%)	R132H- mutated IDH-1 All Patients (%)	HIF-1 α positive/All R132H- mutated IDH-1 (%)
Ganglioglioma WHO grade I/III	1	8 (8)	0/1 (0)	0/0 (0)	0/1 (0)	0/0 (0)
Pilocytic astrocytoma WHO grade I	4	4–20 (9)	0/3 (0)	0/1 (0)	0/4 (0)	0/0 (0)
Diffuse astrocytoma WHO grade II	12	1–58 (7)	0/10 (0)	1/2 (50)	1/12 (8.3)	0/1 (0)
Anaplastic astrocytoma WHO grade III	7	2–59 (24)	0/3 (0)	1/4 (25)	1/7 (14.3)	1/1 (100)
Glioblastoma (primary) WHO grade IV	37	1–76 (50)	0/8 (0)	2/29 (6.9)	2/37 (5.4)	2/2 (100)
Glioblastoma (secondary) WHO grade IV	7	14–64 (46)	0/1 (0)	2/6 (33.3)	2/7 (28.6)	2/2 (100)
Oligodendroglioma WHO grade II	14	3–54 (17)	0/7 (0)	5/7 (71.4)	5/14 (35.7)	2/5 (40)
Anaplastic oligodendroglioma WHO grade III	16	6–64 (48)	0/2 (0)	10/14 (71.4)	10/16 (62.5)	4/10 (40)
Mixed glioma WHO grade II	7	2–59 (16)	0/5 (0)	1/2 (50)	1/7 (14.3)	0/1 (0)
Anaplastic mixed glioma WHO grade III	10	8–80 (50)	0/1 (0)	3/9 (33.3)	3/10 (30)	3/3 (100)
TOTAL	115	1–80 (40)	0/41 (0)	25/74 (33.8)	25/115 (21.7)	14/25