

## Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers

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Missense somatic mutations in *IDH1* gene affecting codon 132 have recently been reported in glioblastoma multiforme (GBM) and other gliomas. The recurrent nature of the *IDH1* mutations in the same amino acid strongly suggests that the mutations may play important roles in the pathogenesis of glial tumors. The aim of this study was to see whether the *IDH1* codon 132 mutations occur in other human cancers besides glial tumors. We also attempted to confirm the occurrence of the *IDH1* mutations in GBM of Korean patients. We have analyzed 1,186 cancer tissues from various origins, including carcinomas from breast, colon, lung, stomach, esophagus, liver, prostate, urinary bladder, ovary, uterine cervix, skin and kidney, and malignant mesotheliomas, primary GBM, malignant meningiomas, multiple myelomas and acute leukemias by single-strand conformation polymorphism analysis. We found four *IDH1* codon 132 mutations in the GBM (4/25; 16.0%), two in the prostate carcinomas (2/75; 2.7%) and one in the B-acute lymphoblastic leukemias (B-ALL) (1/60; 1.7%), but none in other cancers. The *IDH1* mutations consisted of five p.R132H and two p.R132C mutations. The data indicate that *IDH1* codon 132 mutations occur not only in GBM, but also in prostate cancers and B-ALL. This study suggests that despite the infrequent incidence of the *IDH1* mutations in prostate cancers and B-ALL, mutated *IDH1* could be therapeutically targeted in these cancers and in glial tumors with the *IDH1* mutations.

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**Key words:** IDH1; glioblastoma multiforme; mutation; IDH1 codon 132; cancer

For the comprehensive elucidation of genetic alterations in glioblastoma multiforme (GBM), Parsons *et al.*<sup>1</sup> recently analyzed 20,661 genes (approximately two-thirds of total human genes) by a direct DNA sequencing method. In GBM tissues, they identified 685 genes that contained at least one nonsilent somatic mutation(s). In addition to the genes with known mutations in GBM such as *TP53*, *EGFR*, *PTEN*, *NF1*, *RB1* and *PIK3CA*, two genes *isocitrate dehydrogenase 1 (IDH1)* and *phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1)* harbored mutations at high incidences.<sup>1</sup> Somatic point mutations of *IDH1* were detected in 12 of 105 GBM (11.4%). Of note, all of the 12 mutations were predicted to substitute an Arg residue in position 132 of amino acid sequences. A following study using various brain tumors detected the *IDH1* codon 132 mutations not only in GBM (primary and secondary), but also in other glial tumors.<sup>2</sup> Despite the high incidence of the *IDH1* mutations in the brain tumors, the functional roles of the mutations in cancer development remain unknown.

One of the main concerns in cancer genetics is as to whether any mutation found in cancer is specific to few cancer types or is widespread in many cancer types. For example, *EGFR* and *JAK2* mutation is specific to few cancer types,<sup>3,4</sup> whereas *K-RAS* and *TP53* mutations are common to many cancer types.<sup>5,6</sup> To see if any other types of human cancers besides glial tumors carry the *IDH1* codon 132 mutations, we have analyzed the *IDH1* gene in various types of human cancers in this study.

### Material and methods

Cancer tissues (1,186) from Korean patients (carcinomas from breast, colon, lung, stomach, esophagus, liver, prostate, urinary

bladder, ovary, uterine cervix, skin and kidney, and malignant mesotheliomas, primary GBM, malignant meningiomas, multiple myelomas and acute leukemias) were used for this study (Table I). All of the cancers analyzed were primary cancers, but not metastatic cancers. We did not include cell lines in this study. For the solid cancers, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides using a 30I/2 gauge hypodermic needle affixed to a micromanipulator, as described previously.<sup>7</sup> Approval for this study was obtained from the Catholic University of Korea, College of Medicine's institutional review board.

Up to now, all of the *IDH1* mutations have been detected at nucleotide sequences 394 or 395 in exon 4, which would result in amino acid substitutions at 132.<sup>1,2</sup> Thus, we analyzed a part of the exon 4 of *IDH1* gene by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) analysis. Genomic DNA each from tumor cells and normal cells of the same patients were amplified by PCR with one primer pair (5'-AAACAAATGTGGAAATCACC-3' and 5'-TGCCAACATGACTTACTTGA-3'; product size 166 base pairs). Radioisotope (<sup>32</sup>P)dCTP was incorporated into the PCR products for detection by autoradiogram. Other procedures of the PCR-SSCP were described in our previous studies.<sup>7-9</sup> After SSCP, direct DNA sequencing reactions were performed in the cancers with the mobility shifts in the SSCP according to the manufacturer's recommendation (ABI Prism Genetic Analyzer, Applied Biosystem, Foster City, CA). As potential positive controls for the SSCP, we included GBM tissues with known *IDH1* mutations.

### Results and discussion

PCR-SSCP analysis of the exon 4 of *IDH1* gene in the 1,186 cancers identified aberrant bands in seven cancers (Fig. 1). There was no visible aberrant band in the other 1,179 cancers. Direct DNA sequencing analysis of the PCR products in the seven cancers [four GBMs, two prostate cancers and one B-acute lymphoblastic leukemia (B-ALL)] with the aberrant SSCP bands led to the identification of seven *IDH1* mutations (Fig. 1; Table II). None of the normal samples from the same patients showed evidence of mutations by the SSCP and direct DNA sequencing (Fig. 1), indicating the mutations had arisen somatically. The *IDH1* mutations consisted of five c.395G>A (p.R132H) and two c.394C>T (p.R132C; Table II). We repeated the experiments twice, including PCR, SSCP and sequencing analysis to ensure the specificity of the results and found that the data were consistent (data not shown).

Grant sponsor: Korea Science and Engineering Foundation (KOSEF); Grant number: R01-2008-000-10014-0.

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Received 15 December 2008; Accepted after revision 10 February 2009

DOI 10.1002/ijc.24379

Published online 23 February 2009 in Wiley InterScience (www.interscience.wiley.com).



Publication of the International Union Against Cancer

The SSCP of all of the seven cancers with the *IDH1* mutations at the mutation sites showed both wild-type and aberrant bands (Fig. 1a), and direct sequencing analysis also

TABLE I – *IDH1* MUTATIONS IN 1,185 CANCERS

Type of cancers	Number of cancers	<i>IDH1</i> codon 132		
		Wild type	Mutation	Mutation (%)
Primary GBM	25	21	4	16
Prostate carcinoma	75	73	2	2.7
B-ALL	60	59	1	1.7
T-ALL	26	26	0	0
Acute myelogenous leukemia	100	100	0	0
Multiple myeloma	30	30	0	0
Malignant meningioma	29	29	0	0
Malignant mesothelioma	6	6	0	0
Non-small cell lung cancer	179	179	0	0
Gastric carcinoma	101	101	0	0
Colorectal carcinoma	97	97	0	0
Breast carcinoma	94	94	0	0
Hepatocellular carcinoma	81	81	0	0
Hepatoblastoma	56	56	0	0
Esophageal squamous cell carcinoma	71	71	0	0
Urothelial carcinoma	28	28	0	0
Uterine cervical carcinoma	10	10	0	0
Ovarian carcinoma	85	85	0	0
Squamous cell carcinoma, skin	19	19	0	0
Renal cell carcinoma	14	14	0	0

revealed both mutant and wild-type sequences (Fig. 1b). We used microdissected tissues that had purity of cancer cells over 90%.<sup>7</sup> Thus, both SSCP and direct sequencing data indicate that the *IDH1* mutations may be heterozygous in the seven cancers.

We compared the incidences of the *IDH1* mutations in primary GBM between the previous (7/99)<sup>1</sup> and our data (4/25). There was no statistical difference between them (Fisher's exact test,  $p > 0.05$ ). The prostate carcinomas used in the present study consisted of 55 TNM II, 19 TNM III and 1 TNM IV cancers. According to the Gleason score, the prostate carcinomas were graded as Gleason score 6 ( $n = 18$ ), Gleason score 7 ( $n = 47$ ), Gleason score 8 ( $n = 8$ ) and Gleason score 9 ( $n = 2$ ). However, there was not any significant association of the *IDH1* mutations either with the Gleason scores or the TNM stages ( $\chi^2$  test,  $p > 0.05$ ).

Recurrent genetic alterations have been used as therapeutic targets in cancer treatment. For example, recurrent mutations of *EGFR* in lung cancers, *BCR/ABL* translocations in chronic myelogenous leukemias and *HER2* amplifications in breast cancers are being used as therapeutic targets of Gefitinib (Iressa), Imanitib (Gleevec) and Trastuzumab (Herceptin), respectively.<sup>4,10-12</sup> Recurrent *IDH1* mutations in a specific amino acid (Arg 132) suggested a possibility that targeting of the *IDH1* mutation could be used in the treatment of cancers harboring the mutations as in the cases of *EGFR*, *BCR/ABL* and *HER2*.<sup>4,10-12</sup> In the current study, we found that prostate cancers and B-ALL harbored the *IDH1* codon 132 mutations. Also, we confirmed the occurrence of the *IDH1* mutation in the GBM of Korean patients. Despite the low frequency of the *IDH1* mutations in these cancers compared with that of glial

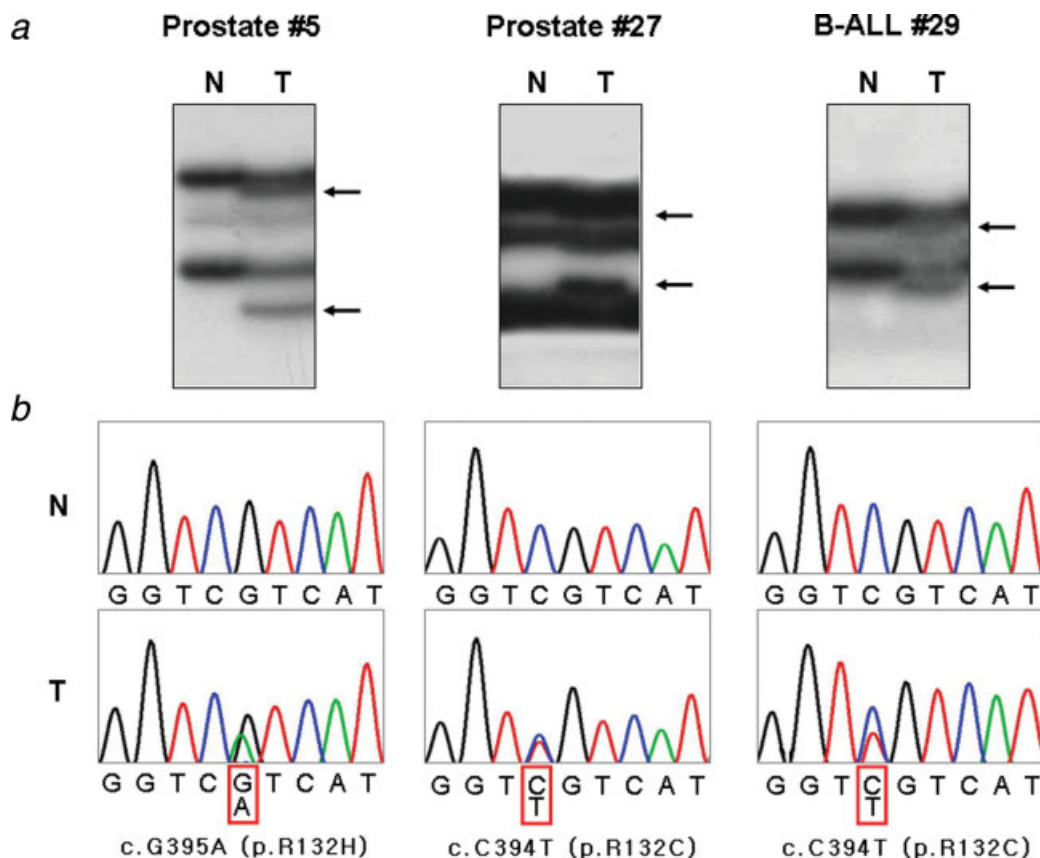


FIGURE 1 – Mutations of *IDH1* gene in prostate cancers and B-ALL. SSCP (a) and DNA sequencing analysis (b) of DNA from tumor (Lane T) and normal tissues (Lane N). (a) Arrows (Lane T) indicate aberrant bands compared with the SSCP from normal tissues (N). SSCPs of DNA from tumors (T) show wild-type bands and additional aberrant bands. (b) Direct DNA sequencing analyses from prostate cancers #5 (left) and #27 (middle) and B-ALL #29 (right). There are nucleotide substitutions (red box) in tumor tissues (T) as compared with normal tissues (N). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

TABLE II – SUMMARY OF THE IDH1 MUTATIONS IN THE CANCERS

Case no.	Type of cancers	Nucleotide change (Predicted amino acid change)	Other clinicopathologic characteristics
GBM 6	Primary GBM	c.395G>A (p.R132H)	–
GBM 23	Primary GBM	c.395G>A (p.R132H)	–
GBM 27	Primary GBM	c.395G>A (p.R132H)	–
GBM 31	Primary GBM	c.395G>A (p.R132H)	–
PS 5	Prostate adenocarcinoma	c.395G>A (p.R132H)	TNM stage II, Gleason score 7
PS 27	Prostate adenocarcinoma	c.394C>T (p.R132C)	TNM stage III, Gleason score 8
Leu 29	B-ALL	c.394C>T (p.R132C)	BRAF mutation (c.486G>A), no cytogenetic abnormality

tumors, the *IDH1* mutation may play an important role in the development of the cancers with the mutations, given that the *IDH1* mutations provide cancer-related functions with the affected cells. Also, the data suggest a possibility that not only glial tumors, but also prostate and B-ALL should be considered as candidate cancer types for future therapies targeting the *IDH1* mutations. Recently, Bleeker *et al.*<sup>13</sup> analyzed the *IDH1* mutation in a large series of tumors, and observed comparable results to our data. They found no *IDH1* mutation in other tumors besides GBM.

To date, there have been six types of the *IDH1* codon 132 mutations (p.R132H, p.R132C, p.R132S, p.R132G, p.R132L and p.R132V) detected in human cancers.<sup>1,2</sup> In glial tumors, p.R132H is the most common *IDH1* mutation and p.R132C is the second one.<sup>1,2</sup> In agreement with these, the *IDH1* mutations detected in our study were either p.R132H or p.R132C. However, functional role of the *IDH1* mutations in cancer pathogenesis depending on the subtypes remains to be clarified.

IDH1 is an enzyme that catalyzes the oxidative decarboxylation of isocitrate into  $\alpha$ -ketoglutarate utilizing either NAD or NADP as cosubstrates.<sup>14,15</sup> IDH1 is mainly involved in metabolic processes and its roles in cancer biology are largely unknown. Thus, it is

crucial to discover the functions of mutant IDH1 in cancer development. However, before the identification of functions, its normal physiological functions must be found out first. One of the cancer-related functions of IDH1 is the cellular control of oxidative damages.<sup>16</sup> Whether alteration of its oxidative damage response by the *IDH1* mutations is crucial in cancer development, and/or whether alterations of other unidentified functions are crucial, should be further clarified. In addition, analysis of alterations of tissue expression of *IDH1* gene together with the *IDH1* mutations could further elucidate roles of IDH1 in cancers.

In summary, this study identified that few types of human cancers harbored *IDH1* codon 132 mutations, suggesting some overlap of pathogenesis among glial tumors, prostate cancers and B-ALL. Despite the infrequent incidence of the *IDH1* mutations in prostate cancers and B-ALL, mutated IDH1 could also be therapeutically targeted in these cancers with the *IDH1* mutations.

#### Acknowledgement

The prostate cancer tissues were supplied from 'The Prostate Bank of Korea' supported by KOSEF.

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