Isocitrate Dehydrogenase 1 (*IDH1*) Mutation in Breast Adenocarcinoma Is Associated With Elevated Levels of Serum and Urine 2-Hydroxyglutarate

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Key Words. Isocitrate dehydrogenase 1 • 2-Hydroxyglutarate • Breast adenocarcinoma • Targeted therapy

ABSTRACT

Mutations in the *IDH1* and *IDH2* (isocitrate dehydrogenase) genes have been discovered across a range of solid-organ and hematologic malignancies, including acute myeloid leukemia, glioma, chondrosarcoma, and cholangiocarcinoma. An intriguing aspect of *IDH*-mutant tumors is the aberrant production and accumulation of the oncometabolite 2-hydroxyglutarate (2-HG), which may play a pivotal oncogenic role in these malignancies. We describe the first reported case of an *IDH1* p.R132L mutation in a patient with hormone receptor-positive (HR+) breast adenocarcinoma. This patient was initially treated for locally advanced disease, but then suffered a relapse and metastasis, at which point an *IDH1*-R132 mutation was discovered in an affected lymph node. The

mutation was subsequently found in the primary tumor tissue and all metastatic sites, but not in an uninvolved lymph node. In addition, the patient's serum and urine displayed marked elevations in the concentration of 2-HG, significantly higher than that measured in six other patients with metastatic HR+ breast carcinoma whose tumors were found to harbor wild-type *IDH1*. In summary, *IDH1* mutations may impact a rare subgroup of patients with breast adenocarcinoma. This may suggest future avenues for disease monitoring through noninvasive measurement of 2-HG, as well as for the development and study of targeted therapies against the aberrant *IDH1* enzyme. *The Oncologist* 2014; 19:602–607

Implications for Practice: Isocitrate dehydrogenase (*IDH*) mutations have been identified in various hematologic and solid-tumor malignancies. To date, there are no published reports of these mutations in breast cancer. With this article, we describe a case of hormone receptor-positive adenocarcinoma of the breast with an *IDH1* (p.R132L) mutation. The impacted patient had markedly elevated levels of serum and urine 2-hydroxyglutarate, an oncometabolite that accumulates as a result of the neomorphic activity of the altered *IDH* enzyme. *IDH1* mutations may impact a rare subgroup of patients with breast adenocarcinoma, and these findings may carry future therapeutic implications, given the emergence of targeted therapies against the altered *IDH* protein.

INTRODUCTION

The recent identification of isocitrate dehydrogenase (*IDH*) mutations in human malignancies has generated a significant amount of excitement, as it serves as a rare example of a cancer mutation in a metabolic enzyme that confers neomorphic activity and may be a target of therapy [1–3]. Normally, *IDH1* and *IDH2* are essential enzyme components of oxidative phosphorylation and catalyze the decarboxylation of isocitrate to α -ketoglutarate (α -KG) in the cytosol and peroxisomes (in the case of *IDH1*) and in mitochondria (in the case of *IDH2*), leading to the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH). These enzymes are encoded by their respective genes (*IDH1* and *IDH2*). In contrast to

normal *IDH* enzymes, the mutant forms catalyze an NADPH-dependent reduction of α -KG to the metabolite 2-hydroxyglutarate (2-HG), in place of the normal process of isocitrate decarboxylation, leading to accumulation of 2-HG. High levels of 2-HG are thought to drive the tumorigenic process through the modulation of a number of α -KG-dependent dioxygenases that control DNA methylation, histone methylation, hypoxic response, and a number of other cellular processes [4–6].

Parsons et al. first identified *IDH1* mutations in 12% of patient samples of glioblastoma multiforme (GBM) [3]. The mutations were more common in younger patients and in those whose disease had emerged from lower grade gliomas.

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Those with *IDH1*-mutant GBM displayed a significantly longer median survival compared with those displaying wild-type *IDH1* [3]. Additional studies have confirmed that *IDH1* mutations also occur commonly in low-grade gliomas [7, 8]. Mutations in *IDH1* as well as *IDH2* were subsequently discovered in various myeloid malignancies [9, 10], including acute myeloid leukemia (AML) [2, 11], in which case they have been associated with intermediate-risk cytogenetics and concurrent *NPM1* mutations [12, 13]. Their prognostic impact in AML is a topic of significant debate, without clear resolution [12–17]. Intriguingly, patients with *IDH*-mutant AML display elevated levels of 2-HG, which are present at low baseline levels in normal human tissue. This observation has led to subsequent studies demonstrating that elevated serum and urine 2-HG may be used as a biomarker of disease activity [18–21].

In addition to gliomas, *IDH* mutations have been discovered in various other solid tumors, including cholangiocarcinoma [1], chondrosarcoma [22, 23], prostatic adenocarcinoma [24], and melanoma [25], among others [26–29], with codon R132 of *IDH1* the most commonly mutated site. To date, *IDH* mutations have not been reported in breast carcinomas. With this report, we describe the case of a patient with metastatic hormone receptor-positive adenocarcinoma of the breast, whose tumor exhibited an *IDH1* p.R132L mutation, and whose serum and urine displayed marked elevations of the oncometabolite 2-HG.

METHODS

A total of 454 female patients diagnosed with breast cancer underwent mutational profiling as part of their clinical care received at the Massachusetts General Hospital Cancer Center, from May 2010 to December 2013. Relevant clinical information was gathered from review of the medical record. Retrospective genotyping on additional surgical specimens from a single patient with *IDH1*-mutant breast adenocarcinoma was performed with approval from the institutional review board.

The presence of common cancer gene mutations was evaluated in nucleic acids extracted from patient diagnostic tumor specimens using a custom and clinically validated Applied Biosystems (Foster City, CA, <http://www.appliedbiosystems.com>) Prism SNaPshot Multiplex system, as previously reported [1, 30]. This assay simultaneously queried for more than 130 mutational hotspots across more than 15 cancer genes. Mutational analysis of *IDH1* at nucleotide positions c.394 and c.395 (amino acid position p.R132) and *IDH2* at nucleotide positions c.418 and c.419 (amino acid position p.R140) and c.514 and c.515 (amino acid position p.R172) was incorporated into this clinical platform in May 2010 and November 2012, respectively.

Our mutational profiling of breast cancer patients clinically revealed a single case harboring an *IDH1* c.395G>T (p.R132L) mutation. This finding was made in the patient's diagnostic lymph node specimen containing metastatic, hormone receptor-positive (HR+), adenocarcinoma of the breast. Subsequently, all diagnostic tumor samples previously obtained during the patient's course were retrospectively secured for mutational profiling using the same mutational profiling platform. The available samples containing tumor

tissue included primary tissue from the left breast, central duct, and the sentinel lymph node obtained during initial lumpectomy. In addition, left breast tissue and an area of uninvolved lymph node obtained during a subsequent mastectomy were included in the analysis.

Serum and urine samples were obtained for 2-HG measurement, which was performed through collaboration with Agios Pharmaceuticals, Inc. (Cambridge, MA, <http://www.agios.com>). In brief, labeled 13C5-2HG was obtained from Agios Pharmaceuticals, and 2-HG was obtained from Toronto Research Chemicals (Toronto, ON, Canada, <http://www.trc-canada.com>). Liquid chromatography coupled to tandem mass spectrometry was performed using an AB SCIEX 4000 (Framingham, MA, <http://www.absciex.com>) operating in negative electrospray mode. Magnetic resonance mammography data were acquired for each compound using the following transitions: 2-HG (146.9/128.8 amu), 13C5-2HG (151.9/133.8 amu), and 3HMG (160.9/98.9 amu). Chromatographic separation was performed using an ion-exchange column (Bio-Rad Fast Acid Analysis, 9 μ m, 7.8 mm $\times$ 100 mm; Bio-Rad, Hercules, CA, <http://www.bio-rad.com>). The flow rate was 1 mL per minute of 0.1% formic acid in water with a total run time of 4 minutes.

The study samples were prepared as follows: urine was diluted with 10 $\times$ phosphate-buffered saline, and 30 μ L of diluted urine was extracted by adding 200 μ L of methanol with 200 ng/mL 13C5-2HG as internal standard; 10 μ L of serum sample was extracted by adding 200 μ L of methanol with 200 ng/mL 13C5-2HG. Both sample types were then vortexed, centrifuged at 4,000 rpm at 5°C, and 150 μ L of supernatant was transferred to a clean 96-well plate. The samples were dried down and reconstituted in 200 μ L of 0.1% formic acid in water, and 10 μ L was injected on column.

RESULTS

Case History

At the time of the study, the patient was a 61-year-old female, with a diagnosis of estrogen receptor-positive (ER+), progesterone receptor-positive (PR+), human epidermal growth factor receptor 2-negative (HER2–) breast adenocarcinoma, with metastasis to pleura, bone, mediastinal lymph nodes, and intracranial dura. Approximately 3 years prior, she was diagnosed with stage IIA, T1cN1M0, invasive ductal carcinoma of the left breast after presenting with bloody nipple discharge. A mammogram had initially revealed a hypoechoic nodule in the breast, which was followed by an ultrasound-guided core biopsy, revealing atypical ductal hyperplasia and fat necrosis. One month later, lumpectomy and sentinel lymph node biopsy established the diagnosis of invasive ductal carcinoma with lymph node involvement. Due to positive margins, she underwent a left-sided mastectomy and lymph node dissection, which revealed a 1.2-cm moderately differentiated, invasive ductal carcinoma (ER+/PR+, HER2–), with extensive lymphovascular invasion and metastatic lymph node involvement in 2 of 11 sampled lymph nodes. She underwent adjuvant chemotherapy with cyclophosphamide and docetaxel. She was then prescribed hormonal therapy with letrozole, which she discontinued after a few months because of adverse side effects.

Approximately 3 years after the initial diagnosis, the patient was admitted to the hospital with acute dyspnea and right-sided facial numbness. Imaging revealed diffusely metastatic disease to the intracranial dura, pleura, bones, as well as the mediastinal, hilar, and paracardiac lymph nodes. A mediastinoscopy with lymph node biopsy confirmed metastatic adenocarcinoma, ER+, PR+, and HER2–, consistent with a primary malignancy of the breast. At this time, an *IDH1* c.395G>T (p.R132L) mutation was detected in a lymph node sample through clinical genotyping performed at our institution.

The patient was then treated with the aromatase inhibitor exemestane. A few months later, she developed worsening neurologic symptoms because of progressive dural-based lesions, and she received cranial base irradiation followed by initiation of salvage chemotherapy with vinorelbine. Over the course of the next 3 months, she was managed for worsening pleural effusions as well as neurologic and mental status deterioration. Unfortunately, she expired 14 months after her diagnosis of disease recurrence.

Analysis of *IDH1* Mutational Status

Following the initial identification of an *IDH1* R132L mutation in the patient's lymph node metastasis, additional diagnostic tumor samples, obtained during the patient's initial lumpectomy, were retrospectively tested, including primary tissue from the left breast, central duct, and the sentinel lymph node. Mutational profiling using the same clinical genotyping platform revealed the presence of an *IDH1* p.R132L mutation in all lumpectomy specimens containing tumor tissue. Samples from the subsequent mastectomy were also available, including tissue from the left breast and the lone involved left axillary lymph node, which both again harbored the *IDH1* p.R132L mutation. Finally, lymph node tissue obtained nearly 1 year later to confirm metastatic disease was similarly evaluated, and again was found to harbor the *IDH1* p.R132L mutation (Fig. 1). Of note, an area of uninvolved lymph node was evaluated and found to be *IDH1*-wild-type, indicating that the *IDH1* mutation in the tumor tissue was somatically acquired.

Primary tumor samples from an additional six patients with HR+, HER2 nonamplified, breast cancer were similarly tested and found to be wild-type for *IDH1*. The demographic and clinical features of these patients are described in Table 1.

Measurement of the Oncometabolite 2-HG

Serum and urine samples were obtained from the patient with *IDH1*-mutant malignancy, as well as the six patients with *IDH1*-wild-type tumors, for 2-HG measurement. Serum and urine 2-HG levels were markedly elevated in the *IDH1* p.R132L-mutant patient, with a serum concentration of 1,979 ng/mL and a urine concentration of 22,300 ng/mL. In contrast, the serum and urine concentrations of 2-HG in the *IDH1*-wild-type patients were significantly lower. The median serum concentration for wild-type patients was 104.5 ng/mL (range, 78–135 ng/mL) and the median urine concentration was 1,710 ng/mL (range <1,000–4,700 ng/mL) (Fig. 2; Table 1). Of note, in a previous study, serum 2-HG levels in normal, healthy individuals were at a similarly low baseline level (median 91 ng/mL, range 33–93 ng/mL) [20].

Figure 1. Identification of an *IDH1* p.R132L mutation in tumor tissue from a breast cancer patient. A 61-year-old female patient diagnosed with estrogen receptor-positive, progesterone receptor-positive, human epidermal growth factor receptor 2-negative invasive ductal carcinoma underwent mutational profiling. An *IDH1* c.395G>T (p.R132L) mutation (green peak) was identified in tissue obtained by lumpectomy from the left breast, central duct, and the sentinel lymph node (upper panels). Tissue from a subsequent left-sided mastectomy and lymph node dissection was evaluated and the same *IDH1* mutation was identified in malignant breast tissue (LB) and the left axillary lymph node (middle panels). Conversely, no *IDH1* p.R132L mutation was found in an uninvolved lymph node, where no malignancy was found during pathological review. Metastatic lesions obtained from a later surgery were also identified to harbor the *IDH1* p.R132L mutation from two lymph nodes (LN1 and LN2) (lower panels).

Abbreviations: CD, central duct; *IDH1*, isocitrate dehydrogenase 1 gene; LALN, left axillary lymph node; LB, left breast; LN, lymph node; NM, no malignancy; SLN, sentinel lymph node.

DISCUSSION

To our knowledge, this is the first report of an *IDH1* mutation detected in breast adenocarcinoma. In this case, the patient carried a diagnosis of hormone receptor-positive breast cancer, initially early-stage, but which subsequently recurred as widely metastatic disease. The *IDH1* mutation was initially detected at the time of disease relapse, when serum and urine assays also revealed markedly high concentrations of the oncometabolite 2-hydroxyglutarate. Retrospective genotyping revealed that the same mutation was present in the malignant tissue at both initial diagnosis and relapse. The inability to detect the mutation in lymph node tissue unaffected by metastatic adenocarcinoma in this patient indicates that the *IDH1* mutation was somatically acquired and a specific feature of the tumor.

Over the course of clinical genotyping performed at our institution, this has been the only case of an *IDH1* mutation in breast cancer among 454 total cases tested. Furthermore, the *Cancer Genome Atlas* (<http://cancergenome.nih.gov>) reports only 2 cases of *IDH1*-mutant invasive breast carcinoma among

Table 1. Demographic and clinical characteristics of patients with metastatic hormone receptor-positive adenocarcinoma

Patient	Age at initial diagnosis (yr)	Race	IDH status	ER/PR status	HER2/neu status	Location of distant metastases	Distant metastasis at diagnosis	Serum 2-HG (ng/mL)	Urine 2-HG (ng/mL)
1	61	White	IDH1 p. R132L	ER+/PR+	Nonamplified	Dural, pleuritic, osseous	No	1,979	22,300
2	48	White	IDH1 WT	ER+/PR–	Nonamplified	Pleuritic, osseous	No	114	4,700
3	60	White	IDH1 WT	ER+/PR–	Nonamplified	Hepatic, osseous	Yes	125	<1,000
4	53	White	IDH1 WT	ER+/PR+	Nonamplified	Pulmonary, osseous	No	78	<1,000
5	60	White	IDH1 WT	ER+ /PR–	Nonamplified	Osseous	No	135	1,550
6	50	White	IDH1 WT	ER+/PR+	Nonamplified	Pulmonary, osseous	No	95	1,870
7	48	White	IDH1 WT	ER+/PR+	Nonamplified	Osseous	No	82	4,350

Abbreviations: ER+, estrogen receptor-positive; HER2/neu, human epidermal growth factor receptor 2; PR–, progesterone receptor-negative; PR+, progesterone receptor-positive; 2-HG, 2-hydroxyglutarate; IDH1, isocitrate dehydrogenase 1 gene; WT, wild-type.

Figure 2. 2-HG measurements in samples from patients with a wild-type IDH1 ($n = 6$) versus the patient harboring the IDH1 p.R132L mutation in serum (A) and urine (B). Each point represents an individual patient sample, with blue dots depicting IDH1-wild-type samples and the red dots depicting IDH1-mutant samples. The left column in each figure represents the wild-type samples and the right column represents the mutant sample. Horizontal bars indicate the median, and vertical lines indicate the lower and upper quartiles. All figures describe a statistically significant difference in 2-HG levels between the IDH1-mutant sample relative to the wild-type samples ($p < .05$).

Abbreviations: 2-HG, 2-hydroxyglutarate; IDH1, isocitrate dehydrogenase 1 gene; WT, wild-type.

825 tested. One case was an ER+, PR+, HER2+ invasive ductal carcinoma that harbored a rare IDH1 Y235C mutation. The other case was a mixed infiltrating ductal and mucinous carcinoma carrying an IDH1 R132C mutation, but the ER, PR, and HER2 statuses were not indicated. We could therefore infer that mutations in IDH1 are a rare finding in breast cancer.

We, along with other groups, have previously demonstrated that elevated 2-HG levels are a specific feature of IDH-mutant AML [19, 20]. However, up to the time of preparation for this manuscript, elevations in serum and urine 2-HG have not been reported in IDH-mutant solid tumors. Levels of intratumoral 2-HG have been shown to be elevated in IDH1-mutant glioma tissue [18, 31] using both in vitro and in vivo techniques [32, 33]. Nevertheless, an earlier small study of IDH1-mutant gliomas failed to detect an elevation in circulating 2-HG levels in sera of

affected patients [34]. In IDH-mutant AML, intracellular 2-HG levels are measured at approximately 50-fold higher levels in affected malignant cells compared with their IDH-wild-type counterparts [21]. Serum levels of 2-HG in IDH-mutant AML patients are above a concentration of 1,000 ng/mL in the vast majority of cases, decrease upon cytoreduction with antileukemic therapy, and again increase with relapse [20, 21].

Serum and urine samples, obtained from the above-described patient with IDH1-mutant breast carcinoma, revealed markedly elevated 2-HG levels, likely reflective of her widely disseminated, metastatic disease. These levels were significantly higher than those measured in six other patients with metastatic HR+ breast cancer whose tumors displayed wild-type IDH. This supports the hypothesis that the elevation in 2-HG was related to the marked neomorphic activity of the

altered IDH1 enzyme, rather than being a feature of metastatic breast adenocarcinoma.

It has been reported in glioma that *IDH1* mutations are early events in the carcinogenic process [35], with the pathogenesis and transformation of *IDH1*-mutant gliomas linked to suppressed cellular differentiation, with 2-HG implicated as a mediator in these processes [11, 18]. In AML, *IDH* mutations have been associated with the aberrant hypermethylation of promoter sites involved in myeloid differentiation [36], an underlying pathogenic process in forms of myelodysplasia and AML [36–39]. Emerging evidence suggests that 2-HG may be a key leukemogenic oncometabolite impacting such epigenetic modulation [21, 40, 41]. Although the tumorigenic role of the *IDH1* mutation in this patient with breast carcinoma is not known, the presence of the mutation at all known metastatic sites and the dramatic accumulation of the 2-HG oncometabolite in the serum suggest that it was likely an important event in the underlying etiology of this malignancy.

CONCLUSION

We here describe an intriguing case of *IDH1*-mutant breast cancer. Apart from the novelty of this mutation in this malignancy, the concurrent marked elevation of the metabolite 2-HG in the serum and urine is similarly intriguing. As described in other tumors to date, it will be of interest to determine whether 2-HG may play an important role in the genesis and perpetuation of malignancy in breast cancer through its impact on epigenetic and metabolic modulation. In addition, 2-HG measurement may provide a noninvasive manner of diagnosing disease and following disease activity during treatment and remission. Our patient had hormone receptor-positive disease, and whether *IDH1* mutations are a feature of a rare subset of this category of breast cancer requires further study. Finally, targeted therapies, in the form

of IDH inhibitors, have shown great promise in preclinical models [42], and are currently entering early-phase clinical trials. The therapeutic impact of such agents in *IDH*-mutant disease, across the spectrum of solid-tumor and hematologic malignancies, is awaited with significant hope and anticipation.

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DISCLOSURES

Amir Fathi: Agios Pharmaceuticals (C/A); **David Schenkein, Katharine E. Yen, Kimberly Straley:** Agios Pharmaceuticals (E, OI); **Sam Agresta:** Agios Pharmaceuticals (E, OI); **Darrell Borger:** BioReference Laboratories, Inc. (C/A). The other authors indicated no financial relationships.

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