IDH2 Mutations Define a Unique Subtype of Breast Cancer with Altered Nuclear Polarity


Abstract

Solid papillary carcinoma with reverse polarity (SPCRP) is a rare breast cancer subtype with an obscure etiology. In this study, we sought to describe its unique histopathological features and to identify the genetic alterations that underpin SPCRPs using massively parallel whole-exome and targeted sequencing. The morphologic and immunohistochemical features of SPCRPs support the invasive nature of this subtype. Ten of 13 (77%) SPCRPs harbored hotspot mutations at R172 of the isocitrate dehydrogenase IDH2, of which 8 of 10 displayed concurrent pathogenic mutations affecting PIK3CA or PIK3R1. One of the IDH2 wild-type SPCRPs harbored a TET2 Q548* truncating mutation coupled with a PIK3CA H1047R hotspot mutation. Functional studies demonstrated that IDH2 and PIK3CA hotspot mutations are likely drivers of SPCR, resulting in its reversed nuclear polarization phenotype. Our results offer a molecular definition of SPCR as a distinct breast cancer subtype. Concurrent IDH2 and PIK3CA mutations may help diagnose SPCR and possibly direct effective treatment. Cancer Res; 76(24): 7118–29. ©2016 AACR.

Introduction

Breast cancer is a heterogeneous disease at the clinical, morphologic, and genetic level (1–3). The current World Health Organization (WHO) classification recognizes 21 histologic types (1), but few have been found to harbor specific genetic alterations, such as CDH1 alterations in invasive lobular carcinoma (4) and recurrent ETV6–NTRK3 and MYB–NFI fusion genes in secretory and adeno-cystic carcinomas, respectively (5–7). Additional rare breast cancers with distinctive morphologic features also exist, but have not yet been included in the WHO classification. One such malignancy has been described as “breast tumor resembling the tall cell variant of papillary thyroid carcinoma (PTC)” (8, 9), which has a unique histologic appearance consisting of solid, circumscribed nodules of epithelial cells, many of which have fibrovascular cores, resulting in a solid papillary growth pattern. Thirteen such tumors have been reported to date (8, 9), of which approximately half lack estrogen (ER) and progesterone receptor (PR) expression, and all lack HER2 overexpression (8, 9). These tumors are generally associated with a favorable prognosis (8, 9); however, two of 13 patients have been reported to develop metastatic disease, one to an intra-mammary lymph node and another to the bone (8, 9).

Although these tumors have some histologic similarities to PTC, they consistently lack expression of thyroid-specific markers, including TTF-1 and thyroglobulin (8, 9). RET rearrangements and BRAF exon 15 mutations commonly found in PTC have also not been detected in these tumors (9). Given the morphologic overlap with other papillary lesions of the breast and lack of immunohistochemical and genetic evidence of an association...
with PTC, it has recently been suggested that these neoplasms should be considered morphologic variants of papillary breast carcinoma (9, 10).

In this study, we sought to characterize the morphologic and genetic landscape of this rare and morphologically unusual breast tumor and determine whether it represents a distinct subtype of breast cancer underpinned by disease-specific genetic alterations. To this end, we performed an extensive immunohistochemical characterization and whole exome (WES), targeted, and Sanger sequencing of 13 previously unreported tumors. We found that 10 of 13 (77%) tumors harbored R172 IDH2 mutations, of which eight had a concurrent pathogenic mutation affecting PI3K pathway canonical genes (i.e., six PIK3CA hotspot mutations and two PIK3R1 likely pathogenic mutations). In addition, a PIK3CA-mutant but IDH2-wild-type tumor was found to harbor a TERT Q548* truncating mutation. Functional studies using nonmalignant breast epithelial cells demonstrated that IDH2 and PIK3CA mutations constitute likely drivers of this tumor and contribute to its characteristic phenotype. Because of their unique histologic and genetic properties, we redefine these tumors here as a discrete subtype of breast carcinoma: solid papillary carcinoma with reverse polarity (SPCRP).

**Materials and Methods**

**Patient selection**

Between 2005 and 2014, 13 SPCRP1s were identified by two of the authors (S.J. Schnitt and E. Brogi) on the Breast Pathology Consultation (11 cases) or Breast Pathology (1 case) Services at Beth Israel Deaconess Medical Center (Boston, MA), and the Breast Pathology Consultation Service (1 case) at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY). Hematoxylin-and-eosin–stained sections were reviewed to delineate the morphologic features of these lesions. Available clinical data were reviewed for demographics, presentation, clinical and family history, treatment, and outcome. The study was approved by the Institutional Review Boards from the respective authors’ institutions, and all samples were anonymized prior to analysis.

**Immunohistochemistry and Western blotting**

Primary antibodies for IHC were as follows: calponin, smooth muscle myosin-heavy chain (SMHHC), p63, AE1/AE3, cytokeratin (CK) 7, CK5/6, CK34βE12, ER, PR, androgen receptor (AR), HER2, mammaglobin, H3K27me3, gross cystic disease fluid protein-15 (GCDP-15), TTF-1, thyroglobulin, E-cadherin, and MUC1 (Supplementary Table S1). For H3K27me3, we semiquantitatively assessed its nuclear expression using the H-score system (17). For three cases (SPCRP1, 3, and 6) with very limited tissue availability, DNA was subjected to PCR amplification using primer sets encompassing the IDH2 R140 and R172 hotspot residues and the PIK3CA E542/545 and H1047 hotspot residues (Supplementary Table S3), followed by standard Sanger sequencing as previously described (14). All analyses were performed in duplicate. Sequences of the forward and reverse strands were analyzed using MacVector software (MacVector, Inc; ref. 14).

**Methylation profiling**

Methylation profiling was performed using the Infinium MethylationEPIC Kit (Illumina). DNA samples from five IDH2-mutant SPCRP1s (cases 3–5, 7, and 9), one TET2-mutant SPCR (case 11), and two invasive ductal carcinomas of no special type (IDCs, one ER-positive/HER2-negative and one ER-negative/HER2-negative) were bisulfite–converted using the EZ-96 DNA Methylation Kit (Zymo Research), restored using the Illumina Infinium HD FFPE DNA Restore Kit, and whole-genome amplified (Supplementary Methods). The BeadChips were scanned, and the raw data files containing the fluorescence intensity data for each probe were generated. Data analysis was performed using RnBeads (Supplementary Methods; ref. 19).

**DNA transfections and analysis of transgene expression**

The human IDH2 (NM_002168) cDNA ORF clone pCMV6-IDH2::Myc-DDK was purchased from Origene (RC201152), and
In addition, cells were tested for mycoplasma infection using (Promega) in the IGO at MSKCC three months after receipt.

Cell proliferation and cell migration assays were performed basically as previously described (25). Vector-, IDH2- or IDH2 R172S-expressing MCF10A and MCF10A H1047R were grown overnight on a 96-well plate at a density of 1 x 10^4 cells/well, treated with 1 mmol/L hydrogen peroxide (H2O2) in serum-free medium for 48 hours at 37°C, and cell viability was assessed using CellTiter-Blue as described above and reported as compared with absorbance measured in untreated control cells. Fluorometric detection was carried out in triplicate in two independent experiments.

Results

Clinicopathologic characteristics of SPCRPs

SPCRPs have a very distinctive histologic appearance that allowed us to identify a series of 13 such tumors from our consultation and institutional files. They consisted of solid, circumscribed nodules of columnar epithelial cells, some exhibiting a geographic, jigsaw-like growth pattern (Fig. 1A–C). Many nodules contained fibrovascular cores with foamy histiocytes and a double-layered epithelium without a clear lumen, resulting in a solid papillary appearance (Fig. 1B). The cells in many nodules appeared back-to-back and, in particular, the nuclei were present at the apical rather than basal pole of the cells, creating the impression of reverse polarization (Fig. 1C).

In all cases, all tumor nodules lacked a surrounding myoepithelial cell layer as assessed by immunohistochemical analysis
MUC1 staining, which highlights the apical membranes of columnar epithelial cells, was identified at the end of the cell closest to the nucleus, indicating that the nuclei were in an abnormal location (apical rather than basal) and creating the impression of reversed cell polarity (Fig. 1H). Clinical data are summarized in Table 1.

**SPCRP harbors IDH2 hotspot mutations and mutations affecting PI3K pathway canonical genes**

Two SPCRPs (cases 10 and 13), for which sufficient tumor and normal DNA samples were available, and two SPCRPs (cases 5 and 11) with sufficient tumor DNA were subjected to WES and the MSK-IMPACT sequencing assay (15), respectively. In cases subjected to WES (cases 10 and 13), the median sequencing depth of coverage was 127× (range, 111–212×), and 45 and 24 nonsynonymous somatic mutations were identified (Supplementary Tables S4 and S5). Cases 5 and 11, subjected to MSK-IMPACT sequencing at a coverage if 734× and 573×, respectively, were found to harbor eight and 26 nonsynonymous somatic mutations, respectively (Supplementary Tables S4 and S5). Sequencing analysis revealed that three of these cases harbored somatic single nucleotide variants (SNV) affecting the R172 residue in the substrate-binding pocket of the isocitrate dehydrogenase 2 (NADP+) mitochondrial (IDH2) gene (Fig. 2A; Supplementary Table S5), the same IDH2 codon targeted by recurrent hotspot mutations in glioma, acute myeloid leukemia (AML), cholangiocarcinoma, and chondrosarcoma (26). In SPCR11, which lacked an IDH2 somatic mutation, a TET2 Q548R truncating mutation was detected. We also identified mutations affecting canonical genes of the PI3K/AKT/mTOR pathway in all four SPCRPs, with two cases harboring PIK3R1 frameshift mutations and two cases harboring PIK3CA somatic mutations (Fig. 2A; Supplementary Table S5). No other recurrent somatic mutations were found. To validate these findings, we subjected DNA derived from an additional six SPCRPs (cases 2, 4, 7–9, and 12) to SNaPshot profiling assessing mutations in 22 cancer genes (Supplementary Table S2) and from three SPCRPs (cases 1, 3, and 6) to IDH2 and PIK3CA hotspot Sanger sequencing (Supplementary Fig. S1, Supplementary Table S3), which confirmed the presence of IDH2 R172 hotspot mutations in seven cases (70%), and PIK3CA H1047R and E542K hotspot mutations in six cases and one case (78%), respectively. No other hotspot mutations were identified in the SPCRPs subjected to SNaPshot profiling. In total, 10 of 13 (77%) SPCRPs analyzed harbored an IDH2 R172 mutation, of which five had a concurrent PIK3CA H1047R hotspot mutation, one a concurrent PIK3CA C420R mutation, and two a concurrent PIK3R1 frameshift mutation (Fig. 2A). Given that one of the IDH2 wild-type SPCRPs harbored a truncating mutation in TET2, which encodes for an α-ketoglutarate (αKG)-dependent enzyme that catalyzes cytosine 5-hydroxymethylation resulting in demethylation of DNA (27), we sought to define whether the IDH2 wild-type cases SPCR1 and SPCR6 would also harbor mutations affecting this gene; however, both were found to be TET2 wild-type. No histologic differences were observed among the IDH2-mutant, TET2-mutant, and IDH2/TET2 wild-type SPCRPs.

To date, IDH2 mutations have not been described in breast cancer, and a reanalysis of breast cancers reported by The Cancer Genome Atlas (TCGA) revealed that only one of the 971 invasive ductal and invasive lobular carcinomas harbored an IDH2...
mutation, however affecting a different codon (E345K, Fig. 2B; www.cBioPortal.org, accessed on November 30, 2015; Fig. 2B; refs. 4, 28). Taken together, our results indicate that SPCPs are uniquely characterized by highly recurrent IDH2 R172 hotspot mutations often in combination with mutations affecting PIK3CA H1047R hotspot mutations, and suggest that at least in a subset of SPCPs lacking IDH2 mutations, somatic genetic alteration affecting TET2 may be present. These observations suggest that SPCPs may constitute an example of a convergent phenotype stemming from alterations of genes leading to similar epigenetic defects and potentially function genetically in the same pathway (26, 29), as IDH1/IDH2 mutations inhibit TET2 function and are mutually exclusive with TET2 mutations in hematologic malignancies (30).

**IDH2 and PIK3CA mutations constitute likely drivers of SPCR**

IDH2 hotspot mutations are enzymatic gain-of-function alterations that lead to an increased conversion of αKG to 2HG. Increased levels of 2HG result in hypermethylation of epigenetic regulatory factors, such as TET2, leading to the loss of DNA methylation and eventually to genomic instability (31). We next sought to determine whether IDH2 mutations result in an accumulation of 2HG oncometabolite in SPCPs, as reported in glioblastoma (33) and AML (34) harboring this mutation. We measured intratumoral 2HG by HPLC and found 2HG in the IDH2-mutant SPCPs (case 12), where adequately preserved tissue was available, and in five IDCs (two ER-positive/HER2-negative and two ER-negative/HER2-negative) compared with IDH1 wild-type IDCs, IDH2-mutant SPCRPs displayed significantly higher levels of trimethylation of H3K27 (H3K27me3, P = 0.029, Mann–Whitney U test; Fig. 2E), consistent with the results reported in IDH1-mutant gliomas (31).

We next tested the functional impact of the IDH2 R172S mutation on the growth and phenotype of nonmalignant breast epithelial cells and investigated potential epistatic interactions between IDH2 R172S and the most frequent PIK3CA (H1047R) mutations concurrently detected in SPCRPs. We used the MCF10A model system, including parental MCF10A cells and MCF10A cells harboring a stable knock-in of the PIK3CA H1047R mutation. As expected, forced expression of wild-type IDH2 (IDH2WT) and R172S mutant IDH2 (IDH2R172S) in parental MCF10A cells (MCF10AH1047R) resulted in increased IDH2 mRNA expression (Supplementary Fig. S3A) and IDH2 protein mitochondrial localization (Supplementary Fig. S3B). When expressed at similar levels, IDH2WT resulted in significantly higher neomorphomic activity than IDH2WT on the analysis of SPCRPs, for which DNA samples were available. Genome-wide DNA methylation analysis using the Illumina Infinium MethylationEPIC BeadChip revealed that IDH2/TET2-mutant SPCRPs showed a genome-wide hypermethylation profile as compared with IDH2 wild-type IDCs, consistent with the hypermethylation profile reported in IDH1/IDH2-mutant cancers (Fig. 2D and Supplementary Fig. S2; refs. 30, 35). In fact, hierarchical clustering revealed that IDH2/TET2-mutant SPCRPs clustered together based on their methylation profile, and separate from the two IDH2/TET2 wild-type IDCs analyzed (Fig. 2D). Finally, the protein expression levels of H3K27me3 were assessed by IHC in four IDH2-mutant SPCRPs for which adequate histologic sections were available, and in four IDCs (two ER-positive/HER2-negative and two ER-negative/HER2-negative). Compared with IDH2 wild-type IDCs, IDH2-mutant SPCRPs showed significantly higher levels of trimethylation of H3K27 (H3K27me3, P = 0.029, Mann–Whitney U test; Fig. 2E), consistent with the results reported in IDH1-mutant gliomas (31).

**Table 1. Clinicopathologic characteristics of SPCRPs analyzed in this study**

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Abbreviations: AR, androgen receptor; C, chemotherapy (carboplatin and taxol); CK, cytokeratin; ER, estrogen receptor; GCDFP-15, gross cystic disease fluid protein-15; L, left; LN, lymph node; mo, months; NED, no evidence of disease; NP, not performed; PR, progesterone receptor; R, right; S, surgery; SMHC, smooth muscle myosin heavy chain; TTF-1, thyroid transcription factor-1; U, unknown; XRT, radiation; y, year.
of the production and secretion of 2HG, regardless of the presence of the H1047R PIK3CA mutation (Fig. 3A).

**IDH1** and **IDH2** mutations have been shown to constitute drivers of glioma (36, 37), AML (34, 38), and spindle cell hemangioma (39, 40) among others, resulting in a differentiation block and tumorigenesis (31, 32). Although in our transient transfection system, no statistically significant differences in the expression levels of H3K27 and H3K9 trimethylation were observed upon forced expression of IDH2WT or IDH2R172S as compared with cells expressing empty vector (Supplementary Figure 2).

**Figure 2.**

**IDH2 and PI3K pathway mutations, 2HG levels, and methylation profiles of SPCRP.** **A,** Nonsynonymous somatic IDH2, TET2, PIK3CA, and PIK3R1 mutations identified in the 13 SPCRP studied here by massively parallel sequencing (WES or MSK-IMPACT), SNAPSHOT, or Sanger sequencing. **B,** Mutation plot shows the domain structure of IDH2 and the nonsynonymous IDH2 mutations identified in SPCRP and in common forms of breast cancer published by TCGA available from cBioPortal (28). **C,** 2HG analysis in IDH2-mutant SPCRP (case 12) and IDH wild-type invasive ductal carcinoma of no special type (IDC). **D,** IDH2/TET2-mutant SPCRP display global DNA hypermethylation assessed by Infinium MethylationEPIC BeadChip as compared with IDH2/TET2 wild-type IDCs (left). Unsupervised hierarchical clustering using all methylation values/genes of six IDH2/TET2-mutant SPCRP and two IDH2/TET2 wild-type IDCs using complete linkage and Euclidean distance (right). The 1,000 genes with the highest variance across samples are displayed. Methylation status is color coded according to the legend. **E,** H3K27me3 immunohistochemical analysis of four IDH2-mutant SPCRP (top) and four IDH2 wild-type IDCs (bottom). Nuclear immunoreactivity for H3K27me3 was quantified using the H-score. *, P < 0.05; Mann-Whitney U test.
Fig. S4), expression of IDH2R172S resulted in changes in expression of cell-cycle and epithelial–mesenchymal transition (EMT) markers in the nonmalignant breast epithelial cells, MCF10A. Forced expression of IDH2R172S in MCF10A$^{H1047R}$ cells led to significantly increased phosphorylation of RB1 protein compared with empty vector, as detected by immunoblot (Fig. 3B). Consistent with these observations, we observed a significant increase in cell growth upon forced expression of IDH2WT and IDH2R172S in MCF10A$^{H1047R}$ cells (Fig. 4A), as well as a significant increase in migration of MCF10AWT and MCF10A$^{H1047R}$ cells expressing R172S-mutant IDH2 (Fig. 4B). Furthermore, we observed that forced expression of IDH2WT and IDH2R172S led to a reduction of E-cadherin protein expression in MCF10A$^{H1047R}$-mutant IDH2 (Fig. 4B). Given that cancer cells can avoid cell death by inhibiting the initial deleterious effects of oxidative stress (25, 41), we sought to define the impact of IDH2R172S on the viability of nonmalignant breast epithelial cells. In MCF10A$^{R}$ cells treated with exogenous H$_2$O$_2$, forced expression of wild-type, but not mutant IDH2, displayed a higher viability than empty vector expression (Fig. 4D). In H$_2$O$_2$-treated MCF10A$^{H1047R}$ cells, however, forced expression of both wild-type and mutant IDH2 resulted in increased viability compared with the empty vector control (Fig. 4D). These results suggest that IDH2R172S may protect against reactive oxygen species (ROS) in nonmalignant breast epithelial cells, consistent with observations described previously for IDH1 and IDH2 in other nonneoplastic tissue types (25, 41).

We next sought to define the impact of IDH2R172S on the growth and glandular architecture of MCF10A cells grown in a 3D model system, which has been previously used to assess the oncogenic properties of somatic mutations (13, 23, 42). Forced expression of IDH2R172S in MCF10A$^{R}$ and MCF10A$^{H1047R}$ cells resulted in acinar structures that were significantly larger than those observed in MCF10A cells transfected with empty vector or IDH2WT (Fig. 5A), a phenotype previously reported to be elicited by the expression of bona fide oncoproteins in this model system (13, 23, 42). In fact, IDH2R172S expression in MCF10A$^{H1047R}$ cells...
led to the formation of anastomosing solid cell nests/cell nodules (Fig. 5A and Supplementary Fig. S5). Consistent with the reverse polarization observed in the primary tumors, IDH2R172S expression in MCF10A cells grown in the 3D system also resulted in an increase in cells displaying a reversed nuclear polarity, as defined by expression of adhesion molecule E-cadherin (CDH1) and apical Golgi marker GM130. This reversed nuclear polarity phenotype was particularly evident in MCF10AH1047R cells expressing IDH2R172S (Fig. 5B). Taken together, our data provide evidence that IDH2R172S and PIK3CAH1047R hotspot mutations constitute likely drivers of SPCRP, and that together these somatic genetic alterations are likely sufficient, but not necessarily required, to cause its unusual reverse polarization phenotype.

**Discussion**

Here, we characterize the morphologic, immunohistochemical, and genomic profile of SPCRP, a rare and histologically distinct subtype of invasive breast carcinoma. Through whole exome and targeted massively parallel sequencing analysis, we have identified IDH2 hotspot and TET2 truncating mutations in 77% and 8% of these cases, respectively. In fact, SPCRP’s may constitute an example of a convergent phenotype as IDH2 and TET2 mutations lead to similar epigenetic defects (26, 29). This is the first report of IDH2 hotspot mutations detected in breast cancer, expanding the spectrum of solid tumors and hematologic malignancies in which IDH
mutations may drive tumorigenesis (43). The high frequency of IDH2 mutations found in SPCRP highlights the association between genotype and the unique tumor morphology (29). In addition, mutations affecting canonical genes of the PI3K pathway were found in 85% of SPCRPs, including 54% harboring PIK3CA H1047R mutations, a common genetic alteration in breast cancer that may be enriched in this subset of tumors (44).

A striking feature of SPCRP is the unique epithelial morphology of a double layer of columnar cells with apical nuclei. The absence of expression of E-cadherin, a protein known to play an important role in cell polarity, in apical and/or basal membranes combined with strong MUC1 staining of apical membranes indicates that the cells are indeed polarized. The presence of nuclei near the apical membrane indicates reverse nuclear polarization, and loss of apical polarity was also

Figure 5.
Impact of IDH2 mutations on glandular architecture and polarity of nonmalignant breast epithelial cells. A, Impact of forced expression of IDH2WT or IDH2R172S on glandular architecture of MCF10A (top) and MCF10A (R1047R) (bottom) cells grown in 3D basement membrane cultures (scale bar, 0.1 mm). The volume of the MCF10A and MCF10A (R1047R) acinar structures expressing IDH2WT and IDH2R172S was quantified; *, P < 0.05; **, P < 0.001; P < 0.0001; error bars, SD of mean. B, Immunofluorescent analysis of MCF10A (top) and MCF10A (R1047R) acinar structures expressing IDH2WT or IDH2R172S with antibodies against E-cadherin (red), cis-Golgi marker GM130 (green), and nucleus (DAPI, blue; scale bars, 25 μm; left). Arrows, cells with reverse polarity. MCF10A and MCF10A (R1047R) acinar structures showing either apical or reversed polarity were quantified. MCF10A (R1047R) IDH2R172S acini showed significantly more frequently inverted polarity than MCF10A (R1047R) vector control and MCF10A (WT) IDH2WT acini (*, P < 0.05 each; t test).
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observed in our cell line models, in particular in cells expressing mutant forms of IDH2 and PIK3CA, suggesting that this phenotype may result from epistatic interactions between mutations affecting these genes. Although our findings provide a novel example of a genotypic–phenotypic correlation in breast cancer, further analyses are required to define the genetic basis of the reverse polarity in SPCRPs lacking IDH2, TET2, and/or PI3K pathway gene mutations.

The mechanism by which IDH1 or IDH2 mutations cause human cancer remains to be fully elucidated. It has been found, however, that these alterations lead to a gain-of-function enzymatic activity that allows NADPH-dependent reduction of eKg to 2HG within tumor cells (33, 34), which inhibits eKg-dependent dioxygenases and alters genome-wide histone and DNA methylation, cell differentiation and survival, and extra-cellular matrix maturation (26, 31, 45). High 2HG concentrations disrupt TET2 catalytic function and prevent hydroxylation of 5-methylcytosine, resulting in attenuated TET2-dependent demethylation of DNA (30, 45). Akin to IDH1-mutant glioma, AML and common forms of breast carcinoma harboring IDH1 mutations (33, 34, 46), we found high concentrations of intratumoral 2HG in the IDH2-mutant SPCR successfully tested; the use of archival material makes this analysis challenging as formalin fixation and paraffin embedding may lead to oncometabolite loss (21). In addition, we observed global DNA hypermethylation and H3K27 trimethylation in IDH2/TET2–mutant SPCRPs as compared with IDH2 wild-type IDCs, consistent with those reported in IDH1/IDH2–mutant cancers (26, 30, 31, 35). IDH1 and IDH2 have been described previously to protect cells from ROS (25, 41). We observed that IDH2R172K provided ROS protection in MCF10A^{H1047R} but not in MCF10A^{H540E} cells. Further studies are warranted to assess the potential mechanistic interaction between mutant IDH2, mutant PI3K and ROS, in particular because intracellular ROS levels have been reported to affect the PI3K pathway (for a review, see ref. 47).

IDH2 mutations in SPCR may serve as a novel target for therapeutic intervention in breast cancer. Acquired enzymatic activity resulting in 2HG accumulation is specific to tumor cells, making mutant-specific small-molecule inhibitors an attractive alternative to chemotherapy regimens with systemic toxic effects. IDH2 inhibitors have now entered phase I clinical trials for the treatment of AML with favorable safety profiles (29, 48). Clinical benefit in the solid tumor patient population has yet to be demonstrated.

This study has important limitations. First, owing to the rarity of SPCR, we studied 13 bona fide cases of this entity. Despite the small sample size, this is the largest collection of SPCRPs reported to date. Second, most samples were individual contributions from different institutions; hence, we were unable to perform a survival analysis to ascertain the impact of IDH2 mutations on the outcome of SPCR. Importantly, given that none of the patients included in this study developed a distant relapse during the follow-up period available and that less than 25% of SPCRPs lack IDH2 hotspot mutations, it is unlikely that these mutations would be of prognostic significance. Third, we were unable to identify the driver genetic alterations in two SPCRPs lacking IDH2 hotspot and TET2 mutations by Sanger sequencing, however, due to the rarity of IDH2/TET2–wild-type SPCRPs and the limited material available from the cases analyzed, we were unable to sequence the entire coding region of TET2 in the remaining cases. Further studies of IDH2 wild-type SPCRPs are warranted.

Despite these limitations, here we have identified somatic IDH2 hotspot mutations or TET2 mutations in conjunction with mutations affecting PI3K pathway genes in SPCR, validating this rare breast cancer as a unique clinicopathologic entity underpinned by a distinctive constellation of somatic mutations. Detection of IDH2 mutations may serve as an ancillary marker for the diagnosis of SPCR. Broad-base genetic profiling in breast cancer patients who develop progressive disease with this tumor type may efficiently identify those eligible for clinical trials for IDH inhibition.

Disclosure of Potential Conflicts of Interest

S. Pusch is a patent holder of 2 HG assay patent. A.J. Iafrate has ownership interest (including patents) in ArcherDX and is a consultant/advisory board member for Roche. No potential conflicts of interest were disclosed by the other authors.

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References


