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ARTICLE

Immunohistochemical analysis of *IDH2* R172 hotspot mutations in breast papillary neoplasms: applications in the diagnosis of tall cell carcinoma with reverse polarity

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Abstract

Tall cell carcinoma with reverse polarity is a rare subtype of breast carcinoma with solid and papillary growth and nuclear features reminiscent of those of the tall cell variant of papillary thyroid carcinoma. These tumors harbor recurrent *IDH2* R172 hotspot mutations or *TET2* mutations, co-occurring with mutations in PI3K pathway genes. Diagnosis of tall cell carcinomas with reverse polarity is challenging in view of their rarity and the range of differential diagnosis. We sought to determine the sensitivity and specificity of IDH2 R172 immunohistochemistry for the detection of *IDH2* R172 hotspot mutations in this entity. We evaluated 14 tall cell carcinomas with reverse polarity (ten excision and five core needle biopsy specimens), 13 intraductal papillomas, 16 solid papillary carcinomas, and 5 encapsulated papillary carcinomas by Sanger sequencing of the *IDH2* R172 hotspot locus and of exons 9 and 20 of *PIK3CA*, and by immunohistochemistry using monoclonal antibodies (11C8B1) to the *IDH2* R172S mutation. The 14 tall cell carcinomas with reverse polarity studied harbored *IDH2* R172 hotspot mutations, which co-occurred with *PIK3CA* hotspot mutations in 50% of cases. None of the other papillary neoplasms analyzed displayed *IDH2* R172 mutations, however *PIK3CA* hotspot mutations were detected in 54% of intraductal papillomas, 6% of solid papillary carcinomas, and 20% of encapsulated papillary carcinomas tested. Immunohistochemical analysis with anti-IDH2 R172S antibodies (11C8B1) detected IDH2 R172 mutated protein in 93% (14/15) of tall cell carcinomas with reverse polarity samples including excision ($n = 9/10$) and core needle biopsy specimens ($n = 5$), whereas the remaining papillary neoplasms ($n = 34$) were negative. Our findings demonstrate that immunohistochemical analysis of IDH2 R172 is highly sensitive and specific for the detection of *IDH2* R172 hotspot mutations, and likely suitable as a diagnostic tool in the evaluation of excision and core needle biopsy material of tall cell carcinomas with reverse polarity.

Introduction

Solid papillary carcinoma with reverse polarity [1] also known as breast tumor resembling the tall cell variant of papillary thyroid carcinoma is a rare histologic subtype of breast cancer [2, 3], with a distinctive morphology which is reminiscent of that of the tall cell variant of papillary thyroid carcinoma [1, 2, 4–7]. To address issues and possible ambiguities in the aforementioned designations, the World Health Organization recently introduced the designation of "Tall Cell Carcinoma with Reverse Polarity" for this entity, and this is the terminology we adopt in this report. Tall cell carcinoma with reverse polarity are invasive carcinomas

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with papillary, solid and follicular architecture, composed of columnar epithelial cells displaying reverse polarization with abundant cytoplasm and apically rather than basally located nuclei [1, 2, 4]. The nuclei of tall cell carcinomas with reverse polarity are characterized by optical clearing, grooves, and occasional pseudoinclusions [1–4], akin to the nuclei of papillary thyroid carcinomas. Tall cell carcinomas with reverse polarity are HER2-negative and estrogen receptor-negative or express estrogen receptor weakly in 1–10% of tumor cells [1, 2, 4]. Although tall cell carcinomas with reverse polarity usually have an indolent behavior, rare cases with nodal involvement, local recurrence, or distal metastasis have been reported [2, 8, 9].

Tall cell carcinomas with reverse polarity are underpinned by recurrent *IDH2* R172 hotspot mutations, most of which are in the form of R172S or R172T mutations, or *TET2* inactivating mutations. These mutations often co-occur with genetic alterations affecting PI3K signaling pathway-related genes [1, 4, 10]. Functional studies using benign breast epithelial cells grown in three-dimensional culture revealed that forced expression of *IDH2* R172S in *PIK3CA* H1047R knock-in cell models resulted in reverse polarization of the epithelium, recapitulating the morphology of tall cell carcinomas with reverse polarity, suggesting that *IDH2* and *PIK3CA* hotspot mutations are likely drivers of these tumors, establishing a novel genotypic-phenotypic correlation in the context of breast cancers [1].

Even though *IDH2* mutations appear to be pathognomonic for tall cell carcinomas with reverse polarity in a breast-specific context [1], malignant neoplasms affecting other organ systems, such as gliomas [11], myeloid and lymphoid leukemias [12], sinonasal undifferentiated carcinomas [13], chondrosarcomas [14], and cholangiocarcinomas [15], also harbor *IDH2* and/or *IDH1* mutations. Therefore, there is an increasing interest in the application of *IDH2*- and *IDH1*-mutation specific antibodies in diagnostic pathology [16–20]. Given the rarity of tall cell carcinomas with reverse polarity, their differential diagnosis from other mammary lesions, especially papillary neoplasms, may be challenging, particularly in the diagnostic assessment of core needle biopsy material.

In this study we sought to determine the sensitivity and specificity of the immunohistochemical analysis of *IDH2* R172 hotspot mutations using a monoclonal antibody (clone 11C8B1) raised against IDH2 R172S in breast papillary neoplasms for the detection of *IDH2* R172 hotspot mutations.

Materials and methods

Slides and formalin-fixed paraffin-embedded tissue blocks of breast papillary neoplasms were retrieved from the authors' institutions following local Institutional Review Board approval. Patient consents were obtained as required by Institutional Review Board protocols. Samples were anonymized prior to analysis. Our series included 48 breast papillary neoplasms including 14 tall cell carcinomas with reverse polarity, 13 intraductal papillomas, 16 solid papillary carcinomas, and 5 encapsulated papillary carcinomas. Two pathologists (FP and EB) reviewed all cases and confirmed the diagnosis according to criteria put forward by Eusebi et al. [3] and by the World Health Organization 5th edition to be released in 2019. The clinicopathologic characteristics and *IDH2* and *PIK3CA* mutation status of five tall cell carcinomas with reverse polarity and nine solid papillary carcinomas included in this study had been previously reported by Lozada et al. [4], Bhargava et al. [21], and Zhong et al. [10] Estrogen receptor status was assessed by immunohistochemistry in accordance with the American Society of Clinical Oncology/College of American Pathologists guidelines [22].

Tumor tissue was microdissected from consecutive 8-mm-thick formalin-fixed paraffin-embedded sections under a stereomicroscope (Olympus SZ61) to ensure a tumor cell content >80%, as previously described [23]. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) according to manufacturers' instructions. Sanger sequencing using primers sets encompassing the *IDH2* R172 hotspot locus and exons 9 and 20 of *PIK3CA* was conducted as previously described [4]. Sequence electropherograms of the forward and reverse strands were analyzed with Mutation Surveyor (SoftGenetics) and identified mutations were manually curated.

All breast papillary neoplasms included in this study ($n = 48$; including both paired core needle biopsy and excision specimens of TCCRP20) were subjected to the immunohistochemical analysis of IDH2 R172 using the monoclonal antibody raised against IDH2 R172S (clone 11C8B1; NewEast Biosciences, Malvern, PA). We also conducted the IDH2 R172 immunohistochemical analysis of 226 triple-negative breast carcinomas available as triplicate cores in tissue microarrays. The characteristics of the samples included in these tissue microarrays were previously reported elsewhere [24].

Immunohistochemistry for IDH2 was performed as previously described [16] using a Leica-Bond-3 automate stainer platform (Leica, Buffalo Grove, IL). In brief, following heat-based antigen retrieval using high pH retrieval buffer (ER2, Leica) for 30 minutes, tissue sections were incubated with the monoclonal antibody clone 11C8B1 to IDH2 R172S (NewEast Biosciences, Malvern, PA) at a concentration of 0.5 mg/ml (1:2000) for 30 minutes. The primary antibody was detected by a polymer-based secondary kit (Refine Detection kit, Leica). The chondrosarcoma cell line SW1353 which harbors an *IDH2* R172S mutation [25] and a panel of normal tissues stemming from various organs were used as positive and

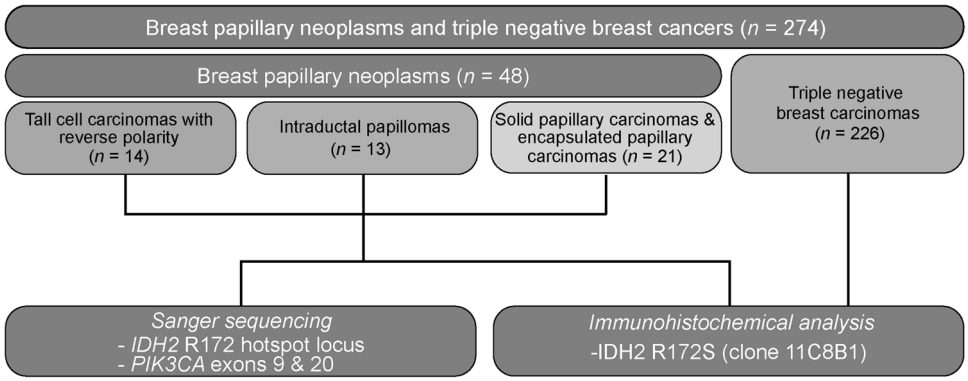

Fig. 1 Schematic representation of the cases included and analyses conducted in this study. Depiction of the cases evaluated in this study, encompassing breast papillary lesions including tall cell carcinomas with reverse polarity ($n = 14$), intraductal papillomas ($n = 13$), solid papillary carcinomas ($n = 16$), and encapsulated papillary carcinomas ($n = 5$) subjected to Sanger sequencing analysis of the *IDH2* R172 hotspot locus and of exons 9 and 20 of *PIK3CA*, and to the immunohistochemical analysis for IDH2 R172 (clone 11C8B1). Triple-negative breast cancers ($n = 226$) were subjected to immunohistochemical analysis of IDH2 R172 expression using tumor microarrays.

negative controls, respectively. Immunohistochemistry for calretinin was performed on a BenchMark ULTRA platform (Roche), following antigen retrieval with a cell conditioning solution (CC1, Roche). Tissue sections were incubated with the monoclonal antibody clone SP65 (Roche, Basel, Switzerland) at a 'ready-to-use' dilution, which was detected using the iVIEW DAB detection system (Roche). Immunohistochemistry for androgen receptor was performed following antigen retrieval (ER2, Leica), using the monoclonal antibody clone SP107 (Abcam, Cambridge, United Kingdom) at a 1:250 dilution for 30 minutes on a Leica-Bond-3 platform (Leica). The primary antibody was detected using the Refine Detection kit (Leica).

Results

Forty-eight breast papillary neoplasms, including tall cell carcinomas with reverse polarity ($n = 14$), intraductal papillomas ($n = 13$, including three intraductal papillomas with focal epithelial atypia), solid papillary carcinomas ($n = 16$), and encapsulated papillary carcinomas ($n = 5$) were included in this study (Fig. 1 and Supplementary Table 1). The median age of the patients with tall cell carcinoma with reverse polarity was 63 years (range, 46–85) and the median tumor size was 1.3 cm (range, 0.6–2.6; Supplementary Table 2). The tall cell carcinomas with reverse polarity included in our series displayed solid, papillary, and follicular growth patterns and were composed of cuboidal to columnar tumor cells with apically located nuclei (i.e., reverse polarization; Fig. 2a, b). The nuclei showed optical clearing and grooves (Fig. 2b). All tall cell carcinomas with reverse polarity were of histologic grade 1. No ductal carcinoma in situ or atypia was observed in these cases (Supplementary Table 2). None of the patients for whom clinical information was available ($n = 7$) had a prior cancer history, and one of them had a synchronously diagnosed contralateral invasive ductal carcinoma of no special type (Supplementary Table 2). None of the patients with available information had nodal involvement (0/6) or metastatic disease (0/6; Supplementary Table 2). In addition to IDH2 R172S, other immunohistochemical analyses had been performed in some tumors: 62% (8/13) were estrogen receptor-negative, whilst 38% (5/13) displayed focal estrogen receptor-positivity; 13/13 cases were diffusely and strongly positive for CK5/6. All (14/14) tall cell carcinomas with reverse polarity lacked a myoepithelial cell layer, as revealed by the immunohistochemical assessment for p63 (Supplementary Table 2). We conducted the immunohistochemical analyses for calretinin and androgen receptor in cases with available material. In agreement with Alsadoun et al. [26], the majority (90%; 9/10) of tall cell carcinomas with reverse polarity analyzed showed either diffuse ($n = 5$) of focal ($n = 4$) immunoreactivity for calretinin, and most cases (81%; 9/11) displayed either absent ($n = 3$) or low (≤10%; $n = 6$) androgen receptor expression (Supplementary Fig. 1A–D and Supplementary Table 2). Out of six cases with available treatment information, five patients underwent a partial mastectomy, two of which received radiotherapy, and one patient underwent a total mastectomy and received no adjuvant therapy (Supplementary Table 2).

We analyzed 15 samples corresponding to 14 tall cell carcinomas with reverse polarity, including core needle biopsy ($n = 5$) and excision specimens ($n = 10$). For one case (TCCRP20) we analyzed paired core needle biopsy and excision specimens. We subjected tumor-derived DNA to Sanger sequencing of the *IDH2* R172 hotspot locus and of exons 9 and 20 of *PIK3CA* (Fig. 1). Our analysis revealed that all (14/14) tall cell carcinomas with reverse polarity in this series harbored *IDH2* R172 hotspot mutations, including R172T ($n = 6$), R172S ($n = 7$), and R172I ($n = 1$) mutations. Overall, 50% of tall cell carcinomas with reverse polarity (7/14) harbored *PIK3CA* hotspot mutations

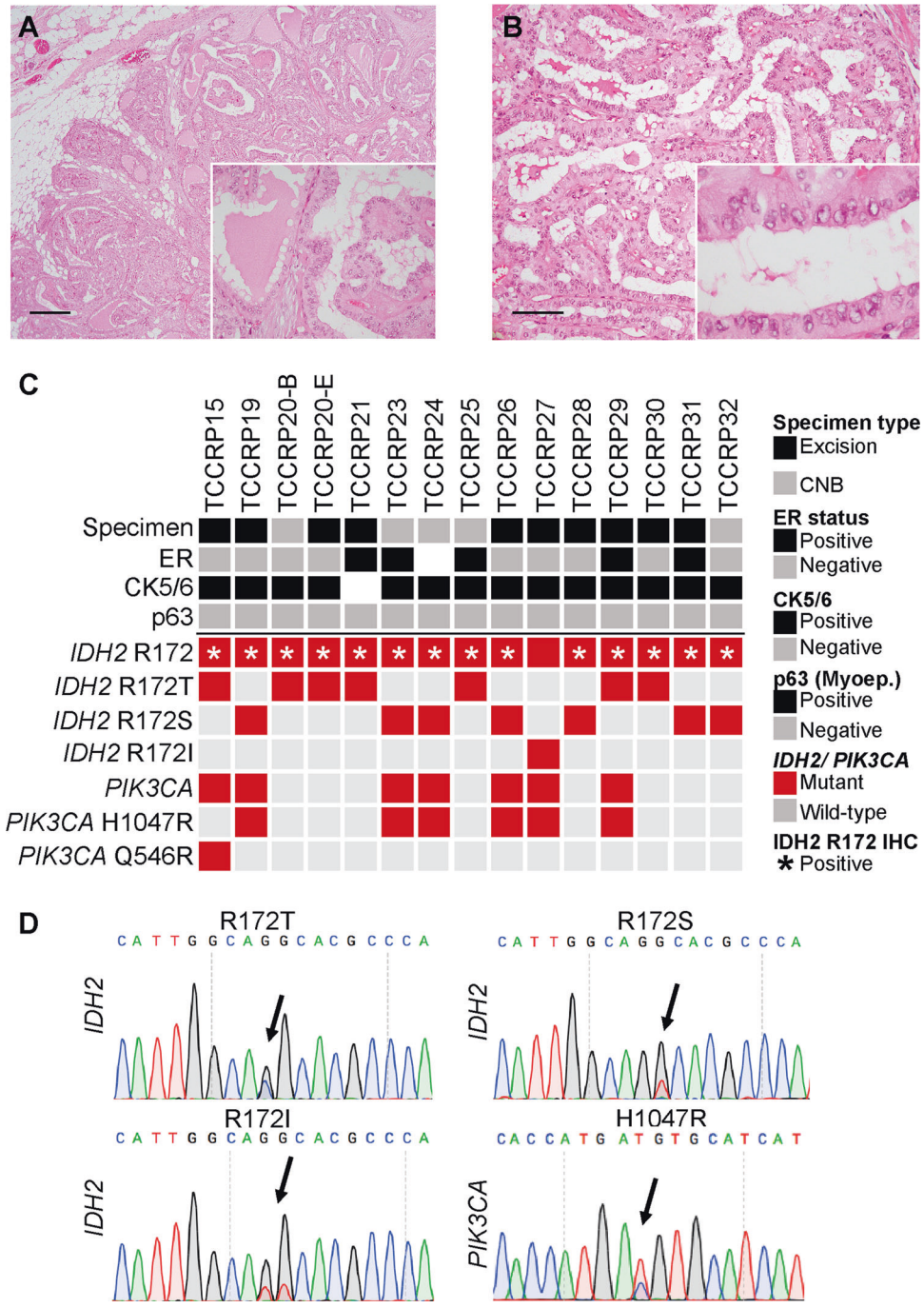

Fig. 2 Tall cell carcinomas with reversed polarity harbor recurrent *IDH2* R172 hotspot mutations which are detectable by immunohistochemistry, frequently co-occurring with *PIK3CA* hotspot mutations. **a, b** Representative hematoxylin and eosin photomicrographs of TCCRP15 (**a**) showing solid and papillary growth patterns with follicule-like structures with colloid-like secretion (inset) and (**b**) composed of tumor cells displaying reverse polarity (i.e., nuclei localized in the apical aspect of the cell) and occasional nuclear grooves (inset). Scale bars in **a** 200 µm and in **b** 50 µm. **c** Heatmap depicting the clinicopathologic characteristics, specimen type, genetic alterations and immunoreactivity for IDH2 R172 of samples including ten excision specimens and five core needle biopsy specimens corresponding to 14 tall cell carcinomas with reverse polarity included in this study. Cases are shown in columns and genes and genetic alterations in rows. Clinicopathologic characteristics and specimen type are depicted in phenotype bars (top). *IDH2* and *PIK3CA* mutations and immunoreactivity for IDH2 R172 are color-coded according to the legend. **b** Representative Sanger electropherograms of *IDH2* R172T, R172S, and R172I hotspot mutations and a *PIK3CA* H1047R hotspot mutation identified in tall cell carcinomas with reverse polarity.

in exons 9 or 20, including H1047R ($n = 6$) and Q546R ($n = 1$) mutations (Fig. 2a, b).

Next, we sought to determine whether the presence of *IDH2* R172 hotspot mutations could be detected by immunohistochemistry. For this, we conducted the immunohistochemical analysis of all cases using the monoclonal antibody 11C8B1 raised against IDH2 R172S (Fig. 1) which revealed that 93% (14/15) of tall cell carcinoma with reverse polarity samples, including core needle biopsy ($n = 5$) and excision specimens ($n = 9/10$) were immunoreactive (Figs. 2a and 3a–h). Interestingly, both *IDH2* R172S ($n = 7$) and R172T ($n = 7$) mutations were detected by our immunohistochemical analysis despite the use of anti-IDH2 R172S antibodies (Fig. 2a). The immunoreactivity for IDH2 R172 in all tall cell carcinomas with reverse polarity analyzed was strong, diffuse and cytoplasmic and showed a granular pattern (Fig. 3a–h), which might be related to the fact the *IDH2* encodes for the mitochondrial enzyme isocitrate dehydrogenase 2 [27]. In contrast, the IDH2 R172I mutation identified in case TCCRP27 (Fig. 2a, b) was not

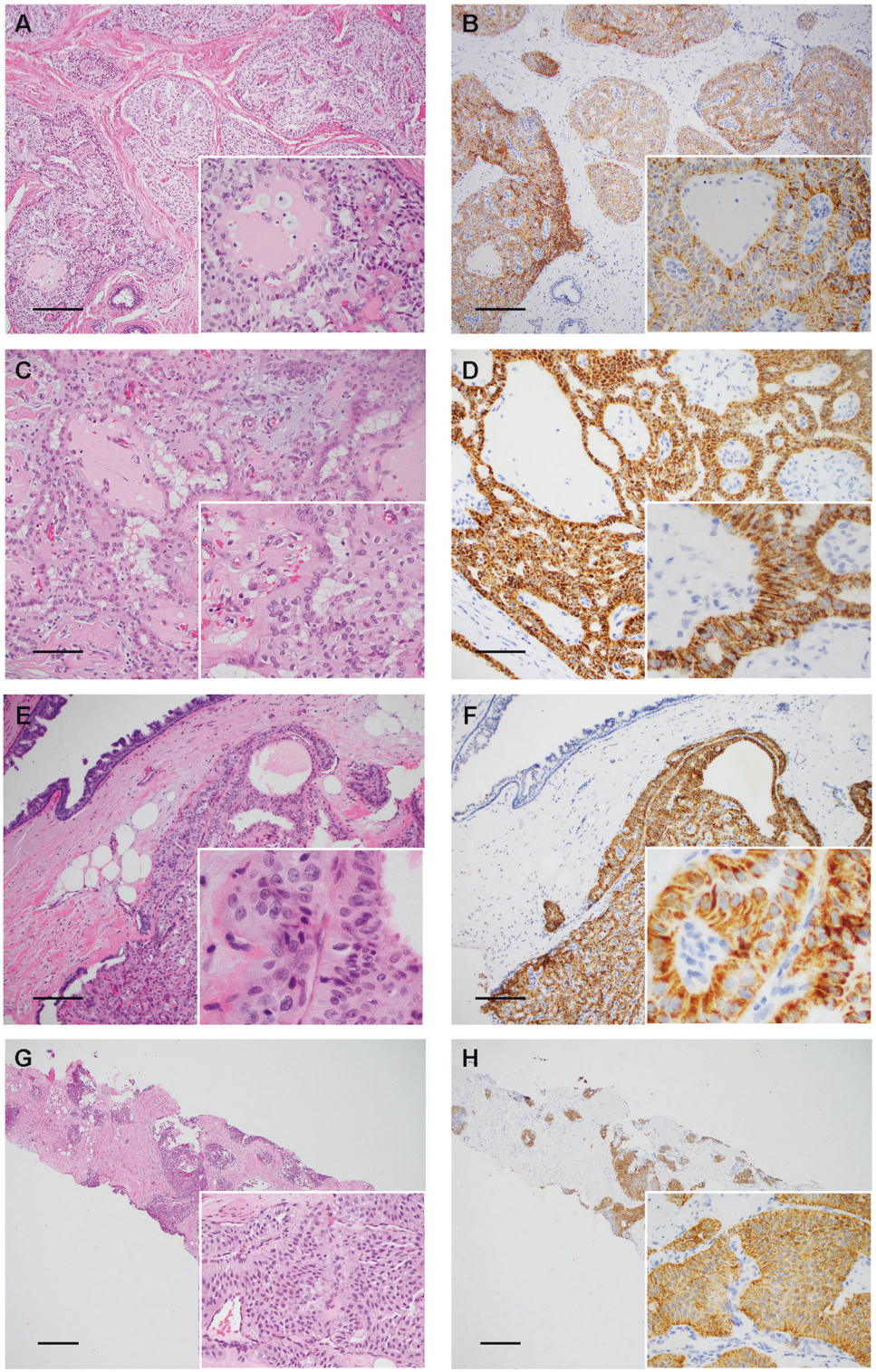

Fig. 3 Detection of *IDH2* R172 hotspot mutations by immunohistochemistry is feasible in excision and core needle biopsy specimens. Representative hematoxylin and eosin (**a, c**) and corresponding IDH2 R172 immunohistochemical expression micrographs of TCCRP30 (**a, b**) and TCCRP19 (**c**, **d**) showing strong and diffuse immunoreactivity for IDH2 R172 (**b**, **d**) with a cytoplasmic granular pattern (**b–d**, insets). Representative (**e**) hematoxylin and eosin and (**f**) IDH2 R172 expression photomicrographs of TCCRP20 displaying strong immunolabeling for IDH2 R172; in contrast the adjacent benign breast epithelium shows no immunoreactivity; (**g**, **h**) the core needle biopsy specimen of case TCCRP24 shows strong cytoplasmic and granular IDH2 R172 immunoreactivity. Scale bars in **a**, **b** 100 μm; **c–f** 50 μm; **g**, **h** 200 μm.

detectable by immunohistochemistry. Of note, this case did not display unusual histologic features that distinguished it from the other tall cell carcinomas with reverse polarity. Notably, the benign breast epithelium surrounding the tumors was negative for IDH2 R172 by immunohistochemistry in all cases, a finding that supports the specificity of the immunoreactivity observed (Fig. 3e, f). Importantly, we observed strong and diffuse cytoplasmic immunoreactivity for IDH2 R172 in all (5/5) core needle biopsy specimens analyzed (Fig. 3g, h).

Overall, these findings support the contention that *IDH2* R172S and R172T hotspot mutations in tall cell carcinomas

with reverse polarity are detectable by immunohistochemistry using the monoclonal antibody raised against IDH2 R172S. Moreover, although we did not interrogate the presence of genetic alterations affecting *PIK3CA* in exons other than 9 and 20 and in other PI3K genes, our data lend additional support to the notion that tall cell carcinomas with reverse polarity are underpinned by recurrent *IDH2* R172 hotspot mutations which oftentimes coexist with genetic alterations affecting the PI3K pathway. Importantly, our findings indicate that detection of *IDH2* R172 hotspot mutations in tall cell carcinomas with reverse polarity by immunohistochemistry using IDH2 R172S monoclonal antibodies (clone 11C8B1) is feasible in excision and core needle biopsy material.

To determine whether immunohistochemical assessment of *IDH2* R172 mutations could be used to discriminate tall cell carcinomas with reverse polarity from other breast papillary neoplasms, we subjected 13 intraductal papillomas, of which three displayed epithelial atypia, 16 solid papillary carcinomas, and 5 encapsulated papillary carcinomas to Sanger sequencing of the *IDH2* R172 hotspot locus and of exons 9 and 20 of *PIK3CA*, and to immunohistochemical analysis of IDH2 R172 (Fig. 1 and Supplementary Table 1). Our analyses revealed that 54% (7/13), 6% (1/16), and 20% (1/5) of the intraductal papillomas, solid papillary carcinomas and encapsulated papillary carcinomas in our series, respectively, harbored *PIK3CA* hotspot mutations, whilst none of the intraductal papillomas ($n = 13$), solid papillary carcinomas ($n = 16$), and encapsulated papillary carcinomas ($n = 5$) studied harbored *IDH2* R172 hotspot mutations (Fig. 4a, b and Supplementary Table 1). Our immunohistochemical analysis using the *IDH2* R172S antibody revealed the lack of IDH2 R172 expression in all intraductal papillomas ($n = 13$), solid papillary carcinomas ($n = 16$) and encapsulated papillary carcinomas ($n = 5$) analyzed (Fig. 4a–f). Given that tall cell carcinomas with reverse polarity generally display a triple-negative phenotype, we sought to assess IDH2 R172 expression in triple-negative breast cancers by immunohistochemistry as an additional validation step. For this purpose, we subjected 226 triple-negative breast cancers to immunohistochemical analysis of IDH2 R172 using tissue microarrays (Fig. 1) and observed that none of the triple-negative breast cancer cores analyzed (0/226) expressed IDH2 R172 by immunohistochemistry (results not shown).

Taken together, our findings indicate that the immunohistochemical evaluation IDH2 R172 mutations using the monoclonal antibody 11C8B1 has a sensitivity of 93% and specificity of 100% for the detection of *IDH2* R172 hotspot mutations in tall cell carcinomas with reverse polarity and constitutes a useful tool for the differential diagnosis of tall cell carcinomas with reverse polarity from other papillary lesions of the breast.

Discussion

In this study we conducted the genetic analysis of *IDH2* R172 and *PIK3CA* hotspot mutations and the IDH2 R172 immunohistochemical evaluation in breast papillary neoplasms to assess the performance of immunohistochemistry employing the monoclonal antibody 11C8B1 for the detection of *IDH2* R172 hotspot mutations in tall cell carcinomas with reverse polarity. Consistent with prior reports by our group [1, 4] and others [10, 21, 26], we observed a high frequency of *IDH2* R172 hotspot mutations in tall cell carcinomas with reverse polarity, which coexisted with *PIK3CA* mutations in 50% of cases. Whether the tumors found to lack *PIK3CA* hotspot mutations analyzed here harbor other genetic alterations affecting genes of the PI3K signaling pathway, such as loss-of-function mutations targeting *PI3KR1*, as previously reported in *PIK3CA*-wild type tall cell carcinomas with reverse polarity [1], was not investigated in this study.

Importantly, our findings show that only tall cell carcinomas with reverse polarity, which were all *IDH2* R172-mutant, were positive for IDH2 R172 by immunohistochemistry whereas all other breast papillary neoplasms studied lacked *IDH2* R172 hotspot mutations and were negative by immunohistochemistry. Furthermore, none of the triple-negative breast cancers analyzed expressed immunoreactivity for IDH2 R172. These findings are in agreement with the notion that *IDH2* R172 hotspot mutations are pathognomonic for tall cell carcinomas with reverse polarity in the context of breast neoplasms. Indeed, our reanalysis of 971 breast invasive ductal and invasive lobular carcinomas [1] revealed only one case harboring an *IDH2* mutation, which did not target the R172 hotspot locus, but instead affected a different codon (*IDH2* E345K) [1], and the reanalysis of 1918 primary and metastatic breast cancer samples [28] revealed only five cases with *IDH2* mutations, none of which affected the R172 locus. Although most *IDH2* R172 mutations in tall cell carcinomas with reverse polarity reported to date were either R172T or R172S, *IDH2* R172G [4, 26] and R172W [10] mutations have also been identified. We now report a tall cell carcinoma with reverse polarity harboring an R172I mutation. Interestingly, our immunohistochemical analysis using monoclonal antibodies to IDH2 R172S detected *IDH2* R172S and R172T mutations in tall cell carcinomas with reverse polarity, which is in agreement with the study by Dogan et al. [16] on sinonasal undifferentiated carcinomas using the same anti-IDH2 R172S monoclonal antibody as in this study (11C8B1). The cross-reactivity observed might be due to the structural similarities between serine and threonine, as both are polar amino acids [29]. However, the *IDH2* R172I mutation identified in TCCRP27 was not detected by immunohistochemistry using IDH2 R172S

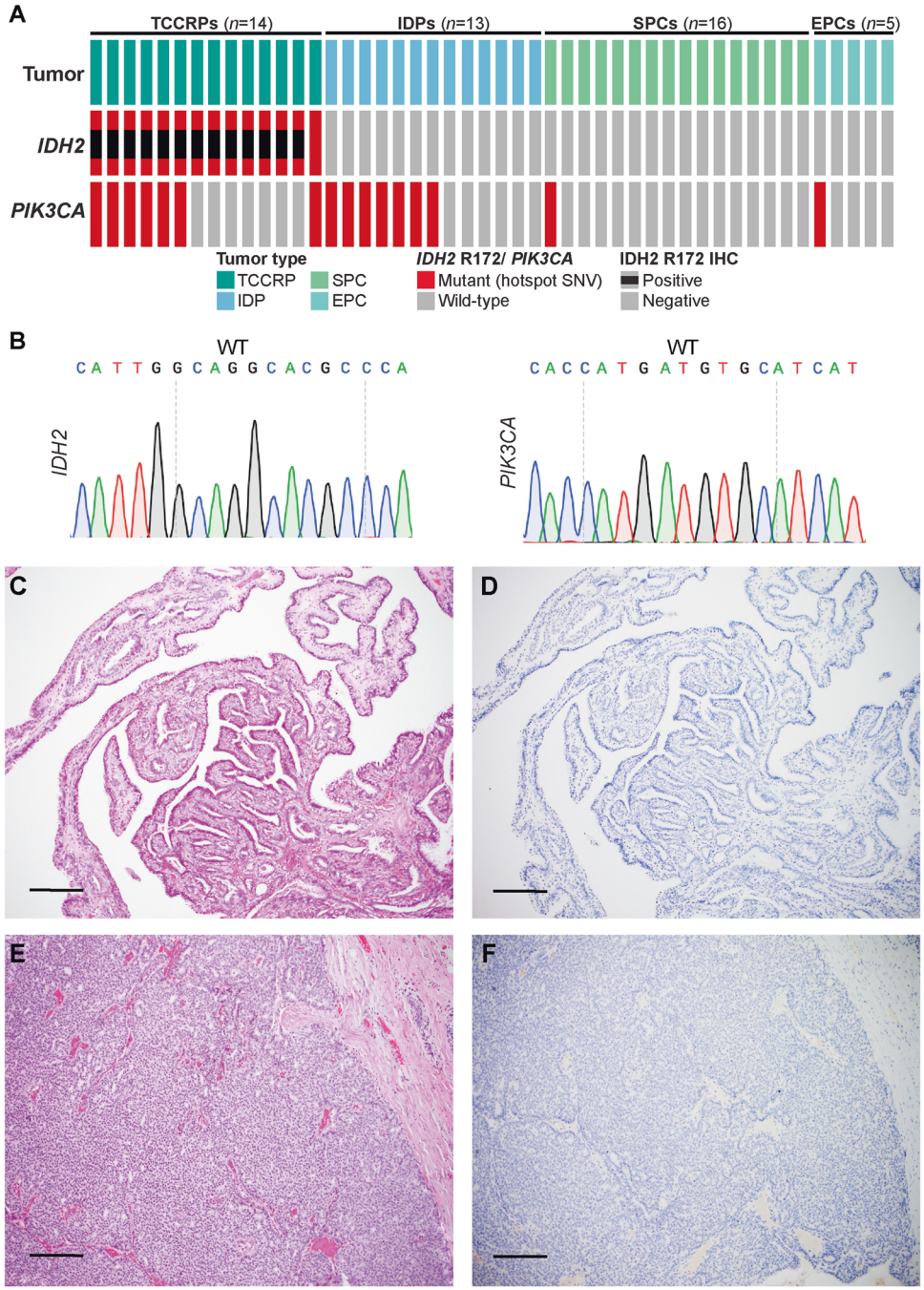

Fig. 4 Immunohistochemical analysis of IDH2 R172 is highly sensitive and specific for the detection of *IDH2* R172 hotspot mutations in tall cell carcinomas with reversed polarity. a Frequency of *IDH2* R172 and *PIK3CA* hotspot mutations and expression of IDH2 R172 by immunohistochemistry in tall cell carcinomas with reverse polarity ($n = 14$), intraductal papillomas ($n = 13$), solid papillary carcinomas ($n = 16$) and encapsulated papillary carcinomas ($n = 5$). *IDH2* R172 and *PIK3CA* mutational status and immunoreactivity for IDH2 R172 are color-coded according to the legend. **b** Representative Sanger sequencing electropherograms of the *IDH2* R172 (left) and *PIK3CA* H1047 (right) hotspot loci in intraductal papilloma IDP12. Representative (**c**, **e**) hematoxylin and eosin and (**d**, **f**) IDH2 R172 expression photomicrographs of (**c, d**) the intraductal papilloma IDP12 and (**e**, **f**) solid papillary carcinoma PC14.

mutation specific antibodies, which might be due to the fact that isoleucine is a nonpolar amino acid [29]. Given that not all *IDH2* mutations can be detected by immunohistochemistry and that cases lacking *IDH2* mutations have been reported [1], the diagnosis of tall cell carcinoma with reverse polarity should not be ruled out in cases displaying the typical histologic features of this entity that are negative for IDH2 R172 by immunohistochemical analysis. Taken together, these results indicate that the immunohistochemical assessment of *IDH2* R172 hotspot mutations is a sensitive and specific test for the identification these mutations in tall cell carcinomas with reverse polarity.

Our findings are in agreement with those reported in the study by Alsadoun et al. [26], who subjected nine tall cell carcinomas with reverse polarity to whole-exome sequencing and to immunohistochemical analysis using *IDH1*/*IDH2* mutant (R132/R172) antibodies in excision specimens. They reported that six of seven tall cell carcinomas with reverse polarity harboring *IDH2* R172 hotspot mutations were immunoreactive for IDH2 R172. Our cohort

consisted of both core needle biopsy and excision specimens and IDH2 R172 immunoreactivity was observed in both specimen types, suggesting that the immunohistochemical analysis of *IDH2* R172 hotspot mutations is a useful and reliable diagnostic tool in the work-up of breast lesions devoid of myoepithelium and displaying the distinctive morphologic features of tall cell carcinomas with reverse polarity in core needle biopsies as a surrogate of genetic analysis or to triage tumors for sequencing of the *IDH2* R172 locus.

Our study has limitations. The relatively limited number of tall cell carcinomas with reverse polarity included in our series reflects the rarity of these tumors. We did not investigate the presence of genetic alterations affecting other PI3K pathway genes in these cases, and our series did not include any *IDH2*-wild type tall cell carcinoma with reverse polarity harboring *TET2* mutations, which might presumably be negative for IDH2 R172 by immunohistochemical analysis. No clinical follow-up information was available for our cases, which for most part were diagnosed only recently. Despite these limitations, our study lends further support to the notion that *IDH2* R172 hotspot mutations are pathognomonic for tall cell carcinomas with reverse polarity in a breast-specific context and demonstrates that the immunohistochemical analysis of IDH2 R172 employing the monoclonal antibody 11C8B1 is a sensitive and specific test for the identification of tall cell carcinomas with reverse polarity harboring *IDH2* R172 hotspot mutations. Detection of *IDH2* R172 hotspot mutations by immunohistochemistry has practical applications in the diagnostic evaluation of this exceedingly rare tumor type, especially in core needle biopsy material when an unequivocal diagnosis is difficult by morphologic evaluation alone due to limited sampling.

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Author contributions JSR-F and EB conceived the study. EZ, FD, TMD, HYW, DG, MMH, GK, RB, JPP, EAR, SH, MS, LC, SS, Y-YC, and EB contributed with cases. FP and EB reviewed the cases. FP, EdS, and DF conducted the experiments. FP, EdS, DF, FCG, JRL, TB, ADCP, BW, and AAJ analyzed and interpreted the data. FP wrote the first manuscript that was reviewed by all co-authors.

Compliance with ethical standards

Conflict of interest JSR-F reports receiving personal/consultancy fees from Goldman Sachs and REPARE Therapeutics, membership of the scientific advisory boards of VolitionRx and Page.AI, and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Ventana Medical Systems, Novartis, Genentech, and InVicro, outside the scope of this study. All other authors declare that they have no conflict of interest.

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