Histopathology

Histopathology 2018, **73**, 339–344. DOI: 10.1111/his.13522

SHORT REPORT

Solid papillary breast carcinomas resembling the tall cell variant of papillary thyroid neoplasms (solid papillary carcinomas with reverse polarity) harbour recurrent mutations affecting *IDH2* and *PIK3CA*: a validation cohort

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Date of submission 6 January 2018
Accepted for publication 23 March 2018
Published online *Article Accepted* 30 March 2018

Lozada J R, Basili T, Pareja F, Alemar B, Paula A D C, Gularte-Merida R, Giri D D, Querzoli P, Cserni G, Rakha E A, Foschini M P, Reis-Filho J S, Brogi E, Weigelt B & Geyer F C (2018) *Histopathology* **73**, 339–344. https://doi.org/10.1111/his.13522

Solid papillary breast carcinomas resembling the tall cell variant of papillary thyroid neoplasms (solid papillary carcinomas with reverse polarity) harbour recurrent mutations affecting *IDH2* and *PIK3CA*: a validation cohort

Aims: Solid papillary breast carcinoma resembling the tall cell variant of papillary thyroid neoplasms (BPTC), also known as solid papillary carcinoma with reverse polarity, is a rare histological type of breast cancer that resembles morphologically the tall cell variant of papillary thyroid carcinoma. BPTCs are characterised by *IDH2* R172 hotspot somatic mutations or mutually exclusive *TET2* somatic mutations, concurrently with mutations affecting PI3K pathway-related genes. We sought to characterise their histology and investigate the frequency of *IDH2* and *PIK3CA* mutations in an independent cohort of BPTCs, as well as in conventional solid papillary carcinomas (SPCs).

Methods and results: Six BPTCs, not previously analysed molecularly, and 10 SPCs were reviewed centrally. Tumour DNA was extracted from microdissected histological sections and subjected to Sanger sequencing of the *IDH2* R172 hotspot locus and exons 9 and 20 of *PIK3CA*. All six BPTCs were characterised by solid, papillary and follicular architecture with circumscribed, invasive tumour nodules composed of epithelial cells with reverse polarity. *IDH2* mutations were identified in all six BPTCs (three R172S, two R172T and one R172G), four of which also harboured *PIK3CA* mutations (two H1047R, one Q546K and one Q546R). By contrast, all SPCs lacked *IDH2* mutations, while

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one of 10 harboured a *PIK3CA* mutation (H1047R).
Conclusion: We validated the presence of *IDH2* R172 hotspot mutations and *PIK3CA* hotspot mutations in 100% and 67% BPTCs tested, respectively, and documented absence of *IDH2* R172 mutations in SPCs. These findings confirm the genotypical–phenotypical correlation reported previously in BPTC, which constitutes an entity distinct from conventional SPC.

Keywords: breast cancer, IDH2, sanger sequencing

Introduction

Solid papillary breast carcinoma resembling the tall cell variant of papillary thyroid neoplasms (BPTC),[1] also known as breast tumour resembling the tall cell variant of papillary thyroid carcinoma[2,3] or solid papillary carcinoma with reverse polarity,[4] is a rare and distinctive subtype of invasive breast cancer. First recognised because of striking similarities with papillary thyroid carcinomas (PTCs),[3] including nuclear diagnostic features (e.g. nuclear grooves and intranuclear pseudo-inclusions), BPTCs were catalogued further under different terminologies, all of which are synonyms. Despite histological similarities to papillary thyroid neoplasms, clinical, immunohistochemical and genetic data indicate that BPTCs are primary breast tumours, given the lack of expression of thyroid-specific markers, including thyroid transcription factor 1 (TTF1) and thyroglobulin,[3,5,6] and somatic genetic alterations typically found in PTCs, including those affecting *RET/PTC* and *BRAF*[3,6–8]

Histologically, BPTCs are characterised by circumscribed tumour nodules lacking a peripheral layer of myoepithelial cells growing in solid, papillary and/or follicular architectural patterns.[1,5,6] The term 'reverse polarity' stems from the tumour cells harbouring nuclei at the apical rather than the basal pole, thereby creating the impression of reverse polarisation.[4] The polarisation of the cells, however, is preserved, with expression of mucin 1 (MUC1) being found at the apical luminal border.[4] To date, 40 such tumours have been reported.[1–6] BPTCs are mainly of triple-negative phenotype, with a third being weakly oestrogen receptor (ER)-positive/human epidermal growth factor receptor (HER2)-negative.[1,3–6] Clinically, the majority of BPTCs display an indolent biological behaviour with an excellent prognosis; only two reported cases displayed axillary lymph node metastasis.[1,3,5,6]

We have demonstrated previously that BPTCs constitute a discrete subtype of breast cancer underpinned by highly recurrent *IDH2* R172 hotspot somatic mutations or *TET2* loss-of-function somatic mutations, alongside PI3K pathway-related genes.[4] As in leukaemias,[9] *IDH2* and *TET2* mutations were found to be mutually exclusive in BPTCs,[4] functioning as alternative genetic drivers. *In-vitro* studies demonstrated that expression of *IDH2* mutations in non-malignant breast epithelial cells results in a phenotype closely recapitulating that of BPTCs. While Bhargava *et al.* recently described *IDH2* R172 mutations in two of three additional BPTCs,[2] to the best of our knowledge, *IDH2* R172 mutations, which are common in hematological malignancies and brain tumours,[10] have not been described in other breast cancer subtypes.[11–13] Here, we sought to characterise the unique histology of BPTCs further and investigate the frequency of *IDH2* and PI3K catalytic subunit alpha (*PIK3CA*) hotspot mutations in an independent series of BPTCs, as well as in conventional solid papillary carcinomas (SPCs) to define their potential genetic relatedness.

Methods

COHORT

After obtaining approval by the Institutional Review Boards and the local research ethics committees from the authors' institutions, six BPTCs, not previously analysed molecularly, and 10 SPCs were retrieved, reviewed centrally by three breast pathologists (F.C.G., F.P. and E.B.) and anonymised prior to analysis. Three cases were reported previously by Foschini *et al.*[1] (BPTC14, BPTC15, BPTC17).

MICRODISSECTION AND DNA EXTRACTION

Representative sections of formalin-fixed paraffin-embedded BPTCs and SPCs were subjected to microdissection to ensure > 80% tumour content, as described previously,[14,15] and DNA was extracted.

IMMUNOHISTOCHEMISTRY (IHC)

IHC data [cytokeratin (CK) 5/6, p63 and/or calponin] from BPTC14, BPTC15 and BPTC17 was retrieved from Foschini *et al.*[1] Representative histological sections of BPTC18, BPTC19 and BPTC20 were analysed

for CK5/6, p63 and/or smooth muscle myosin heavy chain, as described previously[16] (Supporting information, Table S1). Positive and negative controls were included in each slide run. ER, PR and HER2 IHC data were retrieved from original pathology reports for all cases.

SANGER SEQUENCING ANALYSIS

The *IDH2* R172 hotspot residue and exons 9 and 20 of *PIK3CA* were investigated in the cohort of BPTCs and SPCs by Sanger sequencing, as described previously (Supporting information, Table S2).[15,17]

Results

CLINICOPATHOLOGICAL CHARACTERISTICS OF BPTC

The six BPTCs occurred in female patients, with a median age at diagnosis of 60 years (range = 58–85 years). Tumour size ranged from 0.6 to 5.0 cm. The histological features were consistent throughout all cases (Figure 1, Supporting information, Figure S1). All BPTCs were multilobulated and characterised by solid and papillary architecture, with circumscribed and invasive tumour nodules composed of epithelial cells displaying reverse polarity. Follicle-like structures with colloid-like secretion were also focally present. The nuclei displayed grade 2 pleomorphism, with occasional grooves and intranuclear pseudo-inclusions. The six BPTCs were of histological grade 1 and of triple-negative phenotype (Supporting information, Table S3), expressed CK5/6 diffusely, and lacked a myoepithelial cell layer (Figure 1I–L; Supporting information, Table S3).

BPTCS HARBOUR HIGHLY RECURRENT MUTATIONS AFFECTING *IDH2* AND *PIK3CA*

Given that our previous study[4] identified highly recurrent mutations affecting the *IDH2* R172 hotspot residue and *PIK3CA* hotspots in BPTCs, we sought to define the frequencies of these mutations in an independent set of BPTCs. Sanger sequencing analysis revealed high mutation frequencies in both genes (Figure 2). *IDH2* R172 hotspot mutations were found in 100% of BPTCs (six of six; three R172S, two R172T and one R172G). *PIK3CA* hotspot mutations were found in 67% of BPTCs (four of six; two H1047R, one Q546K and one Q546R). These findings are consistent with our previous study, which identified *IDH2* R172 hotspot and *PIK3CA* hotspot mutations in 77 and 69% of BPTCs, respectively, and concurrent *IDH2* R172 hotspot and *PIK3CA* mutations in 62% of BPTCs.[4] In contrast, all conventional SPCs lacked *IDH2* R172 hotspot mutations (none of 10 versus six of six BPTCs, $P < 0.001$, Fisher's exact test), and 10% harboured a *PIK3CA* hotspot mutation (H1047R; one of 10 versus four of six BPTCs, $P = 0.035$, Fisher's exact test; Supporting information, Figure S2).

Discussion

Here we confirm that BPTC constitutes a rare histological type of breast cancer, with unique morphological features and underpinned by highly recurrent *IDH2* R172 mutations alongside *PIK3CA* mutations. Yet to be recognised as a special type of breast cancer by the World Health Organisation (WHO) classification of breast tumours,[18] our previous[4] and current findings, together with those by Bhargava *et al.*[2], establish BPTC as a distinct histological type of invasive breast carcinoma, driven by mutually exclusive genetic alterations affecting the *IDH2* R172 hotspot residue or *TET2*, in conjunction with mutations in PI3K pathway-related genes. Using *in-vitro* studies, we have shown previously that expression of mutant *IDH2* in *PIK3CA*-mutant breast epithelial cells results in the characteristic phenotype of BPTC.[4] While PI3K pathway-related gene mutations are common in breast cancer,[11–13] *IDH2* mutations are vanishingly rare or non-existent in other breast tumours.[11–13] Indeed, the conventional SPCs analysed here lacked *IDH2* R172 mutations, consistent with their driver role in the pathogenesis of BPTCs, and with the notion that BPTC should not be perceived as a histological variant of SPC.

The mechanism by which *IDH2* contributes to tumorigenesis has yet to be understood fully. *IDH2* mutations result in loss of catalytic activity and production of α-ketoglutarate (α-KG) and gain of new activity with production of the oncometabolite *R*-2-hydroxylglutarate (*R*-2-HG).[19–22] *R*-2-HG competitively inhibits activity of α-KG-dependent dioxygenases, altering genome-wide histones and DNA methylation and resulting in abnormal epigenetic regulation, cell differentiation and tumorigenesis.[21,22] Importantly, *IDH2*-mutant BPTCs display a hypermethylated profile and higher expression levels of H3K27me3 compared to conventional forms of invasive breast cancer.[4] Importantly, however, the mechanism by which *IDH2* R172 hotspot mutations or *TET2* inactivating mutations alongside PI3K pathway-related gene mutations result in phenotypic

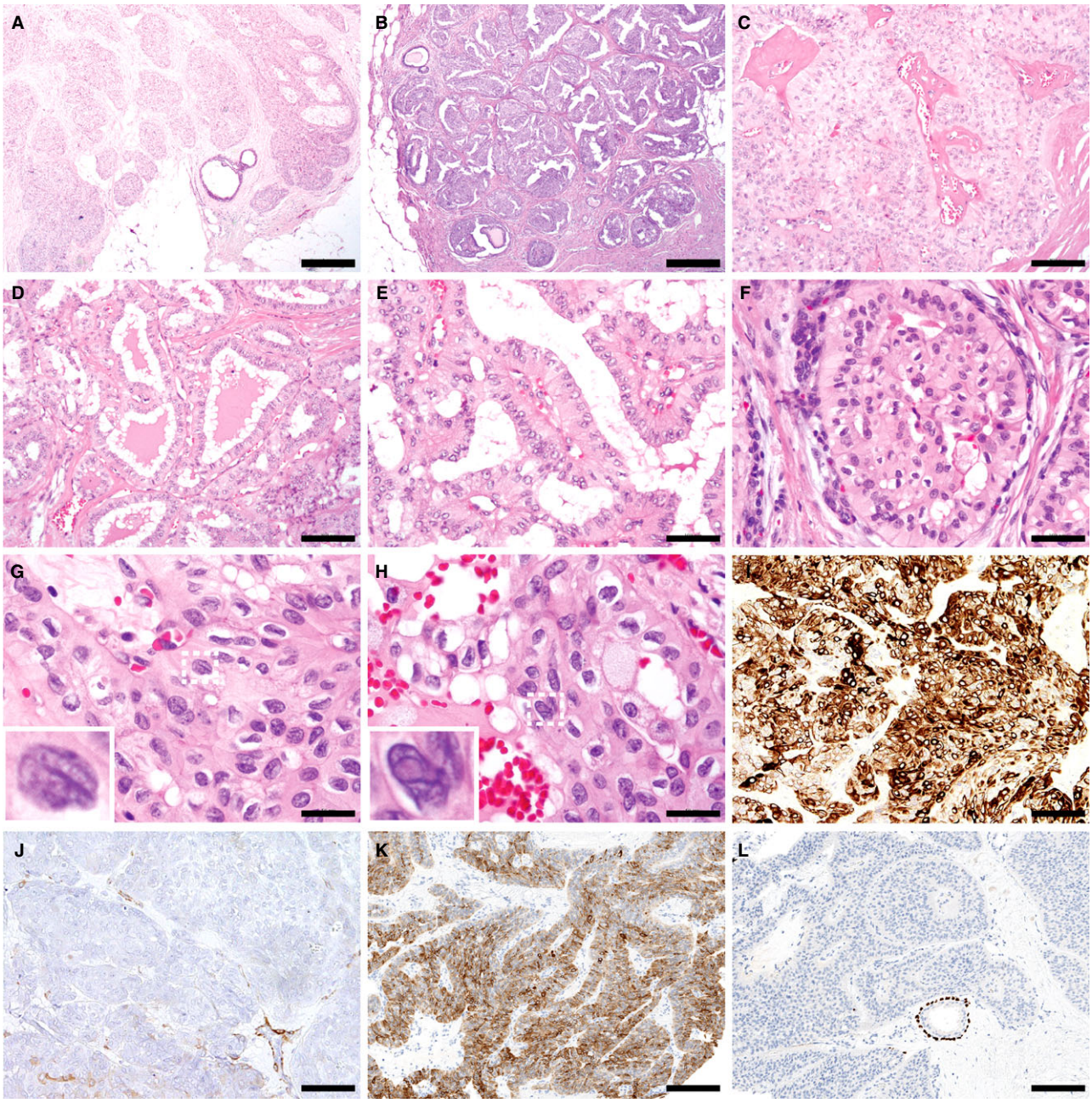

Figure 1. Morphological features of solid papillary breast carcinomas resembling the tall cell variant of papillary thyroid neoplasms (BPTCs). **A**,**B**, Low-power magnification of BPTC18 (**A**) and BPTC17 (**B**), displaying lobulated contours, without an infiltrative pattern (scale bar 1 mm). **C**, Solid and papillary architecture, with evident fibrovascular cores, often with perivascular hyalinised stroma, characterised all BPTCs included in this study (BPTC14, scale bar 200 μm). **D**, Diffuse or focal follicle-like structures with colloid-like secretion were also found in all cases (BPTC15, scale bar 200 μm). **E**,**F**, Reverse polarity, characterised by apical localisation of the nuclei and enhanced cytoplasmic granularity in the basal part, was also observed in all cases (**E**, BPTC15; **F**, BPTC19; scale bar 100 μm). **G**,**H**, All cases displayed grade 2 nuclear atypia, with irregularities of the nuclear membrane, nuclear grooves (**G**, BPTC19, scale bar 50 μm) and intranuclear pseudo-inclusions (**H**, BPTC19, scale bar 50 μm). In **G** and **H**, the highlighted nuclei with dashed lines are shown in the inset. **I**,**J**, BPTC19 diffusely expressed CK5/6 (**I**, scale bar 200 μm) and lacked a myoepithelial cell layer, as shown by immunohistochemistry for smooth muscle myosin heavy chain (**J**, note a vessel wall as internal positive control, scale bar 200 μm). **K**,**L**, BPTC20 diffusely expressed CK5/6 (**K**, scale bar 200 μm) and lacked a myoepithelial cell layer, as shown by immunohistochemistry for p63 (**L**, note the normal duct as internal positive control, scale bar 200 μm).

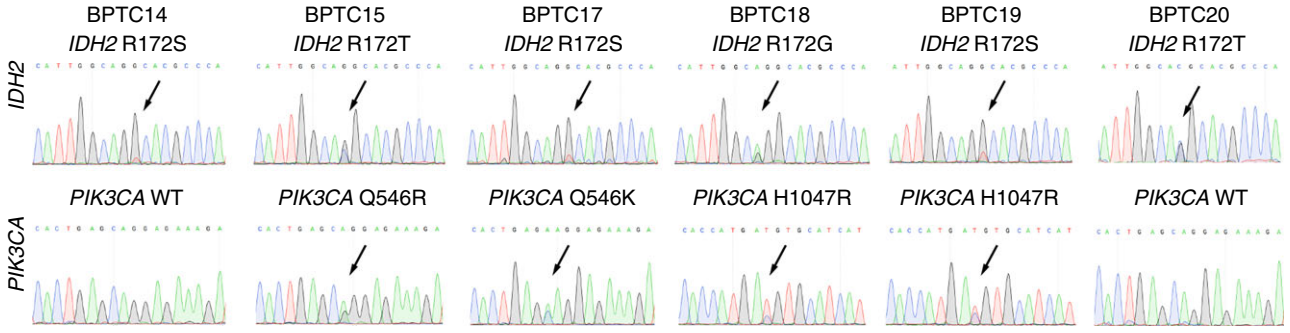

Figure 2. *IDH2* R172 hotspot and *PIK3CA* hotspot mutations identified by Sanger sequencing analysis in solid papillary breast carcinomas resembling the tall cell variant of papillary thyroid neoplasms (BPTCs). Representative sequence electropherograms of the *IDH2* R172 hotspot and exons 9 and 20 of *PIK3CA* identified by Sanger sequencing in the six BPTCs studied.

features similar to those found in BPTC remains to be defined.

The identification of BPTC in clinical practice carries important implications. BPTCs ought to be distinguished from conventional ER-positive SPCs, but still recognised as a primary invasive neoplasm of the breast requiring complete surgical excision. While its infiltrative nature may be best ascertained with immunohistochemistry,[1] its immunoprofile with CK5/6 expression and non-homogeneous cytomorphology may be interpreted as evidence of a hyperplastic process.[2] Interestingly, a link between BPTC and infiltrating epitheliosis, a complex sclerosing lesion with overt, CK5/6-positive epithelial proliferation,[16] has been hypothesised due to similarities in immunoprofile.[2] While recurrent *PIK3CA* mutations have been documented in infiltrating epitheliosis, *IDH2* gene status has yet to be investigated.[16] Furthermore, BPTCs ought to be distinguished from breast secretory carcinomas, which display a similar triple-negative phenotype and CK5/6 expression. Finally, although the number of BPTCs with adequate follow-up is limited, their outcome appears to be excellent despite the triple-negative or weakly ER-positive phenotype.[1,2] Hence, patients with BPTCs are probably managed adequately without adjuvant systemic therapy. Given that *IDH2* R172 mutations are probably pathognomonic of BPTC in the context of breast carcinomas, their identification may be used as a confirmatory molecular finding.

This study has several limitations. First, given the rarity of BPTC, the number of cases analysed is small. Despite this, we validated the high frequency of *IDH2* and *PIK3CA* mutations. Secondly, our results are limited to the selected *IDH2* and *PIK3CA* hotspot residues, hence we cannot exclude the possibility of additional concurrent genetic alterations in BPTCs, such as a concurrent mutation in a PI3K pathway-related gene in the *PIK3CA* wild-type cases. Finally, further analyses of larger cohorts are warranted to define the driver genetic alterations of BPTCs lacking *IDH2* or *TET2* mutations.

Despite these limitations, our study has confirmed the histological and genetic features that characterise BPTCs, validating the high frequencies of mutations affecting *IDH2* and *PIK3CA* reported previously,[4] and demonstrating the genotypical–phenotypical correlation that characterises this unique breast cancer subtype of favourable prognosis. As somatic mutations affecting *IDH2* R172 are vanishingly rare in other breast tumours, they may be employed as ancillary molecular markers in the diagnosis of BPTC.

Acknowledgements

Research reported in this paper was supported in part by the Breast Cancer Research Foundation, a Cancer Center Support Grant of the National Institutes of Health/National Cancer Institute (grant no. P30CA008748), and by the National Research, Development and Innovation Office (Hungary) grant GINOP-2.3.2-15-2016-00020. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of interest

The authors declare no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Representative images of haematoxylin and eosin-stained sections of each case. Cases are reported in rows at 20x magnification (left), 100x magnification (center) and 200 × magnification (right).

Figure S2. Sanger sequencing electropherograms of the region encompassing the genomic position of the *IDH2* R172 hotspot residue (left) and the region encompassing the genomic position of the *PIK3CA* H1047 residue in the conventional solid papillary carcinomas included in this study. The presence of a mutation is highlighted by a black arrow.

Table S1. Immunohistochemistry staining methods.

Table S2. Primers used for Sanger sequencing analysis.

Table S3. Clinicopathologic and immunohistochemical characteristics of six solid papillary breast carcinomas resembling the tall cell variant of papillary thyroid neoplasms included in this study.